

CHEMICAL HERITAGE FOUNDATION

KLAUS BIEMANN

Transcript of an Interview
Conducted by

Michael A. Grayson

at

Alton Bay, New Hampshire

On

29 August 2006

(With Subsequent Corrections and Additions)



Klaus Biemann

ACKNOWLEDGMENT

This oral history is one in a series initiated by the Chemical Heritage Foundation on behalf of the American Society for Mass Spectrometry. The series documents the personal perspectives of individuals related to the advancement of mass spectrometric instrumentation, and records the human dimensions of the growth of mass spectrometry in academic, industrial, and governmental laboratories during the twentieth century.

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KLAUS BIEMANN

1926 Born in Innsbruck, Austria on 2 November

Education

1951 Ph.D., organic chemistry, University of Innsbruck

Professional Experience

1951-1955	University of Innsbruck Instructor
1955-1957	Massachusetts Institute of Technology Research Associate
1957-1959	Instructor
1959-1962	Assistant Professor of Chemistry
1962-1963	Associate Professor of Chemistry
1963-1996	Professor of Chemistry
1996-present	Professor of Chemistry (emeritus)

Honors

1954	Fulbright Fellowship
1962	Honorary Member, Belgian Chemical Society
1962	Stas Medal, Belgian Chemical Society
1966	Fellow, American Academy of Arts and Sciences
1969-1977	Team Leader, Viking Molecular Analysis Team
1970	Tricentennial Medal, University of Innsbruck
1973	Powers Award, American Academy of Pharmaceutical Sciences
1974	Outstanding Spectroscopist Award, Society for Applied Spectroscopy, New York Section
1977	Exceptional Scientific Achievement Medal, National Aeronautics and Space Administration
1977	Fritz Pregl Medal, Austrian Microchemical Society
1978	Newcomb Cleveland Prize (co-recipient), American Association for the Advancement of Science
1980	Fellow, American Association for the Advancement of Science
1981	Honorary Member, Japanese Society for Medical Mass Spectrometry

1983-1984	Guggenheim Fellowship
1986	Frank H. Field and Joe L. Franklin Award for Outstanding Achievement in Mass Spectrometry, American Chemical Society
1989	Maurice F. Hasler Award, Spectroscopy Society of Pittsburgh
1991	Thomson Medal
1992	Pehr Edman Award for Outstanding Achievements in Mass Spectrometry
1993	Elected Member, National Academy of Sciences
1994	Oesper Award, University of Cincinnati
1995	Beckman Award, Association of Biomedical Resource Facilities
2001	Award in Analytical Chemistry, American Chemical Society
2007	The Benjamin Franklin Medal in Chemistry

Memberships

1955-present	Founding Member, American Society for Mass Spectrometry Member, American Chemical Society, Organic and Analytical Divisions
1967-1975	Member, Editorial Advisory Board, <i>Organic Mass Spectrometry</i>
1968-1971	Member, Editorial Advisory Board, <i>Analytical Chemistry</i>
1972-1976	Member, Pharmacology-Toxicology Program Committee, National Institutes of Health
1973-1989	Member, Sub-commission on Mass Spectrometry, International Union of Pure and Applied Chemistry
1975-1985	Member, Editorial Advisory Board, <i>Biomedical Mass Spectrometry</i>
1980-1984	Member, Life Science Advisory Committee, National Aeronautics and Space Administration
1980-1987	Member, Editorial Advisory Board, <i>Fresenius' Zeitschrift für Analytische Chemie</i>
1981-1996	Member, Editorial Advisory Board, <i>Mass Spectrometry Reviews</i>
1982-1988	Member, Board of Trustees, Drug Science Foundation
1983-1986	Member, Board of Scientific Counselors, National Institute of Environmental Health Sciences
1984-1991	Member, Commission V.2 (Microchemical Techniques), International Union of Pure and Applied Chemistry
1985-1989	Associate Editor, <i>Analytical Chemistry</i>
1988-1998	Member, Protein Society
1990-1996	Member, Editorial Advisory Board, <i>Journal of Protein Chemistry</i>
1990-1996	Member, Editorial Advisory Board, <i>Journal of American Society for Mass Spectrometry</i>
1991-1996	Member, Editorial Advisory Board, <i>Protein Science</i>
1991-1998	Member, American Peptide Society

1995-1998

Member, NAS/NRC Standing Committee on Program and
Technical Review, U.S. Army Chemical and Biological Defense
Command

ABSTRACT

Klaus Biemann's oral history begins with a discussion of his youth near Vienna, Austria. As pharmacy was the family profession, Biemann chose to study it at the University of Innsbruck. He soon developed an interest in organic chemistry, however, and shifted his focus, becoming the only graduate student in this field at that time at the University of Innsbruck. Upon finishing his degree, Biemann then received an appointment at the University of Innsbruck, in the context of which he discusses his experiences as well as the post-World War II university environment. After a summer at MIT working with George Buchi, Biemann decided that the American academic system offered more opportunities than the European one and he subsequently accepted a post-doctorate position at MIT. After two years he was appointed to a faculty position in the analytical division by Arthur C. Cope, the Head of the chemistry department. Early in his tenure at MIT, Biemann's research interest shifted from natural product synthesis to the mass spectrometry of peptides and alkaloid structure. He explains how his early work expanded the perceived applications of early mass spectrometry. While talking about his research at MIT, Biemann reflects on the need to develop new experimental approaches to mass spectrometry, using IBM punch cards, writing computer code, etc. It was even difficult to get the structures of new alkaloids published, because of the novelty of the methodology used; he also discusses his funding from NIH, the first NIH Mass Spectrometry Facility grant, and support from NASA during the Apollo and Viking missions. After almost twenty years of transforming the chemistry department, Cope left MIT and Biemann became the only analytic chemistry professor in the department. In 1958, Biemann began attending the annual meetings of the American Society of Mass Spectrometry, to which he and his research group contributed much over the ensuing forty years. Throughout the oral history Biemann discusses many topics relevant to the evolution of mass spectrometry in organic chemistry and biochemistry, including computerization, the environment, and space science.

INTERVIEWER

Michael A. Grayson is a member of the Mass Spectrometry Research Resource at Washington University in St. Louis. He received his B.S. degree in physics from St. Louis University in 1963 and his M.S. in physics from the University of Missouri at Rolla in 1965. He is the author of over forty-five papers in the scientific literature. Before joining the Research Resource, he was a staff scientist at McDonnell Douglas Research Laboratory. While completing his undergraduate and graduate education, he worked at Monsanto Company in St. Louis, where he learned the art and science of mass spectrometry. Grayson is a member of the American Society for Mass Spectrometry [ASMS], and has served many different positions within that organization. He has served on the Board of Trustees of CHF and is currently a member of CHF's Heritage Council. He currently pursues his interest in the history of mass spectrometry by recording oral histories, assisting in the collection of papers, and researching the early history of the field.

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INTERVIEWEE: Klaus Biemann
INTERVIEWER: Michael A. Grayson
LOCATION: Alton Bay, New Hampshire
DATE: 29 August 2006

GRAYSON: You have lived in Alton Bay, New Hampshire, since 1965?

BIEMANN: Yes, and this is our permanent home since 1998, after my retirement.

GRAYSON: Do you stay here in the winter?

BIEMANN: When it gets really tough in the beginning of January, we go down to the Boston area. We have a condo in Concord, Massachusetts. I use the time to go to my office at MIT [Massachusetts Institute of Technology] and try to straighten out the loose ends.

GRAYSON: I read all of the material you sent me,¹ and I was aware of some other things from prior reading. The major points of your career, in your life, are fairly well defined in the literature that exists from the various articles that you have written. I would like to explore things that are a little more intimate and personal that you do not normally put in journal articles.

My understanding is that you were born in 1926 in Austria.

BIEMANN: I was born in Innsbruck, which is in the western part of Austria. At the age of four, my family moved to the Vienna area, which is in the eastern part of Austria. I grew up more or less in a little town north of Vienna, Klosterneuburg, which is a bedroom town of Vienna. I went to school there.

GRAYSON: How did you become interested in science?

BIEMANN: The European system of grammar school, high school, and university is quite different from that in the U.S.

¹The most significant parts of the material are the three papers located in the appendix to this oral history.

My father, Willibald Biemann, was a pharmacist. It was more or less self-understood that I would study pharmacy. My older sister was also a pharmacist. It was a family profession, even though my father was the first. I did not get excited about science for any one reason; it was just understood because my father was a pharmacist, which in Austria and Germany is quite different from what it is here. The social order goes a medical doctor, a lawyer, and then a pharmacist.

GRAYSON: Pharmacist was a higher-ranking social position.

BIEMANN: Also, a professional position. My father owned the pharmacy, which was in the house where we lived. I more or less lived in the apartment above the pharmacy. I had access to all the things, and of course, my father was involved in it. At that time, the pharmacist made many of the prescriptions from individual components.

GRAYSON: How does that compare to the gamut of medicines that would be in a pharmacy today, as opposed to the 1920s and 1930s?

BIEMANN: There were much fewer chemical medicines. Back then, there were many natural products—teas made from plants and extracts of plants—and some chemical things like aspirin, and antipyrin. The doctor prescribed mixtures of those on an individual basis, so the pharmacist had to measure them out, mix them up, and put them into little paper envelopes, or make them into pills. There were lots of chemicals around. In high school, I just turned out to be interested in chemistry, to the point that I always had an “A.” I was ahead of the other kids. That is why I got into science.

Then I went to the university after World War II. My father had died by that time, and it was understood that I would study pharmacy. There was never any question. While doing so, I realized that I was very interested in chemistry. My family had lost the pharmacy at the end of the war because that part of Austria was under Russian control, and my mother, Margarethe Biemann, and my two sisters did not want to live there. They left to stay with a friend of the family who had a large house in a small village near Innsbruck. Although there was no longer a reason for me to study pharmacy, I did so and finished the course with a master’s degree in pharmacy in 1948 from the University of Innsbruck which I had entered in 1945.

GRAYSON: The western part of Austria was not under Russian control?

BIEMANN: Austria was divided into four zones, the east was under Russian control, the southeast under British control, the central part was American, and the western part was under

French control. You could easily travel around in the three western zones but once you entered the Russian zone, crossed the preliminary border, you had to watch out a little bit.

When I finished with my degree in pharmacy, I had become interested in organic chemistry. It was not necessary for me to take a job as a pharmacist, so I continued with chemistry. In the curriculum, the first four semesters for pharmacy and chemistry were practically the same, so it was relatively easy to switch without having to start from the bottom.

GRAYSON: You decided to go into chemistry, specifically organic chemistry?

BIEMANN: Yes. That was closest to my pharmacy beginning. It also was more interesting, or it seemed to be more interesting. My mathematics background and talents were not that great, so I could not go into physical chemistry.

GRAYSON: When did you opt to go for a Ph.D. degree?

BIEMANN: In 1948. Because of the circumstances, after the war it turned out that I was the only graduate student in organic chemistry because, at that time, girls -- now you have to say young ladies -- generally did not go into chemistry; there were lots in pharmacy, but none in chemistry. And the men of my age were still prisoners of war in various places; unfortunately some of them never came back, so I was the only student in organic chemistry.

GRAYSON: Did you get involved in any military activities during that period?

BIEMANN: When I was about sixteen and a half the boys in the school had to man anti-aircraft guns to free the real soldiers who were at the front lines. We attended school with our teachers during the day, except when an alarm sounded and we had to run out. That lasted for a year and then my term was over. After that, I had three months at home because my father had just died. My sister was running the pharmacy, but needed some help. Since I was the next in line to be a pharmacist in the family, I was allowed to help her. At that time, one had to practice two years in a pharmacy before one could study, a practice that was later changed to the old Austrian system.

GRAYSON: You actually had to work as an apprentice before you could study pharmacy.

BIEMANN: Yes. That was the German system. At that time, Austria was part of Germany. After the war, it changed back to the Austrian system where one studied first and then did your

two-year apprenticeship. That was another fortunate thing because otherwise, if I decided to do pharmacy, I would have to spend my first two years in the pharmacy. I spent three months at home, before I was drafted into the army in June of 1944. I was able to survive unscratched. I was in the eastern theatre, and avoided becoming a prisoner of war by, on the last day of the war, putting on civilian clothes and simply walking home. Since I was very young, and looked even younger, I had no troubles getting back to where my mother and my sisters were.

GRAYSON: When the war ended, was it common knowledge that the war was over?

BIEMANN: By that time, we were in a place north of Dresden which is pretty far into Germany so it was quite obvious that the war would be over in a very short time. In fact, it was over on that day.

GRAYSON: You assumed that the war was over to a certain degree.

BIEMANN: Yes, before the Russians arrived.

GRAYSON: So the objective of your family was to avoid being in a Russian occupied area at all costs.

BIEMANN: Both my mother and sisters moved from Vienna to Tyrol, and I managed to get out of what then became East Germany.

GRAYSON: So then, you went to Innsbruck University and pursued a Ph.D. degree in organic chemistry. How did you select the professor you studied under?

BIEMANN: Again, I was the only student at that level at the time. There was only one professor and he was head of the organic chemistry department. I did not have a choice, so I worked for him. He was a pharmaceutical chemist. He spent his career before that in a pharmaceutical company in Hungary, and later on in Germany. Only at the end of the war, he somehow ended up at the University of Innsbruck as a professor.

GRAYSON: What was his name?

BIEMANN: Hermann Bretschneider. It took three years to get it all done, from 1948 to 1951. I worked quite closely with him, even before I was his graduate student when I had to make up the difference between the pharmacy and chemistry curricula. I had to do all the laboratory experiments, which were mainly synthesizing compounds. It was more or less my job to work out those experiments just like a teaching assistant. However, there was no curriculum in that detail and he wasn't familiar with it, so he had to start out making up a curriculum. I was his guinea pig for lots of laboratory experiments. That generated a relatively close relationship with him, and then the obvious choice was to be a graduate student under his mentorship. Once I got my PhD, I was right away appointed as an instructor.

GRAYSON: At Innsbruck?

BIEMANN: At the University of Innsbruck. That involved taking care of the undergraduate laboratory, which had pharmacy and chemistry students combined.

GRAYSON: I want to get a feeling for what the university existence was like then because the war had just finished. There was a great deal of unrest and the normal flow of daily life was really fairly abnormal. There weren't many students at the university, or, as you say, most of the men were still in prisoner of war camps. But the university still tried to go forward.

BIEMANN: Yes. It was mostly filled with people who just graduated from high school and therefore hadn't served in the army. There was not a full set of students around; but everything was in turmoil and parts of the university were destroyed. Things were partly in temporary quarters, and as far as the laboratory goes, it was hard because in a chemical laboratory you have to use gas for Bunsen burners. But there was gas only available for two hours every day, mainly so people could cook lunch. We could only work in the laboratory for those two hours. It was also cold, during the winter of 1945. Our professor told us to bring a brick or two to the lab. He told us that when the gas came on to heat them up over the Bunsen burner, then stand on them, so that the students could at least keep warm and do the experiment. As soon as the gas went off the students could only write up what they did or do anything that didn't need any gas and then go home.

The European – particularly the Austrian-German academic system (and to a certain extent also the French) is very different from the one in the United States. There is no set curriculum. The students would just have to take the required courses and then take the required exams. It was not that in mid-term the students did an exam, and at the end of the term you took an exam and that was it. The students took the exams sometimes a year later after the course. In the United States, if you know when the person is born, you can calculate in 98 percent of the cases, when he or she gets a Bachelors degree; but in Europe, that's not at all the case.

At European universities, the entering students already know what field they want to major in. But in Europe, it's up to the student how they progress; so many students take a long time to finish for all kinds of reasons. But after the war, everything was in reasonable disarray and everybody tried to pick up their life, and usually under quite different circumstances. People worked hard and tried to get things done. This was not only at the university but everywhere—that was how the “Germany economic miracle” after World War II came about—that people were very dedicated to get things rebuilt. Nobody worried about benefits or pensions. Now it's just the opposite.

I was the only student, and in a not rigorously defined system. I could either goof off, or I could get things done, and get ahead, at my own speed. I was fortunate to be in that situation, so, I could do things as fast as possible. It was almost automatic that after I got my Ph.D. that I would be a lecturer. I could have stayed in the academic system, which in the Austrian-German system is somewhat different from here. Namely, that if you kind of do your job, since the universities are run by the government, all the employees are government employees, so if you make a certain step that would be equivalent to getting tenure here, it was even more fixed in what you needed to do to stay on and get your paycheck.

GRAYSON: So I understand that they still do in Germany have this period of *Habilitation*?

BIEMANN: Yes. That is the equivalent of tenure here. I understand that it is now being reconsidered and changed. But I could have stayed on for that.

GRAYSON: How long would that have typically been for the *Habilitation*?

BIEMANN: Between five and ten years. Once when I went over to the dean's office at Innsbruck, I saw a little announcement for a program at the Massachusetts Institute of Technology, which I had no idea what it was, for a summer program for foreign students. I applied for it, and was accepted along with one other Austrian, there were sixty every year.

GRAYSON: Sixty throughout Europe?

BIEMANN: There were sixty at that time throughout the world that had been affected by the war.

GRAYSON: Was this for students only?

BIEMANN: It was for people who had a final degree and had a job at a university or a government research laboratory that could not come to the U.S. for an entire year, but could come for the summer. It was started by a group of MIT undergraduates who had been in Europe during the war, and had seen the destruction of practically everything including universities. They felt that MIT could do something by having people come over to do some of their research that they couldn't do at their home university for lack of instrumentation or facilities. I applied, and came here to MIT by boat, as was common.

GRAYSON: Was that a week to get across the Atlantic?

BIEMANN: It took eleven days, coming and I returned on the *S.S. United States*, which, at that time, had the Blue Ribbon. It was the fastest passenger liner and took only four days to get back, it's a little different than today.

GRAYSON: What summer was that?

BIEMANN: In 1954. I was three years out of graduate school at that time.

GRAYSON: You were teaching organic chemistry at Innsbruck and taking care of teaching assistants during that time?

BIEMANN: I was teaching a course in analysis of pharmaceuticals, which later had some effect.

GRAYSON: What methods were you teaching in that course?

BIEMANN: Wet chemistry, melting points, and color reactions.

GRAYSON: What did you do at MIT when you were there that summer?

BIEMANN: I worked with Professor George Buchi who received his Ph.D. at ETH [Swiss Federal Institute of Technology] in Zurich, Switzerland and was an assistant professor here at MIT. I was assigned to his laboratory because the head of the department Arthur Cope, felt that it would be easier if I was in a laboratory with somebody who could speak German. But one of

the points of coming to the U.S. was to perfect my English, so we really didn't speak much German.

GRAYSON: You had taken English as a language during your schooling?

BIEMANN: Yes. That was automatic. I had eight years of Latin, and five years of English. I can remember more of English than Latin. But Latin helps in foreign languages, because of the grammar, it is related to the grammar of German, French, and Italian, and not so much to English, because English grammar, fortunately, is so simple.

GRAYSON: Summer is not a very long period of time, what were you able to accomplish in that time period?

BIEMANN: I worked on some structural problems, and extended my stay until the end of November because the visa was for six months, so I could stay for that long. I didn't have to be back by the beginning of term. It certainly was a big turning point in my career because Professor Bretschneider was the typical professor in the Austrian-German system, like he is the general and he has some lieutenants, working under him, who take care of the troops. To stray out of that system was not advisable and I applied for the fellowship without his support but it wasn't needed because the application didn't ask for reference letters. The application asked what I was doing and where I lived.

When I came back, he never asked me what I did, or what I learned because it was outside of the box. I could have gone towards *Habilitation* with his blessing, and that required - on paper - that it would be independent research but it was also understood that I could work on some of my own ideas. In general, I had to work on his research, and eventually he would permit me to publish some papers under my own name without his name on it too, to satisfy the requirement. But after seeing that there was another way of academic life in the U.S., I didn't want to go that track. So, I decided to come back to the United States.

GRAYSON: You stayed at Innsbruck until the next summer?

BIEMANN: I came back to Innsbruck in the beginning of December of 1954, and I left end of September of 1955. When George Buchi found out that I was interested in coming back, he offered me a post-doctoral fellowship, which I accepted.

GRAYSON: You expressed an interest in coming back to the United States and then he offered you the position?

BIEMANN: Yes. Originally, there was a little twist to it because when I arrived in 1954, on 1 June, Professor Erika Cremer, the head of physical chemistry at the University of Innsbruck was on a sabbatical at MIT. She knew that I was here, and knew George Buchi. She told him that he had to promise her that he will not keep me here, because she wanted me to stay at the University of Innsbruck. When I left, he didn't say "If you want to come back, you always are welcome here." But I had made contacts with a well-known steroid chemist at the University of Pennsylvania in Philadelphia, at the medical school. So the steroid chemist offered me a job when I met him at ACS [American Chemical Society] meeting in New York, in the fall of 1954.

I wrote a letter to George Buchi telling him that I was thinking about coming back to the U.S. and what did he think? Should I take that job with Professor Ehrenstein? He said, "That would be okay. It would be a good entry into the pharmaceutical industry, but why don't you come back to MIT." Since I decided to leave Innsbruck, he wasn't bound anymore by the request from Erika Cremer. By the way, I should mention that she was the original inventor of gas chromatography. But, that is hardly known, because she did gas solid chromatography as a physical chemistry experiment to determine heats of adsorption. She wrote the paper in 1943, maybe 1944 and sent it in. Her publication was accepted. It was set in print and before the journal was published the publishers' building was bombed, and everything disappeared. Later, after the war, Professor Martin from the UK [United Kingdom] came to her laboratory and saw her experiment. Soon thereafter, he published his paper on gas chromatography with Dr. James Cremer's original paper was finally printed about thirty years later in the journal *Chromatographia* in Germany.

By October 1955 I was back at MIT as a post-doc with George Buchi.

GRAYSON: When you came this time, were you planning on staying in the States?

BIEMANN: Yes, I had an immigration visa, a green card; a legal immigrant.

GRAYSON: MIT was also probably in some form of turmoil as well. Obviously, it wasn't affected by the war physically, but the people's lives had been interrupted, and this was in the early 1950s. Things had probably settled down by then.

BIEMANN: Yes. That summer program started in 1947 or 1948 because those undergraduates who started it had come back to finish their studies. The program was financed by the Alfred P. Sloan Foundation.

GRAYSON: I was wondering where the money came from for that. It's a neat idea but the undergraduates didn't have any money.

BIEMANN: At the time when I came, in 1954, the effect of the war was done and it was an important period for MIT because after World War II the president of MIT wanted to change and modernize the institution. Until then MIT was an engineering school, with science more or less supporting the engineering school. All of the scientific developments generated during World War II because of the Manhattan Project; also because of the need to make synthetic rubber and improve the refining of crude oil, of course, the great thing was medicine. For example, penicillin was discovered in England a few years before. There was a big push to manufacture penicillin so that it could be made chemically and not just biologically for the battlefield. By the end of World War II, chemistry had a much bigger impact on everyday life than it did before. Therefore, it was important to upgrade the chemistry at the educational level.

The president of MIT appointed Professor Arthur C. Cope as a new head of the department with the mandate to put chemistry at the top. He began to hire a completely new group of faculty who were in the line of those new fields. He let the untenured ones go and the tenured ones just stayed and had offices and a laboratory; and of course, salary. George Buchi was one of the new hires because he was a natural products chemist, trained in Switzerland. I should mention Professor John Sheehan who was also an organic chemist and eventually developed a synthetic route to penicillin so that penicillin could be made chemically and not just bacteriologically.

Amongst those new outcomes was to revitalize the analytical chemistry division, and he hired David Hume and Lockhart "Buck" Rogers. David Hume I think was at the Manhattan Project, which produced lots of analytical and physical chemistry and inorganic chemistry. In the process of upgrading the analytical division Cope wanted to have an organic chemist in there because since he was an organic chemist he felt that there had to be an organic chemist in analytical chemistry to make sure that organic chemistry were taken care of. I had taught that course in analysis of pharmaceuticals in Innsbruck and had done a good job in the project I was working on with George Buchi, so Cope felt I might be an appropriate candidate for the position and he offered it to me. I took it because I had to think of what I would do after my two years of post-doctoral work. Staying at MIT was a good opportunity.

GRAYSON: What year was that?

BIEMANN: That was 1 September 1957. I came back on 1 October 1955. While most organic chemists would have wanted to stay in organic chemistry, I felt that I could do just as well in analytical chemistry if I worked on the right thing.

GRAYSON: What did you do for that two-year post-doctoral period?

BIEMANN: I worked on the synthesis of a natural product, called muscopyridine, which is a perfume component. It comes from the perfume gland of the musk deer, which lives in Mongolia and Tibet. Some funds for my post-doctoral position came from a company for which George Buchi was a consultant in Switzerland, Firmenich Company in Geneva. At that time, this compound was not anymore used but it was an interesting structure and George Buchi said it was certainly important to synthesize it, proof of the structure, a ten to eleven step synthesis that a graduate student of his had completed half. When I started, there was no material left to continue the remaining synthesis, so I had to repeat the graduate student's work on a larger scale and carry it through. That got me very well acquainted with physical chemical methods, particularly ultraviolet, and infrared spectroscopy.

GRAYSON: What was the state of the art, of the IR, UV at that period? It was fairly primitive still, wasn't it?

BIEMANN: The UV spectrophotometer we used at the laboratory was still the Beckman model, where you determined point-by-point the spectrum. The earlier infrared spectrograph was the same way. When I finished the work on muscopyridine, we already had a bench top recording, little infrared machine specifically designed for qualitative, organic, infrared spectroscopy.

GRAYSON: Do you recall what that was back then?

BIEMANN: I don't remember.

GRAYSON: It had advanced to a recording bench top instrument.

BIEMANN: Yes. It was about two feet by three feet, and maybe one foot tall. The paper was on a vertical cylinder, so it was sticking out like a smokestack.

GRAYSON: It could be a PerkinElmer [PerkinElmer, Inc]. It sounds a little bit like PerkinElmer design.

BIEMANN: The original big machine was a Baird, built exactly after a prototype that was developed either by duPont or Dow — I don't remember. Again, another development that happened during World War II.

GRAYSON: As an instructor, that's the starting level for a tenure track at MIT at the time. You started doing organic synthesis.

BIEMANN: No, I could not do organic synthesis, which was the area in which I was trained. I had to look for something that was more analytical. I figured out a scheme to determine the C-terminal amino acid in a small peptide. Fred Sanger in the UK had developed a method for marking the N-terminal amino acid of small peptides, which he had used for determining the amino acid sequence of insulin; the first protein – insulin - for which a primary structure was determined. I figured if I could do the same thing with the other end of the peptide, I could simplify a rather tedious work. I planned to use a reaction, which I had developed, while still in Innsbruck, for making certain compounds that may have medical applications. I thought of using it on a micro-chemical level on small peptides.

I sent in a grant application to NIH [National Institutes of Health] that was funded, but in the meantime, I found out about mass spectrometry. That was by accident that was a fortunate happening. While I was still a post-doc, Firmenich, the Swiss company which had financially supported my position, was in the flavor and fragrance business and wanted to find out what happens at a conference in that field in Chicago. Instead of sending someone from Switzerland to Chicago just to attend the conference, nowadays routine, they asked George Buchi to go there and listen to the talks. He was not interested in it because it wasn't anything that really concerned his field. So he asked if I would go for a few days to Chicago and attend a conference and write a report. I did, and took my first airplane ride.

GRAYSON: What did you fly in? Do you remember? Was it the old DC-3?

BIEMANN: No, it was Lockheed. I listened to the talks and one of them was by Dr. Stahl from the Quartermaster Research Center in Natick, Massachusetts. He talked about identification of flavors using a mass spectrometer to identify the compounds. The Quartermaster Corps, that part was involved in the preparation of food rations for the armed forces, so there was a lot of dried food and reconstituting, and the important thing was that it should taste reasonably well. So they had to look at the flavor components. He looked at the volatile stuff that comes off using a mass spectrometer on simple things like methyl butyrate and butyl acetate which he then identified by matching the spectra with the data in the American Petroleum Institute collection. They had compiled mass spectra of all kinds of compounds, mainly hydrocarbons, but also other small, volatile molecules.

At that time, one identified mass spectra by matching them with those of known compound. So, the significance of the method didn't sink in to me right away. Later on, when I was thinking about what to do since I had been working on structure of natural products by synthesis where at each step I had to identify and make sure that what I wanted to make was what I had at the end. That involved a lot of qualitative analysis of the products. During the writing of the NIH proposal on the sequencing of peptides by chemical methods, I realized that

mass spectrometry might be able to do that as well and better. I got the grant for the chemical method but the rules allowed me to use different methods like mass spectrometry. That started my work on mass spectrometry of peptides.

GRAYSON: Was the conference a full week conference or couple of days?

BIEMANN: It was three days.

GRAYSON: Did this fellow at Natick mention the type of instrumentation he used?

BIEMANN: Yes. He used a CEC 21-103.

GRAYSON: Was this in the 1957-58 timeframe?

BIEMANN: It was in the spring of 1957.

GRAYSON: When this grant came through you decided that maybe mass spec has potential, but you didn't have a mass spectrometer, so what did you do?

BIEMANN: That was the problem. I went to Arthur Cope, the head of the department, and asked, "Why don't we have a mass spectrometer?" He said, "Because it's a big expensive instrument, that needs a full time engineer to run it and keep it running, so we don't have one." By that time I had looked into it quite a bit so I said, "No, I think I can keep it running without an electrical engineer." He said, "Okay if you promise that it won't collect dust, I promise to find some money." When he was appointed head of the department, after the war with the task to revamp the department, he said he needed money for that. He had at his disposal an unrestricted amount of money. Not unrestricted in dollars, but unrestricted in what he could do with it; it was completely up to him.

He used \$50,000 of that money towards that instrument, and \$10,000 I got from that company in Switzerland, Firmenich. The reason why he made that comment about collecting dust was that he was worried that we would buy that instrument, and it would be delivered, it wouldn't do what I wanted it to do, and therefore it would just sit there and would be hard to get rid of it. But fortunately, that wasn't the case.

GRAYSON: Then you purchased the 103 instrument?

BIEMANN: The 103C, from CEC, which was delivered in May of 1958.

GRAYSON: You had been working on the chemistry you needed prior to that so you would be able to use the instrument right away?

BIEMANN: Because the peptides are not volatile, I had to devise a method of chemical conversion of the peptide to something that is more volatile -- which I did by converting it to a polyamino alcohol; which is a linear molecular exactly like the peptide, but it was much more volatile. And of course you weren't supposed to put things like that into a mass spectrometer because the reason why the mass spectrometer existed at that time -- the commercial types -- was that the petroleum industry during World War II had to produce more and better fuels, in part, for the Air Force. The analysis of the product from crude oil to gasoline to jet fuel was very important.

Mass spectrometry was both sensitive and highly accurate so it could do that quantitative analysis, but because of the accuracy that was required, when one was dealing with complex mixtures the signals had to be very accurate, and very reproducible, because we had to use the intensities of the signals from standard pure compounds, as a matrix, a mathematical matrix I mean. Since you didn't want to have to run the standards each time that you did an analysis you had to rely on the standard data being very, not only accurate, but also highly reproducible over a long period of time. This required that the mass spectrometer was always in good shape. A crucial part of the spectrometer is the ion source where the ions are produced. It ran at high potentials, like three kilovolts, so the ion source had to be very clean. Otherwise, the potentials through electrical leakage would fluctuate and you couldn't get a good reproducible spectrum.

The rules that could not be violated were that the ion source had to be extremely clean and it had to be at a highly precise temperature, 250 degrees, plus or minus a tenth of a degree. The compounds that I wanted to put in would immediately dirty up the ion source; I mean dirty in the context of quantitative analysis. It always had to be pumped out of the ion source before putting in the next sample. It had to be very easily pumped off and it had to be very volatile; it could not contaminate the surfaces of the ion source. Even the acceptance test, which called for an analysis of ten hydrocarbons in the range from four carbons to eight carbons, was of no use to me because that wasn't what I wanted to do. I asked them that instead of hydrocarbons of that size to use alcohols of that molecular size.

CEC, the manufacturer was very worried about that because they thought it might not work, and then I say it doesn't work and they would have to take it back. But I wasn't interested in a real quantitative long-lasting analysis of that kind. The installation engineer, Don DiQuasie, after he installed it, which took four weeks and two weeks to train us to run it. He said he would do the acceptance test and that it would work. When it was time to do that test he

was called away on an emergency to some refinery, so he couldn't do it, and he said for me to do it.

I ran the acceptance test and was perfectly satisfied with the performance of the instrument. But that was one aspect that sort of helped me, except I didn't know all the rules of the games of mass spectrometry—what you do, what you don't do, and what you never should do.

As I said, it worked out quite well. Also, because one had to keep the ion source very clean, you're not supposed to clean it yourself. It needed cleaning every two or three months perhaps; so you treated the instrument with kid gloves. You had to send the ion source back to the manufacturer in Pasadena who would clean it and send it back. We needed to have two ion sources because it took a while to get it back. Well, we didn't want to have that problem and associated expense. After two or three times of doing that, we just took it apart ourselves, cleaned it and put it back together.

GRAYSON: Did this still have all glass inlet system, glass connections, and glass flight tube?

BIEMANN: Yes, but not the flight tube, which was metal.

GRAYSON: So every time you got into the instrument you had to have a glass blower?

BIEMANN: Yes, you had a glass blower. There was a big ball joint which connected the glass part of the inlet system to the stainless steel part, which was the flight tube, and the part the ion source was in was sealed by Apiezon wax. The glass blower had to come with his torch, heat up that ball joint so that the wax got soft and then we could move the entire inlet system away and get to the ion source, and take it out.

GRAYSON: If they are going to run the ion source at 250 degrees, then that ball joint has to be greased with something that's pretty non-volatile. It sounds like glue I guess.

BIEMANN: You had to heat it up quite a bit to get it flowing enough that you could take it apart. Putting it back together was again a problem. That inlet system could be heated to 250 degrees I think, at least 230 degrees.

GRAYSON: You had a mass spectrometer. You started in on this problem with the sequencing of the small peptides?

BIEMANN: Yes. We started on two separate projects. One was peptide sequencing which required me to work out the chemistry to convert the non-volatile peptide to a more volatile material that retained the sequence information; it retained the backbone but removed the polar groups. That took quite a bit of laboratory work. With the grant from NIH came a post-doc and then I got another grant from NSF that provided for another post-doc; so those two worked on that chemistry at the outset. They were both from the University of Innsbruck because that was my main connection. I wrote to two people, Sepp Seibl and Fritz Gapp, and asked if they want to join me in the U.S. They had just graduated, got their Ph.D. in organic chemistry, and were each working at a different pharmaceutical company in Austria. They saw that I was doing pretty well at MIT, so they agreed to come and work for me.

So I did something else, I started work on the structure of alkaloids that is a completely different field from peptide sequencing, but more closely related to my work with George Buchi, as a post-doc. I had learned a lot about alkaloids and other natural products and there was a compound available to me which was an indole alkaloid called sarpagine. I determined its structure which had been proposed in the literature; but people couldn't prove it because the proof by conventional means was quite complicated and time consuming. I did it by mass spectrometry, where you didn't have to make exactly the same compound that you could use for comparison, but only a similar one. That was a quick project that was successful and led me into the field of structure determination of alkaloids by mass spectrometry.

GRAYSON: You did that independently of the work of the post-docs?

BIEMANN: Yes, because it didn't need much lab work. I just needed to run the spectrum of the compound, then one or two chemical reactions, and the thing was done.

GRAYSON: The whole idea of determining the structure of an alkaloid by mass spectrometry, that came to you as a result of your own background. Also at that time there was some information being published about people understanding how hydrocarbons fragmented. There were some fundamental studies that were being done, and people were trying to understand why certain fragments were formed and others weren't. Did the fragmentation studies that had been done prior give you any help or insight?

BIEMANN: Yes. Those studies were done mainly on small volatile compounds of known structure, and of course, Fred McLafferty was one of those, at that time at Dow Chemicals [The Dow Chemical Company] who was involved with running lots of mass spectra of known compounds, and then trying to correlate the spectra with the structure. Those were quite a few experimental rules available of which bonds cleave and which don't. That information could be used to make sense out of the mass spectra of much more complicated molecules.

GRAYSON: This is probably the first time alkaloids had ever been run by a mass spec?

BIEMANN: Yes.

GRAYSON: Because most people were interested in the simpler compounds or the hydrocarbons.

BIEMANN: The idea of determining the structure of an unknown compound by mass spectrometry was not around. It was mainly determining the mass spectra of known compounds to characterize them, and to be able to identify them when you run across them in another situation. Because of that, you had to have the mass spectrum of the known compound. But that would never have been possible in the case of peptides. Consider the dipeptide, in which two amino acids are linked together; since there are 20 naturally occurring amino acids, this gives you 400 dipeptides that are possible. This is only the smallest one.

Then tripeptides (they are three amino acids long) would be 8,000 so you never could generate a library of authentic spectra to match it. You had to be able to interpret the spectrum from scratch. The same thing was true for alkaloids. There were many known ones, but nobody was interested in those anymore, once you know the structure, that's it. But at that time there were lots of alkaloids of unknown structure around because the pharmaceutical industry was looking for them for medicinal purposes. They all came from plants. Since the determination of such a structure was very complicated, tedious and time consuming, people published the intermediate steps of their work. They did some chemical conversions of the molecule and various reactions on it. From the outcome of some of these reactions you could tell whether it has a hydroxyl group on it or an amino group or things like this. And from the UV spectrum you could tell what aromatic substitution it had.

People published those steps and said that they think it's about this type, and the next time around that it may be this structure, or maybe that structure and now we have to prove that. There was lots of information in the literature about the incomplete structures. I could just read those papers and see with which one, one might be able to do it by mass spectrometry simpler, and that worked out well.

Since I needed some alkaloids of known structures, I wrote to people who had published a real complete structure and asked them to send me a sample of the compound. I need only very, very little which was important in that field. I established contacts, first with Bill Taylor [William Taylor] at Ciba Pharmaceuticals, in Summit, New Jersey. It was a Swiss company but they had a research laboratory in New Jersey. And then shortly afterwards with Norbert Neuss at the Lilly Research Laboratories in Indianapolis and we worked together. They had isolated many new alkaloids but could work only one after the other, the conventional way took a long time.

GRAYSON: You gave them results of your mass spec analyses and studies. Was it a reciprocal arrangement there?

BIEMANN: Yes. I got samples from them of both known ones and unknown ones, and helped them in their work by running the mass spectrum on the ones they were working on. That was a really sort of fruitful collaboration. I became a consultant to Eli Lilly, which lasted for over twenty-five years. Later many of those companies established their own mass spectrometry laboratories; sometimes with my help. Lilly even hired one of my post-docs, John Occolowitz. But the alkaloid work was the type where suddenly it became very fast to determine the structure of the alkaloid, particularly since they all came from tropical plants. Since the plant produces not just one alkaloid but usually a family of alkaloids with related structures, it became easy to determine their structures once you had determined the structure of one of them. You could roll up the rest of it relatively quickly just from the differences in their mass spectra. By the end of the 1950s there were many alkaloids around, particularly in the pharmaceutical laboratories. And by the early 1970s all of their structures were known.

GRAYSON: Am I correct in assuming that this is the first departure of mass spec into an area of chemistry? All the other applications were either in the petroleum industry, or some flavors work was being done. I could go back and look at the early ASMS proceedings, but I think almost everything being done was on fundamental studies. There was some ionization potential work being done. But in terms of moving the analytical capability of the instrument outside of a fairly small niche of organic chemicals this is probably one of the first departures into a different area of chemistry.

BIEMANN: It was an expansion of the analytical side of mass spectrometry into the qualitative identification of structures of natural products; structure determination of molecules of unknown structures. That made it part of the toolbox of the organic chemist. For the biochemist, it took quite a bit longer. Our peptide sequencing method was much more difficult to do experimentally. Also, Pehr Edman in Sweden had developed a chemical method to chip off one amino acid after the other from a large peptide or protein and determine the sequence that way. This technique was then automated and commercialized.

Most of the peptide and protein problems in biochemistry were at that time solved by that method. Only in those situations where it did not work or could not be applied did we use mass spectrometry to solve those problems; but the mass spectrometric peptide sequencing involved complex chemistry on a very small scale so it was not easily adapted by other laboratories. My laboratory was practically the only one that used that chemistry for mass spectrometry. All that changed when Mickey Barber invented fast atom bombardment ionization.

GRAYSON: The limiting problem was the need to get the sample into the vapor phase so that it could be ionized?

BIEMANN: The other problem was that it dealt with a very complex mixture of relatively similar molecules. In order to do a protein structure you have to degrade the protein into small pieces, into peptides. If you have a hundred amino acid long protein, which is not an unusual size, you have, theoretically 99 dipeptides, 98 tripeptides, and 97 tetrapeptides, so the mixture is very complex. Now that part we solved by developing the online GC-MS method because the products had to be relatively volatile to get in the mass spectrometer. So they were also sufficiently volatile to be separated by gas chromatography.

GRAYSON: You did the degradation first and then the chemistry?

BIEMANN: We did the degradation (partial hydrolysis) and the chemistry first, and then the separation afterwards. If we did it the other way around and separated the peptides first -- which was necessary before the advent of gas chromatography; -- for example Fred Sanger used paper chromatography for his insulin work -- then you would end up with up to 99 separate dipeptides, which would have to be chemically converted separately; which would be an enormous task. You had to do the chemistry on the mixture and then separate it and get it into the mass spectrometer. At first we did gas chromatography off-line, and then later on-line.

GRAYSON: When did you start using gas chromatography in your lab?

BIEMANN: Off-line when I started in 1958, on-line probably 1962. It was published in 1964. At first we used it on the CEC 21-110B high-resolution instrument. The first examples were on alkaloids where we separated a mixture of alkaloids from a certain plant by gas chromatography directly into the high resolution mass spectrometer. Since the alkaloids contained carbon, hydrogen, nitrogen and oxygen, the elemental composition -- which you could get from a high-resolution mass spectrometer -- was very helpful.

GRAYSON: You started out with the 103, a CEC instrument. It was designed for petroleum chemists but you modified or simply used it in the way you wanted to use it. Did you use their gas inlet system?

BIEMANN: Yes. Instead of expanding the sample from a glass bulb into the inlet system, we had to inject the sample into the inlet system, through a silicon rubber disk with a needle. We collected it from the gas chromatograph in a melting point capillary and then took it into a

needle or actually we had another little adapter that we could drop the melting point capillary into and heated it to vaporize the sample into the inlet system. There were lots of experimental tricks which I learned and weren't published in a separate paper; but that's all in my 1962 book on mass spectrometry.

GRAYSON: Then did you get a second CEC instrument?

BIEMANN: Then we first got a Bendix Time-of-Flight mass spectrometer which we used to get things directly into the ion source. When we had worked out that way of doing it we adapted it to the CEC instrument because it had much better resolution. We put the sample directly into the ion source of the CEC 21-103C through a vacuum lock.

GRAYSON: So the Bendix Time-of-Flight did have a direct insertion apparatus?

BIEMANN: Actually it came with a pyrolyzing filament, which was sitting right under the ion source. The idea was to pyrolyze non-volatile compounds into the ion source, which of course ruined the compound and you had then to piece the information together to guess what it was. But we converted the Bendix insertion apparatus to make it possible to just vaporize the sample into the ion source.

GRAYSON: So you could bypass the gas inlet system altogether.

BIEMANN: We didn't really use it, because with the Time-of-Flight instrument you saw the spectrum on an oscilloscope screen and you could take a Polaroid picture of that spectrum; which worked okay below mass 150, at best 200; and it had relatively broad and fuzzy peaks. But once we transferred that methodology to the CEC instrument, we got good mass spectra. Then all that had some influence on the manufacturing side of mass spectrometry because now there was an area where high accuracy and long-term reproducibility of the spectra wasn't important anymore. One could scan the instruments faster and record the spectra faster. This made the recording of effluents from a gas chromatograph possible, but since we first used it on a Mattauch-Herzog instrument that recorded the complete spectrum simultaneously on the photographic plate, that wasn't so much of a problem. But, recording fast became important when the photographic plate was not used, so then we developed the online computer recording of GC-MS data. For that we got an IBM 1800 computer in my laboratory which made it possible to record data directly into the core memory.

GRAYSON: I'm a little bit curious about the 110, you were using it for organic applications, so you got an EI source in that instrument?

BIEMANN: Yes.

GRAYSON: But my understanding is that originally the instrument was designed as an inorganic instrument with a spark source.

BIEMANN: A spark source, yes.

GRAYSON: Do you know what motivated CEC to develop an EI source for the instrument?

BIEMANN: That may stem from John Beynon's work at ICI Imperial Chemical Industries. John Beynon had built a Nier-Johnson double-focusing mass spectrometer for ICI. They were interested in trace analysis of organic compounds particularly in order to figure out the processes which their competitors used in synthesis. Dye stuff was ICI's main moneymaker. John Beynon built that mass spectrometer to look at the traces of left over starting materials and intermediates in the final product to figure out how they made it. He felt that to do that he should use high resolution so he could determine the elemental composition, because those were usually aromatic compounds with halogen atoms attached to them.

He built that and the design was taken over by Metropolitan Vickers [Electrical Company] which was a precursor of AEI, Associated Electrical Industries, which later on became Kratos. His instrument was officially named the MS-8, which was never produced because it was not a Metropolitan Vickers product anyway. Then Metropolitan Vickers used that design and concept to build their own high-resolution mass spectrometer, which became the MS-9. It was a Nier-Johnson geometry that didn't have a focal plane, only a focal point. It had to use an electron multiplier to detect the ions and record the spectra. You had to scan it or you had to do what was called "peak matching", namely to put one known ion onto the detector and then switch accelerating potential to get the unknown ion into focus on the detector slit and then calculate the relative mass ratio from the accelerating potential ratio. So you had to do one ion after the other. Since CEC had that double focusing instrument for inorganic analysis, they could just put an EI ion source on it. They could just build an electron ionization source for that and record the entire spectrum on a photograph plate without scanning.

We then developed the means of measuring, in semiautomatic and then automatic ways, the position of all the lines on that plate, and from the position you could calculate the exact mass down to a part per million.

GRAYSON: The photographic plate had an advantage in that it was an integrating detector, whereas you really had a mass spectrograph with the 110 while the MS-9 was a spectrometer

with a point detector. In terms of sensitivity it seems the CEC instrument would be more sensitive since you were able to integrate the signal continuously from all the ions that are produced in the ion source.

BIEMANN: You could of course, the longer you exposed it, the more signal you collected. But the only thing that was very difficult to get was the exact intensity measurements because you had to convert the blackness on the photographic plate to an abundance of the ions striking it, which is not a linear function. But again, we didn't care about whether that peak represented 10,000 ions or 10,050 ions. All we were interested in was the exact mass of that ion and the relative abundance. In other words which ones were the abundant ions, and which ones are minor ions. That was done easily by looking at it on the microscope.

GRAYSON: You bought the 110 instrument sometime in the early 1960s.

BIEMANN: Yes. It was bought 1962, the first one.

GRAYSON: This is at the same time you wrote a book on mass spec?

BIEMANN: Yes.

GRAYSON: Were you teaching any courses?

BIEMANN: Yes. There was a course in analytical chemistry that was in four segments, one each semester, so it was a two-year cycle, and I taught the organic analysis part of that. I had to teach one term every two years. My teaching load was always very light. One reason was the department felt my research was very important and I was doing a good job at it, also not having been brought up in the American strict course system, I wasn't too well adapted to going to class and teaching a lecture, then giving problems, giving exams, all the time. Things worked out well. At one point when I got the NIH facility grant which was a big grant in terms of money, people, instrumentation, and effort which I had to devote to it, I had an arrangement with the Dean of Science, who at that time was Jerry Wiesner, to not do any teaching at all, only once in a while teach a course in high resolution mass spectrometry.

GRAYSON: Did tenure come somewhere in there?

BIEMANN: I was appointed assistant professor in 1959, associate professor in 1962, without tenure, and tenured full professor in 1963.

GRAYSON: This is a fairly long period of time after you started, about eight years.

BIEMANN: Short; from starting as an instructor in 1957 to 1963, six years. From assistant professor it was only four years.

GRAYSON: I'm sure writing the book helped.

BIEMANN: Yes.

GRAYSON: What was the inspiration for that?

BIEMANN: First, I was asked in 1960 to write a chapter on mass spectrometry in organic structural chemistry. I wrote that chapter and it became obvious that there was a need for a book on mass spectrometry. I had all this information, because most of it was done in my laboratory. McGraw-Hill, a publisher that had just done a book on NMR, a fledgling technique at that time, and they did Djerassi's optical rotatory dispersion, suggested to me after they found out about that chapter, to write a book. That was a *tour de force* thing of writing a book. It got published almost four years after I had run my first mass spectrum.

GRAYSON: That probably helped with your tenure position and established you pretty well in the field. I know McLafferty had done an interpretation book, was that out by then, do you recall?

BIEMANN: No, it was not.

GRAYSON: Because it's used primarily as a textbook.

BIEMANN: That was quite a bit later.

GRAYSON: I understand that you did have problems publishing the alkaloid work because of the fact that you were determining a structure by a new method and not the old method. Were

there any other details about trying to get through that publication that you left out of your literature?

BIEMANN: No, I think that was probably about it. Except that there was the question in general of how mass spectrometric data should and could be used instead of the then obligatory element analysis. I had some correspondence with Dr. Richard Gates who was the editor of the Journal of the American Chemical Society, so I came up with some rules or definitions as to what has to be recorded if one wants to use mass spectral data, particularly to establish a molecular weight or molecular composition. That was before high resolution mass spectrometry. That alkaloid work came a little bit later because we already used elemental compositions there.

GRAYSON: Normally papers go out to reviewers, but you're saying the reviewers were okay with what you'd done, but the editor of the Journal of the American Chemical Society objected?

BIEMANN: Yes. An associate editor of the Journal gave me a hard time because I guess he was worried that now everybody would say "This is a mass spectrum of that compound and therefore the structure is such and such -- that's it -- believe me". Originally, he wanted us to provide melting points and combustion analyses for each one of the alkaloids, which we had found in that plant, and claimed that we actually have isolated and determined the structure of. The entire idea of using mass spectrometry was to eliminate all those steps, and all that wasteful burning of valuable compound just to get the carbon, hydrogen, nitrogen and oxygen values. The oxygen number was a difference anyway, so it didn't really mean much.

Eventually, he was convinced and softened it a little bit, but one of the things he indirectly complained about was that we didn't have the compound in a bottle, nicely crystalline, to take a melting point. It was one relatively valid point at that time and since it was very common that if you had a compound to determine the structure and thought it was identical to one which had been found before, that you wrote to that person and said "Please send me a small sample so that I can do a mixed melting point."; which at that time was the absolute criteria for identity or non-identity. Since we never had it in a bottle and there were only a few of them that we could crystallize, that wasn't possible. So he said "How would people be able to tell that they had isolated the same known compound for which you had already determined the structure because you can't send him the sample and he may not have a mass spectrometer." Few laboratories had a mass spectrometer at that time

I said that the person could send me a very small sample, much less than was required for a melting point, and we could run the spectrum for him, and show whether it was identical or not. That finally set the associate editor's mind to rest. In addition to the fact that the reviewers who were more familiar with what we were doing. In the alkaloid community word got around very quickly about our work, because they either read my papers or I had contacted them for samples of some known compounds.

[END OF AUDIO, FILE 1.1]

GRAYSON: We are talking about the mid-1960s and you have really pretty much established your career at MIT and now you say you actually had a second 110 instrument purchased at this time.

BIEMANN: Yes.

GRAYSON: And you had the 1800 IBM computer?

BIEMANN: We got that because of the importance of mass spectrometry in organic and biochemistry, particularly in organic chemistry and natural products chemistry and this became obvious to NIH. They thought that other chemistry departments would want to buy mass spectrometers, but the problem there was that nobody was trained in the use and operation of the mass spectrometer, and particularly not in the interpretation of the data. So NIH came to me and said that they wanted me to apply for a training grant, which NIH just started, a program to improve training in various biomedical sciences. I told them to explain to me what's involved and then I said yes. Then I need another high resolution mass spectrometer at that time. And I needed my own computer. Up until then we put all the data, particularly from the high resolution mass spectrometer, onto IBM cards, took them to the MIT computer center and had them processed and picked up the processed data. With more people working in my laboratory that wouldn't work. Of course, I wanted to have my own computer.

GRAYSON: When you say IBM cards, you mean IBM punched cards. At this point in time, the technology was such that each line of code and each piece of data required one punched card.

BIEMANN: Yes. We had automated our photoplate reader. We attached to it a cardpunch, which could run automatically. And the line positions and intensities were punched as maybe four or five data pairs on one card, that was as much data as could fit on a card. All that ran up the required budget quite a bit, and a training grant really was more to support graduate students and post-docs, not instrumentation. But NIH had just started yet another research grant category, the so-called "Research Resource" grant, with which we could buy all kinds of things. So I put in an application for a training grant and for a research resource grant which then provided a second high resolution mass spectrometer, an IBM 1800 computer, and in addition money to put the laboratory space in order, because I needed also more space.

Space is the number one problem in universities. Money is the number two problem. Everything else is below that. But it so happened that a new biology building had just been built and for some technical reasons there was an empty basement. They hadn't planned for the basement so there was no money to put any walls in it, and MIT didn't need it for faculty because the building itself held them. NIH paid for outfitting that part and even for the one room that was completely electromagnetically shielded for one of the two high resolution mass spectrometers. We wanted to do very long exposures for which any influence of outside electric or magnetic fields would be detrimental. It turned out that it was actually necessary because when the subway pulled out of the nearby subway station the current was affecting the entire area around it. We could see that on the mass spectra. It penetrated the shielded room because of the low frequency of that event. By shielding the part of the flight tube between the ion source and the electric sector, which was the longest exposed part of the flight beam, we could handle that.

GRAYSON: You picked up the electromagnetic field from the current draw when the subway went by?

BIEMANN: Yes, when it pulled out of the station.

GRAYSON: This caused the beam to—

BIEMANN: Move.

GRAYSON: Diffuse, to move.

[RECORDER OFF]

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GRAYSON: Diffuse, to move.

BIEMANN: It moved in what's supposed to be a field-free region. It wasn't field-free for maybe 15 seconds every five or ten minutes. Anyway, this large NIH grant provided us with our own computer and the IBM 1800 model was the only one that was actually designed as a process control computer, which wasn't used as much as IBM thought it would. But it had the proper characteristics which lent itself quite well to recording streaming data, which was important for us to record the signal of the microdensitometer which we used to read the plates, and then later to the GC-MS online operation. That led us into lots of computer programming because we needed to write code. And of course, there was none available.

GRAYSON: You had this shop established by the mid-1960s?

BIEMANN: That grant started in 1966, and terminated in 1996. The NIH grant, the peptide grant as we called it, was established in 1958, and terminated in 1996. Those two were some of the longest lasting NIH grants.

GRAYSON: Yes. Was that renewed on an annual basis?

BIEMANN: At first it was three years, and then five years.

GRAYSON: What was the typical budget for those per year?

BIEMANN: The peptide grant started out with \$10,000 a year, and ended up with probably \$250,000 per year. The facility grant in the first year started with a large amount but normally ran about \$500,000 a year, except in those years when -- for example we bought later on in the mid-1980s the Tandem Mass Spectrometer which itself cost over \$900,000 -- in that year it was close to one and a half million dollars. MIT made quite a bit of money on my overhead, much more than it paid me salary. But that's the way it goes.

GRAYSON: It seems like you were able to get funding from NIH fairly readily, they even came to you and offered opportunities, is this a little unusual?

BIEMANN: Yes. Of course, I was really very lucky in that respect, namely that I started my academic career when federal research funding started to be significant. That had to do with Sputnik, when the Russians launched their first satellite. The U.S. was behind, that was a big awakening that something needs to be done and better be done quickly. At that time, money was reasonably available, World War II was long past, Korean War was past, and so there was no serious budget problem. They could put money into NSF and NIH. I rode up that steep slope of the federal funding curve until it leveled off and got tough. But by that time, my research program, after the late 1970s was exclusively NIH. When that leveled off it became difficult to get funding, but I was very well established, with a good track record. By the time I retired I didn't care anymore.

When the NIH came to me, for those two programs my Research Resource grant was the first one in mass spectrometry. And Fred McLafferty got the second one a year later, when he was at Purdue.

The same thing happened with NASA. At one time in the early 1960s somebody from NASA came around. He was sort of a talent scout. It was Jerry Soffen who at that time was at JPL. He came to see what we were doing and I showed him some mass spectra of free amino acids, which we had measured on the Bendix Time-of-Flight instrument. And he said, "Oh, you have data!" because at that time, particularly in the space research area, people were mainly thinking and planning. That led to my involvement in the Space Program, first with the Apollo Project, and then with the Viking Project, which were sort of sidelines of my research. I called it "scientific charity", because it wasn't really something that I was interested in at the outset. But the methods I had developed lent themselves well to that, so if it should be done it might as well be done well and by people who are experts on it.

GRAYSON: The involvement in this Space Program was another situation where they came to you?

BIEMANN: Yes.

GRAYSON: Then you said you had run the free amino acids on the Bendix Time-of-Flight, were these small standard amino acid—

BIEMANN: Yes, a few natural amino acids to see what their mass spectra looked like, and also other things like carbohydrates and nucleosides and nucleotides. We tried to cover the biochemical area from all sides, and all those types of compounds are not volatile so putting them directly into the ion source was the way to do it. We translated that to the CEC instrument to get better spectra. But we never did really anything except show that one can get the mass spectra of those compounds, and what they looked like, and what one can tell from them. But it was only much later that we got into carbohydrate type things.

GRAYSON: I'm familiar with the Viking work but what happened on the Apollo Project?

BIEMANN: We checked whether there were any organic compounds, and if so, what was on the lunar surface, from the samples brought back on Apollo 11, then 12, and of course 13 didn't work, and 14. By that time, we had established that there were no organics on the moon, at least on the surface, and did not continue on 15, 16, and 17. But, because of the worry that there could be pathogenic microorganisms on the moon, everything was quarantined for three weeks including the astronauts. We had a mass spectrometer down at the Houston Space Center, at Johnson Space Center, where the so-called lunar receiving laboratory had been built, and half of it was completely biologically isolated. The astronauts lived there for the first three weeks. We had a number of instruments behind what's called the biological barrier and we had a mass spectrometer there to analyze the samples that they brought back. I was there and two or three of my graduate students and post-docs and a technician —Bob Murphy was one of my graduate students who participated in that.

We had to go through a shower to enter the laboratory, and through a shower to get out of it. We couldn't take anything with us because of this barrier, so the data were Xeroxed through that wall by having a Xerox machine inside but the paper came out on the outside. It was an interesting way of doing things. That was to certify that the samples are not hazardous to humans, so that they could then be distributed to other research laboratories to carry out more detailed and specific investigations on them.

After that, we got the samples into our laboratory and used a high resolution instrument and again Bob Murphy did a lot of that work. There were other problems with the lunar samples; there was worry that they could be stolen and sold for a lot of money. NASA shipped them to an office at MIT and the way of shipping highly classified materials was that they had to put it into a safe and in the morning we had to go get a sample, sign for the sample, weigh it, then take it to the laboratory, and at the end of the day return it there, weigh it again, and the weight difference was what we used in the mass spectrometer.

No sample was ever stolen except I think one which was then put somewhere in a mailbox, and mailed back to NASA just to show them that the security was not that tight. It turns out that they brought back so much material because there was no problem collecting it and bringing it back. I think about 95 percent of the material is still in the vaults at the lunar receiving laboratory.

GRAYSON: What types of analyses did you perform on these samples?

BIEMANN: We directly heated it in the ion source of the high resolution mass spectrometer and did some extracts with solvents and looked whether there was anything. But there wasn't anything.

GRAYSON: That's not terribly surprising considering the environment that the samples experienced for most of their life.

BIEMANN: But of course, nobody knew that; I remember [Harold C.] Urey, who was on the scientific committee and led the planning of what to do. Since one didn't know how much sample would come back, there was the question of who should get which sample, and how much. There was a competition between the organic geochemists, the inorganic geologists, and mineralogists, and at that time there was a hypothesis that the moon could be rich in meteorite material that was called carbonaceous chondrite; because they contained carbon material, and were chondrite type mineralogically. And he (Urey) at one point said, "I think the moon is a carbonaceous chondrite, but if it isn't the mineralogists can have it all." And it turned out not to be a carbonaceous chondrite. We had done, with NASA's support, some studies of meteorites and carbonaceous chondrites; John Hayes was one of those who worked on that.

GRAYSON: There is an interesting little anecdote that I ran across with respect to the Apollo mission. There is an aircraft carrier that is now a museum on the west coast probably, in the Bay Area, and when I was out there visiting my son we went and looked at it. Maybe it was the one that was used to pick up the astronauts after they had come back. I was wandering around and I saw this little plaque on the wall, which I should have taken a photograph of. It was a custom's form about the fact that these astronauts were bringing in material from outside of the country. It's an amazing kind of bureaucratic statement that they had to sign a form they're bringing back rocks from the moon, and bringing them into the country. (Laughter)

BIEMANN: But what did it say in that box where it says "Value?" (Laughter)

GRAYSON: I don't know. But I'm going to have to go back and take a picture of that. I wish I had when I saw it. While you're continuing to do the science that you want to do, you're also doing this science for charity, so to speak.

Then you were involved also in the Mars project, was that the Viking? Were you trying to look for life or signs of organic compounds? This came naturally from your involvement...?

BIEMANN: Involvement in Apollo. In 1968, NASA proposed and was authorized to fly a landing mission to Mars. Before that it was just a Mariner fly-by. One of the objectives was to look for any signs of biology, past or present, associated with a search for organic compounds

because it was not only related to potential biology questions but also, of course, to the origin and past history and present state of the chemistry of Mars. Since no one had an idea what to look for and had to be prepared to find any kind of compound, I proposed to use mass spectrometry because of its wide general applicability and sensitivity; a gas chromatograph for separation and the mass spectrometer for identification. That proposal was accepted in 1969, and the rules of the game required that each experiment had a team of scientists to do the experiment and have the capability of interpreting the data.

That team was constituted in 1969, and I was appointed team leader. We were told that the Jet Propulsion Laboratory had a miniaturized gas chromatograph from earlier lunar proposals, but was never sent, and also a miniaturized mass spectrometer. So, the instrument side was all set and all we needed were people who could make sense out of the data. That turned out to be not really the case. They had built a miniaturized double-focusing Nier-Johnson mass spectrometer planned for some atmospheric studies on earth in rocket flights and it was just sitting there. Al Nier had done very successful flights in the upper atmosphere with his instrument; ironically he used Mattauch-Herzog geometry, not a Nier-Johnson system.

JPL's instrument had to be redesigned because the miniaturized mass spectrometer part was okay but the gas chromatograph was not suitable, nor was the data system suitable. The original plans called for doing all the interpretation of the mass spectra automatically on Mars, and send the results back. The data interpretation was to use our low resolution mass spectral identification algorithms. I said that wouldn't work. We had to get all the raw data back because we had by that time developed mass chromatograms, data interpretation modes which were based on having complete mass spectra of each scan, of each mass spectrum of continuously recorded spectra coming from the effluent of the gas chromatograph.

I wanted to have all the data because only that way could we be sure. The reason for not doing that was the data limitation for recording it on the lander and then sending it back to the orbiter, and from there sending it back to Earth. Fortunately, JPL had developed a tape recorder that didn't use a tape because that couldn't be sterilized. One problem with the entire Space Program at that time was that in order to go to extraterrestrial bodies, the rule was that you could not contaminate that body, like Mars, with terrestrial living things. Therefore, to get the Viking landers there, they had to be sterilized, and that required heat sterilization.

So because you could not heat up and sterilize a tape, JPL had developed for some earlier un-manned lunar missions which were never flown, a recorder that didn't use a plastic tape. It used a metal wire. They could revitalize that recording system, and it was put on Viking. That also made it possible to record a large number of images, which required much more data than a simple GC-MS data set.

GRAYSON: They actually recorded the data on the tape recorder and sent it back?

BIEMANN: You had to wait for the orbiter, which circled around Mars on a one day Mars schedule and was in view of the lander for forty-five minutes. The data had to be stored for that time, and then quickly spewed up in those 45 minutes to the orbiter, which had a more powerful transmitter to send it back to Earth.

GRAYSON: That made it possible to do the whole data set?

BIEMANN: That was the only way it could work reliably, because a compound that was found was perhaps not of the type that an algorithm could interpret. Even on earth such automated systems don't work for everything.

But what was missing was an appropriate gas chromatograph, gas chromatographic column, and the interface to the mass spectrometer. At that time the interface which we had developed, pumped off the helium carrier gas and the one which Ragnar Ryhage in Sweden had developed also had to pump off the helium. There was just no pumping capacity that could handle so much helium, particularly since helium is difficult to pump with a getter pump, an electromagnetic pump, which was the only one we had available. We finally devised an interface where hydrogen was the carrier gas that could be removed from the effluent through an electrolytic process through a palladium wall, that passes hydrogen very easily; but then you still have to pump on the outside.

The pumping was done by an electrical potential pump, and so we could remove 99.9 percent of the hydrogen. Because the instrument had to be pumped down and sealed off in January of 1975, then shipped from JPL to Martin-Marietta in Denver for installation into the lander and then from there to the Kennedy Spaceflight Center to put into the spacecraft in June. Then it had to be launched in September and spend ten months in space, land on Mars, get turned on and work. So it all worked perfectly.

One problem was that all the valves had to be tight, so the hydrogen didn't get lost. Therefore, the hydrogen was sealed off from the tank to the GC with a glass break seal that could be broken with a magnetically held piece of iron. Just before the launch some engineers, who had the task of figuring out all the possible failure modes that could happen, came to the conclusion that the chances that the seal was not going to break was not insignificant and they can't take that chance. They said it must be broken on earth to make sure that it was actually broken because if it wasn't, they had to take it out and put in the spare model. Therefore, the seal was broken and hydrogen was available during the interplanetary flight. We could actually run a blank after it had left earth and before it got to Mars. This turned out to be very fortunate because it showed a spectrum of traces of the solvents remaining from the cleaning of the instrument and it showed that the mass spectrometer worked and showed what the contaminants were. The leak rate of the valves was such that we didn't lose any hydrogen, and we established the instrument background before it ever got into the atmosphere of Mars.

GRAYSON: So what was the column like for this?

BIEMANN: The column was a micro-bore column and the material was developed by Milos Novotny at Indiana University. After the project was approved it was changed while under construction, it turned out that the column that was originally going to be used didn't work quite well, and we needed a Tenax column.

GRAYSON: The selection of the column seemed to be a bit of a challenge since you don't know what you were going to be analyzing . . . was there some pyrolysis involved in this?

BIEMANN: Yes, there were two. Originally, it was designed to have two modes of operation. One was a direct heating into the ion source; which is what we did for the meteorites and the lunar samples. The other was to send the sample through the gas chromatograph. Of course that was a period of the Vietnam War and the federal budget, including NASA's was cut. It turned out that we had to scale down not only our instrument but also others on the lander and drop off one of the biology experiments and delete that from the payload. We also had to reduce the cost of building the GC-MS which meant the deletion of the direct over. So we were left with heating the sample onto the GC column at various temperatures, ambient, 250 degrees, 350 degrees, and 500 degrees Celsius. We could choose various ways of operating the instrument. It also analyzed the atmosphere and that did not go through the GC but directly into the MS.

There was a very complicated valving system, between the GC and the hydrogen separator because we didn't know how much material there was and the ion pump or sputtering pump had to be very small and used the magnetic field of the mass spectrometer, so it was part of its flight tube. It had a very limited pumping capacity. We expected to get at least some water coming off that was absorbed on the material, or in part present as hydrates of minerals, and would overload the pump. This valving system operated by a feedback circuit from the ion pump current to a valving system so if at first the ion pump current became very high that meant that there was a lot of stuff coming into the mass spectrometer, it would open one valve after the other, of a gas dividing circuit, so that we could cut the flow into the MS down to one-third, one-tenth, one-thirtieth up to one-eight thousandth; and then after that, it cut it off completely. Another valve controlled the inlet system for the analysis of the atmosphere.

Since the atmosphere was known to contain mainly carbon dioxide with some carbon monoxide, and we wanted to look at trace constituents like noble gases, we devised a chemical scrubbing system which absorbed the carbon dioxide, oxidized the carbon monoxide with silver oxide to carbon dioxide and created a vacuum—a low pressure in the sample volume so we could open the valve to the atmosphere again to get in more atmosphere; remove the CO₂, and CO repeatedly so we could enrich the trace gases. That way we could measure the amount of nitrogen that was there, and the amount and isotope distribution of the noble gases. All of that could be commanded from earth.

GRAYSON: Isotope distribution would be interesting to the geochemists.

BIEMANN: That isotope distribution is now used to tell whether a meteorite is originally from Mars, by looking at the noble gases, occluded in the mineral particles of the meteorite.

GRAYSON: Al Nier was part of the Viking team, did you guys interact at all?

BIEMANN: Yes. In fact, he had his own experiment, looking at the atmospheric composition during entry and descent of the spacecraft. But, it could not survive to the surface because it was put on the part that had to be thrown off to be able to analyze the upper atmosphere. It was one of his Mattauch-Herzog instruments. He was in the middle of the project and joined my team, the organic analysis team, because some advisory group outside of NASA said that we were flying a complicated instrument, but we had nobody on our team who was an instrumentalist. They wanted Al Nier on the team so he joined our group. He was leading his team and was a member of my team. That led to a close friendship with him, whom I had known before that.

GRAYSON: When did the Viking Project terminate?

BIEMANN: The first lander, landed on 20 July 1976 and the second one on 3 September 1976. We didn't find any organic compounds, which some people still don't want to believe because they want to believe that there is or was life on Mars; and that would not jibe with no organic compounds there. We'll all just have to wait until another lander gets there that's equipped with the appropriate instrumentation or even better, until real samples come back to earth that can be looked at, at leisure, if such a thing exists in science.

[END OF AUDIO, FILE 1.2]

GRAYSON: We're in the middle of your career in the 1970s. Let's explore the business with analytical chemistry in an academic setting. Many schools treat analytical chemistry as a poor stepchild to chemistry. How do you feel about it?

BIEMANN: It depends on which side you view it from. As I mentioned when Art Cope was charged with making a new high power front line chemistry department, it also contained an analytical chemistry division. It flourished for a while. Cope was a dictator of the same class as

my professor in Austria was, but of a completely different mentality. He was not a general, but rather a very benevolent dictator. That was important at that time because if you want to create a new energetic faculty out of a low level one, you can't have faculty meetings to discuss new appointments to replace old ones, etc.

He made his own decisions, and consulted with the new faculty members he brought in because they were the ones making the place run. That kind of a faculty leader is good for that purpose, to generate something new. But when it's all running well then they run into difficulties. If a scientist starts a high tech company, but then wants to be the CEO, the CFO and the human relations person and everything else forever, it goes to pot. He's wise to turn things over to business people.

So Art Cope finally resigned under slight pressure in 1964, after almost twenty years as the Head of the Chemistry Department. Part of the malcontent amongst the faculty was that some didn't like analytical chemistry. Mainly the physical chemists considered the analytical chemists as failed physicists or failed physical chemists, which had some truth to it. Analytical methodology was either physics, or physical chemistry, not even organic chemistry.

The conventional analytical chemistry of that time, in the early 1960s, fifteen years after World War II had ended, was to analyze for elements or molecules in some medium, either qualitatively -- but more quantitatively. That doesn't lend itself to great research advances that catch the public's eye or even the chemists' or scientists' eye.

I was in that group. But, I didn't have that problem because I wasn't pretending to be a physical chemist. I wasn't pretending to be a classical analytical chemist -- because I wasn't even trained in that. I was an organic chemist who had earned his credentials at the Institute as an organic chemist, and I was completely acceptable as a peer to the organic chemist, to the physical chemist, and the inorganic chemist because they couldn't care less. Dave Hume was mainly an electrochemist of inorganic background, and so was Buck Rogers to a certain extent, he was also in separations. Separation at that time just became involved with all the chromatography that was going on.

That was an important field that contributed to lots of fields including biology. But once the inventor developed a procedure, he didn't have anything to do with it -- didn't have any problems to solve with it. He had to turn it over to the physical chemist, to the biologist, or to the organic chemist. That created sort of the aura of service activity; either while you developed it or thereafter because you ran it for those people. Then you just turn it over and start on something new.

Now all that changed with the advent of instrumentation, starting with ultraviolet, infrared, and NMR and then eventually mass spectrometry. People took a physical phenomenon and used it to solve chemical problems. That opened up a new area of activity for analytical chemists and also washed out the lines. They could either turn it over to the end user, who was in the other discipline, or they became part of that other discipline. In that aspect, I am an extreme example of an "analytical chemist" who wasn't affected by that shift in views because I

started out as an organic chemist; had my training in organic_chemistry; but had passed through an analytical division and then established my own discipline almost.

With the resignation of Art Cope, which was somewhat forceful, the anti-analytical forces took over and the non-tenured people were not kept. Fortunately, I had already tenure. If I hadn't, I would probably have gone back into organic chemistry, either at MIT or somewhere else.

There was only Dave Hume and Buck Rogers left. Buck Rogers once wanted to have a substantial salary increase and more space where he had to put his fist down on the table and say, "I want this." This was still under Art Cope at the time and Buck said, "I have an offer from Purdue University." Cope said, "Okay, if that fits you better, you're not out in the cold, it's a very good offer (it was the head of the analytical division there) so why don't you take it." Rogers went to Purdue with the task of re-invigorating the analytical division there, which is now one of the best known in the country.

After quite a few years, he left for Athens, Georgia where he stayed for the rest of his life. He was the one who hired Fred McLafferty because he knew from MIT firsthand what mass spectrometry could do, and he knew Fred who was at that time at Dow Research Laboratory in Framingham, right outside of Boston. So he asked Fred to come to Purdue, and Fred at first said no; but then he went. So that took care of – I shouldn't say took care of -- the leaving of one of the two other tenured people in analytical chemistry at MIT.

Then there was Dave Hume who was more analytical inorganic. He had been working on the Manhattan Project and came to MIT after the War. Until the late 1950s, early 1960s MIT had a large center grant. It was a huge amount of money to establish and run the Research Laboratory for Electronics. After World War II there were lots of electronics, things had been developed, and started so MIT grabbed a big part of that support and had the laboratory where people of any department at MIT could find a funding home by working on something which fell under the rather broad cover of the Research Laboratory of Electronics, and get their research funded from there.

GRAYSON: Was this a part of the chemistry department?

BIEMANN: No, that was an Institute-wide thing. It resided in electrical engineering but not in the department. It was separate, like any other inter-departmental institute wide entity, and was spread over different buildings because they had to find room wherever they had. Most of this research activity was going on in the various departments, and individual faculty members who had something to do with electronics. It was rather broadly defined, so analytical methodology fell under it. Dave Hume had research support from that - besides a small NSF grant – and most of his money came from there. The Laboratory of Electronics was dissolved sometime during the 1960s because funding started to become more difficult, particularly for such a grand scale – I shouldn't say ill-defined, but...

GRAYSON: ...Broadly defined.

BIEMANN: Broadly defined activities. They started to cut down, and of course, chemistry at that time didn't have that much to do with electronics so it was one of the first things that was not renewed. That's when the annual support disappeared. It became more difficult to get new grants for people who hadn't been supported by NSF or NIH, Department of Defense, NASA or someplace like that. Hume had troubles getting research money and his graduate students were working more in civil engineering on water pollution problems. His research declined for various reasons, and MIT had an early retirement program where as soon as you got in the sixty to sixty-five age bracket you could retire. At that time it was mandatory to retire at age 65. So, he decided to take early retirement because it was most beneficial and that was the end of him.

On paper there was an analytical division of which I was the only faculty member. But for practical purposes, it didn't exist anymore. I ended up having all the analytical graduate students. All the lectures in analytical chemistry I had to give, but only in two-year cycles. All the exams, like the general exams, and the various exams the graduate students took, I had to write. I knew exactly what each of those students knew and didn't know. It was very hard to write a three-hour written exam for the class, which was to apply to all of my graduate students.

I finally told the department head at the time that this arrangement didn't make sense anymore. All of my graduate students always came to MIT because they wanted to work in my research group; it was not that I interviewed incoming graduate students and all of them talked to me, and then one made a decision. That was just due to the peculiarity of my research laboratory and its relative uniqueness. Also, whether their undergraduate mentor knew about me and what I was doing. Many of my students came from certain university groups—I had quite a few students from Bowdoin College because one of George Buchi's post-docs joined the faculty there in organic chemistry and so he knew from the beginning what I was doing, and he told them "Go to MIT, work with Klaus Biemann, that's a new field."

That was the end of the analytical division at MIT except the process was slowly spread over a period of fifteen years. Since I came out of the organic group, I was always in communication with everybody in the department because not only organic chemists were interested in mass spectrometry but also biochemists, some physical chemists, and inorganic chemists as well. So I had no problems with communicating within the department, and didn't at all feel isolated. But that's kind of an unusual position.

To get back to analytical chemistry: as I said earlier, I think it's now the instrumentation, from optical to electron spectroscopy, x-ray etc. which provide a wide area of research possibilities. On these you can work in any part of the department. For example, John Waugh in our physical chemistry division was very much in the forefront of the early development of NMR [nuclear magnetic resonance].

GRAYSON: Was he at MIT then?

BIEMANN: Yes. And he still is.

GRAYSON: Okay. He was part of the group, a mainliner but he was actually doing analytical instrumentation work.

BIEMANN: He didn't at all consider himself to have anything to do with analytical chemistry. He was doing research in nuclear magnetic resonance, both in the theory of it, and the instrumentation, building new instruments, or buying new instruments, and modifying them.

GRAYSON: But this is exactly what you were doing in mass spectrometry?

BIEMANN: Yes, I think that's what analytical chemistry has become. A lot of separation science methodology and technique was developed in organic chemistry and in biochemistry, and biology, because you need it. Therefore, you think of ways to do it or ways to improve what has been developed by somebody else. The question really is whether what's called analytical chemistry is needed or viable as its own little box. If you stay in the box it is kind of only making things and sending them out of the box. And IF you go out of the box, then you are not in the box anymore!! Purdue is a perfect example. Graham Cooks did an excellent job in all the ion traps. But, he could have done that just as well in a physics department or in a chemistry department with physical chemists or in a chemistry department, as an organic chemist. The only difference between him and me is that he was more interested in the instrumentation and the technology, and to advance that; while I was more interested in the instrumentation and the technology because I wanted to do something with it. In my laboratory we never built something or made something or invented something if we didn't need it.

For example, the GC-MS was not developed because I wanted to connect a gas chromatograph with a mass spectrometer. I wanted to do it because we had to collect tediously peak by peak in one melting point capillary after the other, put it aside, label it, and make sure we didn't confuse it. Then we put them into the mass spectrometer, one after another. This takes a long time. But it also gives you a lot of time to think about how to convert that tedious manual process into an automatic one, which finally led to Jack Watson's development of the fritted tube connector.

Those are just two different sides of that part of instrumentation. Others are intrigued by the physical principle and want to make it useful for something where they think there is a need for it. Most of the time they are right, that there is a need for it; or perhaps one doesn't hear about the ones that didn't turn out to be of any use. Therefore, there are some universities who

have very well functioning and productive analytical divisions but one wonders whether the existence of that division as a management organization is really necessary to produce that effect; because the advantage of doing it in the other field is that you are much closer to the problems that cry out for a solution or they may be in your own laboratory. They don't have to be found out by chance, like when I went to the meeting in Chicago. They don't sit around unused because it takes a while for people to find out what you did. If you're not actually developing it for a certain purpose you publish it in an instrument journal. You don't publish it in a biology journal or in an organic chemistry journal. So the chances that things are recognized by the beneficiaries of the technique are lower and less quick, than if that development goes on in an environment for which it is actually predestined.

I think that's what one has to think of when one talks about "analytical chemistry" and division of analytical chemistry. As I said, some like the one at Purdue is an excellent one. The one at Northeastern is a very good one. And those are the only two ones, I know of off hand because one is very close and some of my students are there. The other one is well known in the field. Most other universities can do without the specific field labeled as such, without any disadvantage.

GRAYSON: The lab that you started at MIT was the first laboratory that taught mass spectrometry, even in the world.

BIEMANN: You mean as far as—

GRAYSON: In terms of students coming to learn the technique and then to leave your institution and have a career based on their ability to use a mass spectrometer effectively.

BIEMANN: I think it was the first one because there was no university in the United States that even had a suitable mass spectrometer – there were lots of isotope ratio mass spectrometry in biology because of ^{15}N studies. There was one of these at MIT in biology. They had a mass spectrometer but it was a Nier-type isotope ratio machine that could only measure two masses, but very accurately. But of the organic analytic type, my laboratory was the first one at the university, in the United States. There was one group in Australia, but that was a government laboratory.

GRAYSON: Was there anything going on at the Karolinska Institute?

BIEMANN: Yes. The mass spectrometer at the Karolinska Institute was built by Ragnar Ryhage with his own hands. Professor Einar Stenhagen was at the University of Gotheborg. But there were no students involved, certainly not at Karolinska because it was a pure research

institute. Stenhagen was a biologist more or less, and the reason why he was in mass spectrometry was because he wanted to study the fatty acids produced by the tubercule bacillus, in part because his wife had tuberculosis. As far as actually training students—of course that was one of my problems that I had to start out with post-docs because the average graduate student walking into MIT wouldn't have tried to work with me. I needed more well-trained organic chemists, which I fortunately had access to at the University of Innsbruck.

GRAYSON: Most of the training that is going on today in the field of mass spectrometry is spread around the country; Burlingame has an operation on the west coast; McLafferty was at Purdue—

BIEMANN: And then at Cornell—

GRAYSON: Yes, and Cornell. He (McLafferty) wasn't at Purdue very long was he?

BIEMANN: No. All of my early graduate students went into academia and started their own laboratory and trained a lot of students.

GRAYSON: Today there is a reasonable number of places.

BIEMANN: Today's mass spectrometry is so different from what it was fifty years ago, thirty years ago, or even twenty years ago. Now it's so highly automated to the point that when you put in the sample and click the 'on' button it tells you what it is by searching the NIST library right away. Then even in protein chemistry what's now called proteomics, you don't have to interpret anything anymore because it's all automated with the human genome and many other genes of many other classes of organisms known.

You can, as you know, digest the protein with trypsin and separate it on a gel. Then run it either by ESI [electrospray ionization] or MALDI [matrix assisted laser desorption/ionization] get all the molecular weights or at least two-thirds of the molecular weights, and then it's purely a computer problem to match those with all the proteins which the genome information contains, and you'll find out what it is. And then all you need to find out is how it was modified. But again, just look at the shifts in molecular weights to see whether there is a phosphate group or not, and if so, whether it's one or two, and things like that. It's not that you look at the data and have to use your experience to interpret it. It's all very much faster. It produces so much more data that the human mind could not possibly do it in the intellectual way. You have to use computers to help, and it's certainly a great help. It solves the problems and you don't have to have five years experience in basic mass spectrometry.

Most of the people nowadays push the 'on' button, and don't know what's behind the panel. If it doesn't work, you call in the service man or push another button, and it tells you to check this or check that or tells you that you don't have accelerating voltage and you better check that connection. It has changed very much. Now, I think the largest use is routine applications of the method. It's like milk where the cream is on the top. There is a small layer of people who do actual research in mass spectrometry, and it would be an interesting paper study to see in what kind of institutions and departments that layer is. I think that there would be relatively few of them found in an analytical division at a university.

GRAYSON: There was a period when environmental applications dominated the field, at least in terms of the applications I was thinking about. Did you ever do much in the way of environmental mass spectrometry?

BIEMANN: No. Maybe my greatest contribution to environmental mass spectrometry is the connection of the gas chromatograph to the mass spectrometer and to a certain extent the use of high-resolution mass spectrometry. Now in environmental analysis that is mainly used for halogenated compounds, particularly dioxin. That has probably declined in the last twenty years by elimination of the product which was an impurity in 2,4,5-T, the Agent Orange type herbicide. It's kind of unfortunate, because it's a great herbicide. We did the first experiments to look for dioxin in collaboration with Professor Matthew [S.] Meselson, a biochemist at Harvard University. He got involved in the Agent Orange situation and asked me to be part of a group looking into how one could come up with an analytical technique.

We ran some spectra on the 21-110 high resolution mass spectrometer to show that one can obtain a clean signal because of the many chlorines, so different in accurate mass that they can be detected in the presence of lots of other stuff with the same nominal mass. Meselson then had one of his graduate students work out the methodology on the MS-9 which they had at Harvard. Otherwise, I have not done much in environmental chemistry -- except I started Ron Hites in that field. It was his own idea to use mass spectrometry for that purpose, and he went out to collect gunk from the bottom of the Charles River in front of MIT, lowering a bottle down to the bottom of the river from the Harvard Bridge that goes by MIT, and collected a sample and looked at that.

Later on, I was a little bit involved when Ron, then a faculty member in chemical engineering at MIT, had a program that collaborated with other people looking at the combustion products of fossil fuels, mainly looking for carcinogenic polycyclic aromatics. I took that program over from him when he left MIT for Indiana University, for a few years until it ran out. But, it was never close to what I was doing, it was not biological enough to be of lasting interest. It was more of an analytical technique where pushing detection level and accuracy was very important. Then it became so politically enmeshed and legally enmeshed, and I always stayed out of things where I had to appear in court and testify for one side or another.

GRAYSON: Did you ever have to go to court?

BIEMANN: No. Once I came close which is an interesting anecdote. There was a racehorse which belonged to the Aga Khan and it won one of the New York State big horse races. They always tested the horses afterwards and the sample was sent to the laboratory at Cornell.

GRAYSON: Is that where Hunt was? Don Hunt.

BIEMANN: No, no, Jack Henion was there. He did a lot of GC/MS, and LC/MS and was in on the early parts of electrospray which they called a different name. He found one of the doping compounds in the urine sample from the Aga Khan's horse. Not the Aga Khan himself, but his managers said, "No, we didn't dope the horse." So they sent the sample to another laboratory in the Chicago area, and they didn't find anything. A group was convened to sort things out, and I was the mass spectrometry person on it. It turned out that the evidence at that Cornell laboratory was not kept properly. The laboratory was located at Cornell University but it was actually operated for the government of the State of New York by the Department of Veterinary medicine and Jack Henion did the GC-MS analyses.

Unfortunately since the scientist was in academia, it was a side job for him to do those analyses and he didn't do all the blanks necessary to make sense out of it, and did not do those things on which so much rides. You have to have a very detailed flow of evidence and custody, and sign out, when you take the sample out of the refrigerator, and then sign it back in again. Those records were not kept properly so you couldn't even tell if the spectrum was from that sample or from another horse, or another blank or whatever. The state office which had filed the complaint finally gave up, so I didn't have to go to court over it. But in general, I tried to avoid those things since plainly that was the thing to do.

GRAYSON: That has become a fairly important use of mass spectrometry today with the animal and human sports, testing, as we found out with this bicycle race in France.

BIEMANN: And of course the Olympics.

GRAYSON: Yes. This is a very necessary tool, small amounts of material can provide a lot of information quickly, and it's important.

BIEMANN: That reminds me of another project which was of interest in the early part of the 1960s. We developed a procedure for looking for drugs and metabolites in newborn babies and children, mainly the accidental ingestion of things which they found in the bathroom cabinet; overdoses, un-intentioned, rarely any foul play was involved. But it was important to identify it quickly and reliably. That was just after we had developed GC/MS and used it for alkaloids for example. We made a connection with an anesthesiologist at Harvard Medical School and then developed that as sort of emergency service.

We generated a computer program that would look through the GC traces and the mass spectra to identify what didn't belong. We had a 24 hour operation, that if a child was brought into the emergency with some funny symptoms which indicated some toxic problem, they would take the blood and the urine sample, call a cab to send it over to our laboratory and call someone from my laboratory at home and that person then went to the lab. The cab-driver was told exactly where to go. That was at a time when not all the buildings were completely locked and he was told to go to the basement of building fifty-six and hand over the samples. The person on call would quickly extract the two samples, run the GC-MS, figure out what it was and call the physician back and tell them what it was. We did that for two or three years, until it became established. It became so common that analytical laboratories, at least in the greater Boston metropolitan areas, made it a business to run all kinds of analyses; including having mass spectrometrists so that it could be turned over to those commercial laboratories. There was a need for it, with clients that would use it, so they could set up the procedure, and assign or hire someone to do it.

GRAYSON: One of the areas that I discovered where mass spectrometry is used to detect inborn errors of metabolism in newborns. We had a speaker from the state government laboratory in Columbia, Missouri talk to our local discussion group about it a year or two ago. I was really impressed with the way they go about it. I mean they're really not mass spec people. They're more clinicians, but the use of the tool to detect these inborn errors in metabolism is such a life-saving thing and it just gives people information immediately if there's any of these really quirky diseases that even though the baby seems normal at birth, after a short period of time their health may start to deteriorate and it's just a fascinating and wonderful application of the tool.

BIEMANN: One of the pioneers of this field was Isamu Matsumoto in Japan, at Kanazawa Medical University, who really established his entire laboratory that was geared at finding all those errors of metabolism by identifying the products that accumulated because some enzyme was missing. He built a library of mass spectra for that and actually had a nationwide laboratory. That was in the mid-1970s. He died ten years ago. He was a big person in medical mass spectrometry. It then spread all over Japan to different laboratories doing it. It became—not commercialized -- but with the methodology being done by certain central laboratories, like the one you mentioned. But, one can say it was another outcome of our original introduction of mass spectrometry to organic chemistry.

GRAYSON: You talked about moving from the accurate mass determination on the 110 to the use of tandem mass spectrometry. What kind of instrument did you have for that application?

BIEMANN: That was the JEOL HX-110. Tandem mass spectrometry has two forks: one is quadrupoles; the triple quadrupole, and now with ion traps and things like that. The other one was the magnetic instruments which started with two sector instruments where one could use the linked scan mode. It worked quite well but the resolution and sensitivity was quite low. Then one went to three-sector instruments where you used the magnet as a single focusing, first mass spectrometer, and then a double-focusing geometry with an electric and a magnetic sector as a second analyzer; or the reverse. Then the ultimate was a four sector instrument with two high resolution mass spectrometers in tandem and in between the collision chamber.

The first of those were built by VG and went to the National Institute of Environmental Health Laboratory in Research Triangle, North Carolina. It worked reasonably well, but not good. It was two ZAB-SEs. The first one had the magnetic sector followed by the electric sector, but then the second mass spectrometer, for some reason or another, they turned it around so that it had the electric sector first and the magnet second. If the ion optics of the Nier-Johnson geometry would be absolute correct and symmetrical, it should work. But it's not quite perfect and therefore for some ion optical reasons it never worked very well.

So when I wanted a high resolution tandem mass spectrometer, I looked at that instrument but I knew that JEOL also had a high resolution, double-focusing mass spectrometer based on the Nier-Johnson geometry. I happened to be a consultant with JEOL; just starting at that time. They showed me the prototype. So I said, "By the way, you should also think of making it a tandem mass spectrometer" by putting two of them together. But neither of us could understand why there was any reason to turn the second one around, you might as well use it as it is designed and shoot the second ion beam into the second mass spectrometer in the right way.

GRAYSON: Electrostatic and then magnetic?

BIEMANN: Yes. I asked them to build one (tandem mass spectrometer) for me. They first took a step back because they were just getting the single one off the ground, and trying to market it. But to make a double one?? Finally they agreed to do it. But they didn't know how much to charge for it because it was the first one, there was a lot of research to do, so we agreed that they would build it and sell it to me for the same price as the VG tandem mass spectrometer that was on the market. I thought that was fair, so it cost them a lot of money, but I also said I need it in nine months. I explained to them that in nine months my grant year runs out and I had to spend the grant money for that instrument in that grant year. I had money in the grant for the VG instrument and that's the reason I also had that price in mind for the JEOL instrument.

If it wasn't in that year (I think it was 1985), I couldn't pay for it, and of course, they didn't want that to happen. They put together a completely separate group of ion optics people, engineers, manufacturing people, and management people for that project. My grant year ran from 1 August to 31 July and they delivered it on 29 July. They built the entire thing from scratch having just designed a single unit and did not sell any one of those yet, and they pulled it off. They had to do it because the president of the company, Dr. Ito, had talked to his managers, and the top manager said that they could do it, so he promised that to the president. From that point on, in the Japanese system, that had to happen come hell or high water.

They put everything into it and built a marvelous instrument that worked very well from the first day on and I never had any problems. They probably sold between fifteen and twenty instruments having the price increase in the end to about \$1.3 million dollars, so they did make some money back. But what they spent on the first one, who knows? Of course, for them it was also a prestige thing to have it delivered to MIT and to my laboratory. So it was kind of a 'perfect storm' in a positive way; everything came together. Then we put another level of sophistication, performance, and results on to the peptide sequencing because we used a FAB source, and could sequence a peptide, twenty amino acids long, in a single mass spectrum.

GRAYSON: This instrument worked with the FAB source, the first MS (of the tandem instrument) probably had a resolving power on the order of a couple thousand for the purpose of—

BIEMANN: Up to ten thousand, but we set the resolution for whatever was needed to resolve the isotopic multiplet at that mass. That gives a mono-isotopic parent ion species which we used -- although for large molecules it is no longer the biggest signal -- but it gave a simple secondary spectrum. That was the big difference compared with the triple quadrupole which Don Hunt used very successfully; but he had to use the isotopic cluster, therefore the daughter ion spectrum was polyisotopic.

GRAYSON: Much more complicated. The second mass spec (of the tandem instrument) operated at similar resolving power?

BIEMANN: Again, depending on what the mass range was. But you couldn't run it below 1,000 resolving power anyway. So, we ran it at about 2,000

GRAYSON: Then you could ionize the material in the FAB source and got the ion that represented the peptide.

BIEMANN: The molecular ion in very good yield with no fragmentation. Of course fragmentation was irrelevant.

GRAYSON: Right.

BIEMANN: We could do mixtures of ten peptides in one sample, no problem. We did an enzymatic digest of the protein and ran it through an HPLC [high performance liquid chromatograph]. We didn't collect single peaks but groups of peaks, so we ended up with the primary spectrum, maybe ten signals for ten different molecular ions. Then we could just pick off one after the other. We had to reload the sample but that was easy.

GRAYSON: So now you're using a mass spectrometer as opposed to a spectrograph?

BIEMANN: Yes.

GRAYSON: Electrical single point detection.

BIEMANN: As mentioned earlier, since the 1960s we had two CEC 21-110B instruments. In the late 1970s we bought an Atlas MAT 731 mass spectrometer that is not quite the same geometry but a more advanced model, mainly designed for organic compounds.

GRAYSON: Was the 731 Mattauch-Herzog geometry?

BIEMANN: Yes. The 731 was Mattauch-Herzog geometry. [The 711 was also Mattauch-Herzog geometry, but not as well corrected to get a focal plane; it had a focal point. So one used it as a scanning instrument.] The 731 was the one we right away converted to FAB once we read Michael Barber's paper and it became known that the secret to FAB was to add glycerol to the sample. So that instrument remained mainly in that mode until we got the JEOL instrument in 1985. After that we didn't use photo plates anymore.

GRAYSON: Was there an electrical detection system on the 711.

BIEMANN: Yes, with an electron multiplier.

GRAYSON: At one time, weren't you using field ionization/field desorption as an ionization method?

BIEMANN: Yes. The MAT-731 had a field desorption source which we used briefly, but again, a year or two later FAB came about and that was just so superior that we used the field desorption source and the vacuum lock that carried the emitter wire, to convert that emitter wire stage to the stage for FAB. Then all we had to do was put on an argon gun from the top where we had a viewing port anyway, and that was all done. FAB-MS really opened up the field of peptide mass spectrometry to anybody who had a good mass spectrometer. Before that, there was only our chemical method, using GC/MS for it and small peptides and the permethylation method which Dudley Williams and Howard Morris developed in England. Morris came to Don Hunt's laboratory to show him how to do that chemistry because it was even trickier than our reduction method.

The other disadvantage was that the permethylated peptides were not as volatile as polyamino alcohols, which we used so you couldn't use gas chromatography. You had to fractionate or sublime the sample mixture into the ion source and scan continuously and see which peaks came up and which ones disappeared to sort it out. That was quite tedious. Then Don Hunt used LC/MS to do that, but again, since FAB came out, everybody scrapped everything and did FAB -- without any chemistry.

GRAYSON: Yes.

BIEMANN: Since the chemistry was the thing, you had to be very experienced to use it. This prevented the spread of the methodology; but with FAB anybody could do it. And that was really when peptide and protein mass spectrometry took off. But by that time the biochemistry community had already been introduced to mass spectrometry by our work; because there were problems in protein chemistry which Edman degradation could not solve. Those people then had most of it done but then needed to deal with those other problems; like when the N-terminus of the protein was blocked, which is the case in 30 percent of mammalian proteins. These post translational modifications the Edman degradation couldn't do, so then they sent us the N-terminal peptide and we determined that sequence. And that was done.

Or if the peptide was too small and it wouldn't stick in the spinning cup which was the heart of the Edman automatic method then that was duck soup for us. Same thing for hydrophobic peptides like those containing leucine, isoleucine or proline, which if there are too many of them left at the end of the chopped-off peptide makes it very hydrophobic and is then washed out by the solvent. But of course, the more hydrophobic they are, that means the less polar they are, the easier it was for mass spectrometry. So it was a perfect match to do those pieces by our technique.

By that time biochemists already knew about that one can do certain things in peptide chemistry by mass spectrometry so they were ready to get going and use it on a larger scale, which FAB made possible. But even before that, in 1977 Maxam and Gilbert at Harvard developed their DNA sequencing and Sanger in England developed his, so DNA sequencing just came about as a way of indirectly establishing protein sequences by translation. But there were lots of experimental problems which we can't go into in one afternoon.

It so happened, that Paul Schimmel in our biology department at MIT, was interested in a very large protein, perhaps 1,000 amino acids long. It had not just one enzymatic function but more than one, which had to work in concert to recognize the transfer RNA to pick up a specific amino acid and then transfers it to the growing protein chain. That enzyme had to wrap the entire thing around in proper functionalities and sequences to do that, and hand it more or less from one step to the next. These were large proteins. To do that by the Edman degradation, it would have taken a long time, very tedious.

Paul figured out that if he does a DNA sequence he can translate it, so we talked about it and about the problems with DNA sequencing at that time, in concept and practice. I realized that all those problems could be easily solved by our GC/MS method. So we collaborated on that; he was doing DNA sequencing and we were doing mass spectrometric partial sequencing only of the protein. We could tell where there was a mistake in the DNA sequence because a peptide didn't fit anywhere to the translated amino acid sequence. Within one year we finished the work on a protein that was 875 amino acids long. That area of protein chemistry got people perking up and even before that was finished someone else asked to collaborate on a similar protein. But then, even while we worked on that second one, FAB came around and so we just completely switched to FAB. Then we could do big peptides and cover much more of the DNA sequence.

From then on lots of people could start using mass spectrometry for protein work. Of course, we had spent twenty years on that GC-MS method from the first experiment in 1958 to the first real protein that we determined the sequence of just with mass spectrometry in 1976. So over night that methodology became obsolete. Fortunately we were in the position of working on other problems that were waiting to be done right away, and makes things faster.

GRAYSON: Looking back you can see that the real limitation with mass spec was that it could be considered to be a little bit of a niche technique in the analytical scheme because it depended so much on getting the molecule into the gas phase and then doing ionization with electron impact, electron ionization. It wasn't until FAB came along that it just opened up the whole range of molecules that could be studied by mass spectrometry by orders of magnitude. It became a much more universal technique with that simple movement from being free of getting molecules into the gas phase before you could ionize them.

BIEMANN: FAB also was more applicable to polar, particularly basic molecules, and not so much for the fatty acids and fats. But it so happens that biologically significant and important

molecules are polar. Many of them have amino groups, so FAB made it even more useful for that important area, namely biology, than it would have been in any other, vaporizing technique. Of course, electrospray and MALDI [matrix-assisted laser desorption/ionization] were in the same direction; even more applicable to those things. To do fatty things by MALDI is almost hopeless, and very difficult by electrospray. But fortunately, nobody but the food industry was interested in fats. Since you can't even easily distinguish *trans* from *cis* fatty acids, that makes it even less significant for the cooks nowadays.

GRAYSON: Was the tandem instrument the last big purchase for your lab or did you acquire other equipment following it?

BIEMANN: No. The curious thing is that by the time of my retirement in 1996 it was hard to even give it away, because of electrospray and MALDI—well, electrospray was difficult to do on a magnetic instrument. We tried it but because it used 10 kilovolt accelerating voltage the electrical insulation problems were substantial in using electrospray for that kind of instrument. MALDI was also not appropriate because that's a pulse technique and the Time-of-Flight instrument was much more suitable for that. Now the Time-of-Flight instrument had, at the beginning, the problem of low resolving power. Now that I think about it, I should say that we did buy another instrument, namely a Time-of-Flight, MALDI instrument which was the first one that Marvin Vestal built. He knew that I had a renewal application for my research resource grant with NIH and he was a member of the site visit committee, so he knew that I had the money to buy such an instrument.

It took me quite some time to decide which one to buy because the one which Franz Hillenkamp used was really a commercially available instrument for inorganic analysis which he modified. Then that company wanted to make a commercial instrument for organic and biochemical applications. Marvin Vestal had been mainly working on quadrupoles for electrospray. Since he was one of the inventors of chemical ionization, electrospray was a little bit in his line. He had left the University of Utah, and started his own company in Houston, after a short intermediate stay at the University of Houston.

At the same time, or shortly before, Brian Chait at Rockefeller University had built his own instrument, actually put a MALDI ion source into Time-of-Flight instrument which he had. So, he had more or less the design of a MALDI Time-of-Flight instrument, which he had built for his own laboratory, and Marvin Vestal made a deal with him to use his design to make it commercially available. So knowing that I have the money, he called me up and said, "By the way did you buy your Time-of-Flight instrument yet?" I said, "No, we are still thinking." He said, "Why don't I build one for you?" Since Chait had proven that it works and I knew that Marvin Vestal could build instruments I said, "Okay." We ordered it, he built it and in a few months we got that instrument. Later on Perceptive Biosystems in Framingham started to build some and we bought one of those.

GRAYSON: So the Vestal instrument, that was just a standard linear Time-of-Flight.

BIEMANN: Standard linear Time-of-Flight. As far as my laboratory goes there were a number of problems. One is that I had to think about retiring, and I thought that at age 70 I would retire. But one or two years before that MIT decided to completely redo what used to be called the new biology building, which was thirty-five years old. I was in the basement of the building. At first, they said they would only redo the upstairs. But then, as a design and an engineering company was hired and architects plans were drawn up, they figured out that they couldn't completely gut and redo five stories above ground and not disturb things in the basement below. So, eventually they decided I had to move out.

But they realized that there was this huge, heavy big expensive instrument in the basement and they promised to keep it running so as not to disturb my research. But, everything else was to move out. So, it was finally decided that we would move on the fifth floor of the new chemistry building which by that time was twenty-five years old. I was already reducing the number of people in my laboratory because of my impending retirement. So that Time-of-Flight instrument -- actually by that time two Time-of-Flight instruments -- were moved up to the fifth floor. Of course, the big instrument couldn't go on the fifth floor. We moved up there, but then, in one of the boondoggles of MIT, they found out that they had to shut off the air conditioning to gut the building; but this instrument needed air conditioning.

They built a separate air conditioning system for that room so we could run it. But, then it turned out that we would have to wear a hard hat to get in to the room. This escalated to the point that I said, "Let's just put it to sleep, turn it off, and when the construction is finished we'll turn it on again." Everything was sealed off so that no dust got in there. You can imagine dust on a 10 kilovolt instrument. That went on and in the meantime fortunately the MALDI Time-of-Flight methodology improved at a relatively fast pace so that it then got to the point that one could do the delayed extraction and get sort of tandem mass spectra out of it even without having a reflectron, just a linear one. That we could get working, for our purposes of peptide sequencing, pretty close to the data that was more or less equivalent to what we got on the huge four-sector instrument.

While we had to shut down the instrument, it had no impact on my already reduced research activities. Then when they finally were finished it turned out that everything was covered with dust because in order to keep dust out there had to be a hood running in the room adjacent to the instrument itself. Because it used a laser, which had to be filled with fluorine, we had to have a tank of fluorine. However, the safety regulations didn't allow that, even though we only used it once in a while to fill up the laser cavity. If anything leaked, it had to be automatically vented, so it had to be in the hood. The hood had to run next to the tandem mass spectrometer. At one time, the people working on the reconstruction had to turn off the hood fans on the roof of the building.

At first, they didn't know that there was one that they shouldn't shut off, so they did shut it off. But when somebody pointed out to them that that one has to be running, they turned it

back on but in the wrong flow. So instead of having it under over-pressure inside -- which you don't use a hood normally that way, but in this case it had to be over-pressure -- they turned it on the wrong way. It sucked air in from the outside dusty environment. So it ran like that for a few months. Even though the room was sealed pretty well with duct tape, still dust got in. I said, "Let's not even turn it on, because I'm going to retire in a year or less." In the meantime others developed another way, with a much simpler instrument of getting the same kind of data.

For ten years it had served its purpose; let's just not turn it on again. Then we had to look for someone who would use, not buy it, but at least pay for the shipping, which is no simple task for a ten-ton piece of equipment. We finally found a laboratory in Boulder, Colorado, which is a government research laboratory. They only needed one of the two mass spectrometers. They wanted to use it to develop new detectors, so they needed something that generates an ion beam that can go up to eight, ten kilovolts of energy. They took it and I must say that I have never heard of it again. And the same thing happened with others; Catherine Fenselau had exactly the same instrument. It was just like FAB on peptide projects wiped out the need for doing the chemistry and GC-MS; so did MALDI Time-of-Flight, particularly with the reflectron on it, replace the large tandem instruments -- at least for polar molecules that one would use a tandem instrument to analyze.

Within those ten years magnetic tandem mass spectrometry did a great thing, and suddenly it was obsolete. That is one of the things that make chemistry and instrumentation nowadays so rapidly changing because it gets so highly developed; and in the process so expensive. People think of doing it better, cheaper and faster, and the moment that succeeds; that of course makes previous expensive, complex, very sophisticated, and very elegant instrumentation overnight useless. It is left for some very narrow fringe things in which you can do some detailed studies.

GRAYSON: The primary use nowadays for those old sector tandem instruments is if you want to do high-energy collision work. Most of these other instruments the collision cells operate at relatively low energies. That's adequate for most applications. But if you have an interest in high-energy collision then that's where these instruments can still be useful but not a whole lot of people do that kind of work. You retired in 1996 from MIT? You slowly wound down your operation?

BIEMANN: Yes, I wound down the operations over a period of three or four years. We closed down the laboratory in 1999. When my last graduate student graduated in May of 1998, I had two post-docs still for another year and then that was it. I got out of chemistry because the work I was doing, and the way I was doing it and the way it had to be done, you couldn't do it with one or two post-docs, which was the way many of my colleagues at MIT or other places operate. They officially retire because they don't want to teach anymore -- which I didn't have to do much anyway -- but still they can't let go. They continue to work, scrape up some money to pay one or two post-docs -- and it's even harder for those to get research grants because everybody

says, “Why don’t we give it to a young active person, other than to this old person who has gotten his Nobel Prize already?”

I am referring to one of my colleagues, Gobind Khorana, who had a great research career, a great research laboratory. He got the Nobel Prize for synthesizing the first gene in the 1960s. But he still writes research grant applications to carry on with one or two post-docs doing some nice biological experiments; which you can do with even one person to work on a project. He has fun with it. But I don’t know as of today if he still does it, but at least for many years after his retirement he did that. You can do that if you study some biological process where you just need a few test tubes, a spectrophotometer, or an HPLC. But in my business without instruments, you can’t do anything. To keep those instruments, even Time-of-Flight instruments, running you need a number of people to make it worthwhile. I could do some work by borrowing time on some mass spectrometer in the biology department; but it’s not my cup of tea. As you know, just keeping up with the literature these days is a full time job.

GRAYSON: Time to hang it up. I’d like to explore a little bit more the importance of the mass spec meetings; the ASTM E-14 meetings and the ASMS [American Society of Mass Spectrometry] meetings. ASTM E-14 started very close to when you started mass spec back in 1954.

BIEMANN: I went to the first one in 1958, when it was in New Orleans because the instrument was delivered in beginning of May of 1958. I went to the first one when I had a mass spectrometer.

GRAYSON: They were more like Gordon Conferences I would think at the time they were kind of small.

BIEMANN: Small, very informal, and just one speaker at a time. It grew out of CEC users’ meetings. It started by chatting about common problems and solutions, probably not even with a real program. But, in 1958, it had a program for that—

GRAYSON: I have copies of all of those old programs, courtesy of Charlie Judson. It’s interesting to go back and look at some of the titles. There was a lot of work done that you could put in the title of today’s meetings, which show how things have changed -- but they’re the same. The meeting has been, I think, a fairly dynamic event for mass spectrometry. What is your feeling about the conferences? Do you feel that they have contributed?

BIEMANN: You mean today?

GRAYSON: Well just to the evolution of the technique from where it was in the 1950s to where it is today.

BIEMANN: Of course, in the beginning it was a small group of people -- I would think that probably 80 percent of the attendees knew each other very well. To a certain extent it was almost a social get together to chat with old friends. And of course, I was a newcomer. Always at the time, first I should say that I think it is a very good thing, has been over the many years and it's just getting very big. It started out as a users meeting and became just a little bit more organized by the time I joined it in 1958. For me it was an opportunity to meet in person many people whose work I had read about in the previous one and a half years since many of them gave talks at the meeting. I could listen to and talk of course to a lot of CEC people about the instrument. CEC was never an application research entity. They were very good engineers, ion optical people, but application -- they left that to the users.

That was another purpose of the meeting; CEC could harvest last year's 'crop' of ideas. They used it to get new customers and to pick up this or that modification, which the user had come up in his laboratory because he needed it. I learned about most of the people, and could ask all kinds of questions. I told them about how I had just installed a mass spectrometer and they asked what I was going to do with it. When I told them what I was going to do with it they said that I was crazy. One doesn't do that. On the other side were those that thought it was interesting and they wished me good luck. I asked some questions that pertained to what I was going to do.

The next year it was in Los Angeles. It was close to Pasadena and so we visited the plant and met some of the plant people there. I gave a talk about peptide sequencing. People saw me and knew it was my second meeting and noticed that I was already giving a talk, which was unusual. I have given talks ever since. That was one way of showing to that group of people what one can do. My very first paper on peptide sequencing was a brief communication, just one page in JACS [Journal of the American Chemical Society]. I'm sure nobody in that group read it. The alkaloid and some of the alkaloid things were published in Tetrahedron Letters which was almost exclusively a natural products journal for fast publication that people in that group never read, or in Biochemical, Biophysical Research Communications that they also didn't read. So, the meeting provided me the opportunity to bring directly to that community what else one can do with mass spectrometry. And this surely happened with other people coming in from the outside and rounding out the program of the meeting.

Then it became so large that it became an incorporated society and became the ASMS conference. At this point, the last one I attended was in 2002 for the fiftieth meeting. Even before, the program was getting, to a certain extent out of hand, because there are so many things going on in parallel. Everyone has to really make a detailed schedule of what papers to attend. Early on, the question was why not divide it into a biological meeting, an inorganic meeting, and an environmental meeting, and so on.

But I think one of the advantages of keeping it together is that if one wants and makes the effort, one can learn what's going on outside one's immediate field. And I think that's always very important because it's sort of a cross-fertilization process. It does take time and effort and energy to handle this meeting. Then there is the proliferation of posters, which nobody can possibly see, short of just walking by and I think 80 percent are unimportant. It's just a way to justify students and post-docs to attend meetings, so they can charge it to their research, which is sort of an unfortunate formality. In the 1970s or 1980s for a few years there were rules that from each laboratory you can only have so many presentations. Since those are counted by the head of that laboratory whose name is on it, you could easily avoid that rule by just leaving your name off five papers that exceeded the magic number. Those rules were hard to enforce and didn't make too much sense. But I think nobody has come up with a better idea, and of course there's always the argument that give the students and post-docs exposure and people can associate a face with the name.

GRAYSON: It's an opportunity to meet the people.

BIEMANN: You can ask some questions, and sometimes people even get into heated discussions around the poster. But that's fortunately only in a few instances, and it never became physical, but you just have to take it as it is unless somebody comes up with a better way of doing it. But splitting it up into smaller separate meetings would lose that cross fertilization aspect. Of course, one could then go to all of those meetings or at least some of them and that's a possibility. But, anyway, it is a very useful thing, and one just has to manage it.

GRAYSON: It's not perfect but it's the best thing we've got, right now. I know that the program committee does review the abstracts now, and does actually reject some.

BIEMANN: Only a small number are rejected, mainly on technical grounds because it's hard from the short abstract to tell whether it's an also ran or a bombshell, particularly if the person who writes the abstract isn't well-versed in abstract writing. That's a negative aspect which the annual conference has is on the resulting annual cycle of work and manuscript writing in this field, because in the two weeks before the abstracts are due everybody is busy getting some final data which you can just scrape up and write an abstract for it that doesn't commit yourself to the impossible. But sometimes it comes close to it.

Then there is a second peak, the few weeks before the meeting where everybody scrambles to get the data that were promised, plus a few which were not in the abstract just to have a little bit of frosting on the cake. Then you have to create the posters, which is to a certain extent much easier because of all the computer and imaging systems that are available, but also more demanding because now it has to look good, because you have to attract the attention of the people who just have the time to walk by. The people have to be caught not only by the

preliminary abstract, which is in the book which you get two weeks ahead of time, but also by the graphs and pictures in your poster. After a few weeks of recovery, now you can write the manuscript for publication.

Of course, before you get to the meeting you have to write the long abstract, which is the intermediate stage. Then you feel you have to publish it because now that everyone knows what you are doing, you better not be caught by somebody who's doing similar thing, like the three posters down from you. Once those things are published, you really can't say I did this, but I'm too late. So that's one of the scientific management aspects of the annual meeting where everything happens at that time, and all the deadlines are created.

GRAYSON: They do have the Sanibel Conference, the Asilomar Conference and what they call Fall Workshops, which are more focused topic wise. These are all small group meetings. They probably have more of the feel of the early conference. I think that's what the society is doing to try and compensate for the fact that the annual conference has become like an ACS meeting. These smaller conferences are more congenial and people interact better.

BIEMANN: I only went to two Asilomar Conferences I think. And two Sanibel, one which I chaired and the other one the year before to figure out what's going on. And of course they were all very interesting but if I remember correctly, the number of attendees is limited.

GRAYSON: They tend to limit them.

BIEMANN: I think its somewhere along the line of 100, which then lends itself to an in-group mechanism. Depending on the topic, you have to invite certain people who are well known to work in that field, the leaders in that field. And then you have to find the people that maybe are coming up and then you have to get in some students and post-docs and those from other laboratories. With the Asilomar Conference, I don't know the management connections, they just invited me to give a talk. In the first Sanibel I went to see how it's run, and in the second one, I was involved in helping run it.

GRAYSON: Well, I think we've covered pretty much the area that I was interested in trying to talk about. As I indicated at the beginning there's a reasonable amount about your career in the literature that you know is easily accessible. I think this is good because it gets a little bit more personal and down to details that you do not normally publish. This is the philosophizing part of the interview—you've had a career, a very high-powered scientific career, and in a dynamic field. And what does the future look like to you? Where do you think mass spec in particular and science in a broader sense is headed?

BIEMANN: I think mass spectrometry now is obviously about usability to a certain extent, because the more widely it's used the more routine it becomes. Also the pioneering number of events will be the same as it was 50 years ago, and it shouldn't be judged on that part. As we have seen since 1981 when Mickey Barber developed fast atom bombardment ionization, the advances in mass spectrometry have always been caused by new ionization techniques. Even before there was a number of things; chemical ionization of course, and californium plasma ionization, field desorption but they never really caught on because they're too difficult to do and didn't really work that well, or weren't really applicable to many things. Ionization techniques really have opened and enlarged the applicability of the field, and made it even faster.

Computers ran in parallel with that and played a great role in the automation of the instruments. The faster and more easy to use the instruments became, the more data you could generate; particularly in the automated ways that you now can run mass spectrometers. The amount of data generated could not possibly be handled without modern computer techniques. In that respect mass spectrometry without computers wouldn't be where it is now.

That applies to the application on a broad basis in many laboratories where it's just used as a routine instrument. It is hard to say if that will continue at that pace because there's only so much data you can use productively. What it does is it makes the operation of the instrument and the use of the data more generally useful, but it has the danger that the data are generated, produced and consumed by people who don't have the experience to do it right. Now for routine things, that's probably okay because routine means that you do the same thing often enough that it's hard to come up with the wrong answer because if it's really wrong, it would be such an outlier that it would be recognized as such. So, that is a problem that one always has to worry about.

Science in general depends on what new instruments will be developed and what new uses they will find. For example, DNA sequencing, thirty years ago in 1977 -- next year will be thirty years -- the first DNA sequencing method was published. It was very tedious; very error prone. But then, when the human genome project push was on, that provided an impetus to do something to handle the enormous amount of analysis that needed to be done. People at Applied Biosystems and PerkinElmer came up with a new instrument, made 250 units and gave them away to Craig Ventnor's company. They -- just by brute force -- solve the problem swiftly. And here it is. But now there is only nibbling beyond that in other genomes, of other organisms. But the big thing is done. Then the interest always kind of falls off a little bit after something like that.

Certainly computers are important in every respect -- starting from designing an instrument for production, computing ion beams, and then testing the instrument, automatic testing of instruments twenty four hours a day to make sure the parameters are all right when you come in, in the morning and you push a button and it's going. The running of the instruments, the interpretation of the data and distribution of the data, relies on computers. The database searching, that is on the internet. The only trouble nowadays is that the internet is just greatly abused by business, on one side and criminals on the other side, which will eventually

lead to a crash of the system. But, it certainly is part of the rapid development of instrumentation and the development of techniques and methods in all areas of science.

Now whether mass spectrometry is just one of them or for one reason or another unique, I don't know but I really don't think so. I think it had its great exciting times but also in the past some unexciting times; in the 1970s for example we were in a period where not much was happening in mass spectrometry. The instruments were refined, pushing the resolution.

GRAYSON: That was the biggest thing for a while.

BIEMANN: Pushing the mass accuracy was the big thing, but nothing much else happened. To a certain extent, I was lucky because I could devote some of my time to the NASA project, moon and Mars while my laboratory was running smoothly and people worked on the peptide sequencing, refining the methods. But then as we already discussed at length, the jump came with fast atom bombardment.

I don't think any comparable thing happened in the last ten years; comparable to how FAB changed the field and what electrospray and MALDI did -- and the instrumentation aspects that went with it. The driver now is to identify more and more proteins in fewer and fewer spots on a two-dimensional gels with less and less material and finding more and more modifications of those known or predictable proteins. It's a very important thing, no doubt about that.

But it's now at the stage where you need to accumulate more and more information to round out the biological system to understand all the things that go on in the cell at once or consecutively, which is very important. Now mass spectrometry becomes an automatic tool, semi-automatic tool, not quite automatic. You still have to do lots of things with your own hands, with pipettes. But it will be interesting to see what the next thing is that comes along in general. Not just in spectrometry. And often people ask me why MIT did not continue in mass spectrometry. Some people think that MIT closed my laboratory by decree. But that's not at all the case.

When I was asked by department heads and deans what to do when I retired, I said find someone who is going to work on a field that was as new as mass spectrometry when you hired me. Because that's what an academic institution should do with their faculty, move things ahead, and not just continue the things that can be continued just as well elsewhere.

It may be that other factors, unrelated factors, like renovating of the building that my laboratory was in had some effect. I didn't even tell you that where I moved, two years later that building was completely renovated and I had to move at least my office temporarily to another building and then back -- but at least not the laboratory because it's easier to close it than to try to keep it running.

GRAYSON: Are you familiar with this ion mobility spectrometry that's being developed by a handful of young people?

BIEMANN: Yes. And I understand that the person who got the Biemann Medal this year is in that field.

GRAYSON: Yes, David Clemmer I think.

BIEMANN: I was told the name and I sent him a note, but I don't remember it right now. Of course, ion mobility spectrometry is nothing new. But then, mass spectrometry was not new in 1958 either. It just took a new twist. I don't know what those people are doing with it.

GRAYSON: It's a tandem instrument in that you get the ion mobility and the mass spec so you get some information about the—

BIEMANN: The three dimensional—

GRAYSON: . . .molecular conformation with ion mobility and then you get some information about the primary structure at the mass spec end. It's a technique that probably would fit in the category that you're defining, that MIT should find. It's nothing new but the way it's being applied and implemented in the biological community is pretty interesting. That would be an ideal starting point I think. But I'm just curious because it is a really interesting idea. We've got a couple of speakers coming in to talk to our local discussion group about it. It seems like it's going to be quite powerful.

I don't know if there's a whole lot more to cover. We've covered a fairly broad range of topics including most of your career I think. You never really had any quadrupole instrumentation in your laboratory?

BIEMANN: We had a quadrupoles for a short period. It was when Guy Arsenault was in my laboratory. You can look up in the MIT school of mass spectrometry paper for the spelling of his name. In fact, all the names of my graduate students and post-docs are there. But we only worked on positive/negative ion one after the other with the quadrupole. I think that was it. But quadrupoles never reached the performance of magnetic instruments, except speed. So, there was no incentive for me to get involved in a completely different type of instrument; except with Time-of-Flight mass spectrometers in the 1990s. But that was because they had particular performance characteristics which quadrupoles in the early times didn't have compared to other instruments.

We had lots of instruments; of course, we had the first Time-of-Flight mass spectrometer, a Bendix system, as we already discussed, which I got in 1961 or 1962.

GRAYSON: Did you ever go to any of those Bendix symposia conferences that they used to have?

BIEMANN: No. Because again, it was used sort of on the side for things which we tried out and looked promising and then moved to the magnetic instruments.

GRAYSON: So it was kind of test bed instrument?

BIEMANN: Where we couldn't do much damage.

GRAYSON: You couldn't hurt the instrument. So, you tried some weird things and if it looked like it would work, then—

BIEMANN: The only tangent connection with quadrupole mass spectrometers I had was during the Viking project when we needed to see whether the quadrupole instrument would be a better mass spectrometer for a flight instrument. Bendix Aerospace built a miniaturized prototype of one. But it turned out that the power requirements were greater for that than for a magnetic instrument using a permanent magnet. The performance of the magnetic instrument, being a double-focusing one, was much better than the quadrupole which would have required more power. But, they did build one and we looked at it carefully. One of the most important reasons for actually building it was that project management required it. At that time in the early 1970s everyone on the project management was saying that we had to use the quadrupole because it's so simple.

The project management wanted to make sure that they couldn't be blamed if something with the magnetic instrument didn't work either before flight or afterwards. But it was built and tested. Now almost all the mass spectrometers that fly in space are quadrupoles, even the ion traps. And I never had anything to do with ICR [ion cyclotron resonance] or FTMS [fourier transform mass spectrometer].

GRAYSON: The Bendix Time-of-Flight was used quite a bit or at least looked like it would have been a good instrument for GC-MS. It had the speed in terms of the scan speed.

BIEMANN: Yes.

GRAYSON: But it was eventually beat out by quadrupoles?

BIEMANN: And the reason was that the early Time-of-Flight mass spectrometers, and the Bendix was the major commercially available one, had relatively low resolution. When it came out there were no recorders that were fast enough to record the signal. The electronics were of the 1960s or even 1950s. Gohlke used it in McLafferty's laboratory to more or less show that you can record and see the mass spectrum of toluene while it comes off the GC column.

The mass identification was more or less measuring off the oscilloscope screen using a Polaroid camera. So if you know what it is, you can say, this is mass 92 and mass 91. But when it is completely unknown it would have been more difficult. The only advantage it had over magnetic instrument was the speed but it was almost too fast. It could also tolerate a large gas load which with GC-MS was the main problem to overcome before separators were developed. As history shows, aside from that one paper, maybe two, that was it. There were a number of other ways to try to get mass spectra of GC eluates: by complex trapping and then injecting methods, but they never got beyond the demonstrations that it can be done.

GRAYSON: It was too cumbersome. I'm sure there are probably twenty or thirty methods of interfacing an LC to MS prior to electrospray. But everyone had their own method they were trying to develop and once electrospray came along it was like problem solved, and all the other stuff was cast aside.

BIEMANN: Then there was the big comeback of Time-of-Flight mass spectrometry when MALDI was developed because it needed a pulsed instrument. But at that time, the Time-of-Flight mass spectrometer had been around only since Bendix. They were steadily improved for very special things. But then that ionization method made it necessary to build a high performance instrument of that type. By that time electronics and computer control had advanced so much that it was relatively easy to build a high performance instrument almost overnight, and it has been improved since.

For example even at that intermediate time Mamyrin in Russia had developed the reflectron. Nobody was using it, except him and a few other people, until MALDI came around and it was found of great use. So now all MALDI Time-of-Flight mass spectrometers are reflectrons in one form or another.

GRAYSON: I started using Time-of-Flight instrument when I was at McDonnell-Douglas Research labs when I first started there, that's what they had. I've always been fascinated with the fact that today you can get a Time-of-Flight instrument with resolving power of 10 or 20,000

and back with a Bendix machine a couple hundred resolving power was the most you could get. It's just fascinating to me to think that you could improve the technology of that instrument, that analyzer, so much that there's two orders of magnitude improvement in the resolving power, due primarily to developments in electronics.

BIEMANN: Faster electronics and computer control of all the parameters down to high limits made it possible. While on the magnetic instruments computer control wouldn't have done much to the performance. It was the ion optics that had to be tuned. Of course computers helped there by calculating the important parameters. First, Matsuda and then Matsuo, his student, refined ion optics for magnetic sector instruments to achieve higher resolving powers.

GRAYSON: So the Nobel Prize was awarded to Tanaka in Japan for his work on MALDI. I think a lot of people in the mass spec community felt that Hillenkamp and Karas should have been awarded that. Do you have any thoughts on that?

BIEMANN: I completely agree with that. I can't understand the notions behind that decision. The only one that could cause it was that he is not a European or American, but to give it to a Japanese once in a while, which of course doesn't really carry any water. But, I think there were some people who say that Tanaka had the first experiment successfully in that area, and presented it at a meeting in Japan. Many of the advocates of his priority claim are people who attended that meeting and they were quite impressed. But, I think for the Nobel Prize, you have not only to show who published the first paper but also whether he actually did it right and whether it had any practical usefulness and I don't mean in a commercial way but in a scientific way.

The thing that I would fault him on was that he did not disclose what particle size and I don't think he was quite clear whether he used palladium or platinum. But certainly, he did not tell us the particle size, which is the most important parameter of that method. That made it therefore impossible to reproduce and use it in other laboratories. While Franz Hillenkamp laid it out in great detail how to do it and therefore we all could do it. I think since they gave it to Tanaka, they should not have given it to John Fenn, but to Malcolm Dole, who did the first experiments twenty years earlier. I don't remember whether Dole was still alive.

GRAYSON: That is a problem.

BIEMANN: If he wasn't alive, he couldn't get it. Dole published more details in his paper than Tanaka did in his, but he didn't get anywhere because they didn't know what was going on, nor did Tanaka and he didn't carry out the necessary experiments to figure out what was going on. I know that in quite a bit of detail because I was a member of the NIH Study Section when

he applied for a grant to figure out, and develop that method and do it right and show what it can do.

GRAYSON: He being?

BIEMANN: Dole. He was at Northwestern University and was going to retire and move to the University of Texas. The review committee was quite convinced that it's an important thing, very important if it works. Since he was sort of doing the experiments and making the measurements -- he used polyethylene glycols as molecules to measure the molecular weight -- which he might be able to do it and if it worked, it would be great. But since he is going to retire, and he's going to move, the chances are low that he would make it work. It was decided to fund his application because of that slight chance that it might work, and it would be important if it did work, so it was worth some money to fund it. But of course, he moved and then never did anything else.

GRAYSON: What was the year?

BIEMANN: I don't know.

GRAYSON: Around the 1970s?

BIEMANN: Either in the late 1960s or early 1970s. It was around the time when he published those two or three papers. I probably could find out. But then it took someone like John Fenn who came from the other side, mainly the engineering.

GRAYSON: The nozzle design, engineering . . .

BIEMANN: . . . the molecular beam side to figure out what's going on. Sandy Lipsky who was at Yale bumped into Fenn some time after he moved to Yale. Lipsky had read Dole's papers and suggested to John Fenn that he look into it. He might be able to understand what's going on. So Fenn looked at it—

GRAYSON: So Lipsky interacted with Fenn in passing?

BIEMANN: Yes. They never collaborated because Lipsky was in the medical school.

GRAYSON: Fenn was in engineering.

BIEMANN: It was by accident that it happened -- like when I listened to the papers at the flavor conference, and found out about mass spectrometry.

GRAYSON: So one has to take advantage of these opportunities and make the most of them whenever they occur.

BIEMANN: John Fenn could have said, "I'll look into it" and fifteen minutes later forgotten about it. It was a coincidence and then he recognized that there is something that is worth looking at.

GRAYSON: Science is really a gray, non-linear process in many ways. I think the average person just thinks it progresses in a very natural step-wise fashion but it's a very tortured path, and then little things mean a lot.

I've covered I think pretty much of the areas. If there's anything else now's a good time, to leave your thoughts to posterity.

BIEMANN: Something I think about lately is planetary and space research. During the Viking project and before that the Apollo, it was all new and very exciting. You never knew what you would find and you had to keep an open mind. All those factors in addition to the fact that there was money available, for one political reason or another, to do those very expensive things. They had much scientific appeal but much more public appeal which made it possible to scrape up some money. But there was no NASA community. So people from academic institutions and independent or government research organizations were drawn in to do it more or less. None of them had a particular ax to grind. None of them had their scientific livelihood and research funding depending on the project because whatever they did was funded by something else before NASA existed.

The people who got together and volunteered or were drafted to do it, were enthusiastic about it, had nothing to lose, had no ax to grind, and just did it going along with their other work. The Viking project was organized, the experimental science aspect was organized, into teams of scientists for each experiment; and they came from all over the place. On the GC/MS team, one came from the University of Texas, one from the Salk Institute, one from the Geological Survey in Washington, D.C., one from the Low Temperature Research Laboratory here in Hanover, New Hampshire and one was an astronomer from Stony Brook.

We met in person because on the telephone it was hard to do. There was no E-mail. So we just had to get together. We talked things over. One of them was from JPL. A second one was also from JPL who then moved to the UK during the design of the mission. So we had to meet in general at JPL or maybe once or twice in Washington. Once we met at this house here. We had to get together for a few days. None of us knew each other before. It became a very congenial group.

Then there was a science steering committee, which consisted of all of the team leaders where those things were discussed where everybody was affected by it. For example, the data allocation was a big thing, particularly before that wire recorder was brought on board. That lasted from 1969, through the mission at the end of 1976.

The science steering group got to know each other very well, not socially so much, but in terms of science philosophy. The team leader of the imagery on the surface said he needed a picture at that time, on that day, three years from now and therefore we couldn't run a GC-MS experiment on the same day because it would be too much data. Then I knew exactly why he thought it was important to do it on that day, and he knew exactly what we were giving up, and vice versa. Anybody who didn't fit into that way of doing and mutual understanding fell by the wayside in the meantime and was just, either voted off the team or resigned or just was kept on the list but never attended the meeting and for all practical purposes wasn't there. That was from a human and philosophical and scientific aspect.

Maybe what I took away from the Viking project personally, particularly since we didn't find anything, was the fact that not finding anything had no affect on me at all. We went there to find out if there was something and if something is there, what it is. But even to find that there is nothing, at least in the top ten centimeters at the two places we went to; to me that was a full success. But sometimes people thought that I was terribly disappointed that I didn't find anything. And I said no. We answered the question.

GRAYSON: You were just elated that the experiment worked.

BIEMANN: My career wasn't depending on it. That brings me to the opposite side of the situation, which exists now. Now, those missions are run by NASA or ESA [European Space Agency] and the experiments are done by people whose whole career and research funding depends on proving some preconceived notion. They are all government scientists and engineers or are in departments at universities which are solely directed to what's called space research. For them it's a catastrophe if the instrument doesn't work because there was no power; that's one thing. Then it's embarrassing of course to the people who built it. But in spite of all that many of the projects are doing well, and finding something useful and reliable.

But on a recent mission a probe was sent through the atmosphere of Titan, the largest moon of Saturn, and landed on the surface. It happened on 14 January 2005; about one and a half years ago. Things went reasonably well. There is an instrument that is supposed to look at

the chemistry of the haze particles in the atmosphere of Titan -- which is a big mystery, why there is haze on Titan. It was discovered first in 1980 by a Voyager fly-by. So in 1997, the Cassini mission was launched to Saturn and it took the Huygens probe along and then dropped it in the atmosphere.

There was an experiment that was to determine what those haze particles consisted of. In the twenty-five years since Voyager discovered not only the haze but also that the atmosphere consists of mainly nitrogen and a few percent of methane, lots of people have tried to do the Titan equivalent of the Miller-Urey experiment on earth -- which as you may remember sort of shed light on how life on earth got started. But, it was a very famous experiment and of course Stanley Miller got famous and Harold Urey was already famous.

Everybody and his cousin were irradiating over the last twenty-five years mixtures of nitrogen and methane with all kinds of things from UV to microwaves and electrical discharges to particle bombardment. One of the first ones was Carl Sagan at Cornell. In all those experiments a solid deposit formed if you waited long enough. This was an amorphous goo, slightly yellowish brown in all those cases. Carl Sagan with his flare for expression, since he was the first one who made this stuff, or at least the first one who felt that one should give it some name so that we can talk about it -- he called it tholin. It comes from the Greek word for mud because that's what it looked like to him, and what it actually kind of was, not mud in my driveway but mud chemically speaking.

Over those twenty-five years this group of planetary scientists -- some people call them Titan-ologists because they are concerned about what is happening on Titan -- became convinced that that's what is in Titan's atmosphere. One knows that the atmosphere consists of nitrogen and methane, which is irradiated; when methane gets irradiated, it forms ethane; and eventually forms acetylene, which can polymerize and the nitrogen gets involved in it. But nobody ever bothered to analyze what that gunk is. They did carbon/hydrogen analyses, and nitrogen, and they came out all over the place depending on what you irradiated and what temperatures and pressures you used in the experiment.

Recently a group in Texas did a FT-ICR MS measurement on such a tholin and got the elemental composition of all that stuff. It turned out that it's about at least one hundred and eight different things, as far as elemental composition goes; not even counting isomers. It's fifteen series of different carbon-hydrogen-nitrogen compositions. But nobody ever tried to separate it or do anything but run an ultraviolet spectrum of tholin. But people were sort of convinced that this was there and called it "Titan" tholins.

That experiment was designed by a group in France because it was an international effort, mainly run by the European Space Agency. The Cassini mission was mainly run by NASA and JPL. The Huygens probe to Titan was mainly European with some Americans involved in it. The mission was to collect the sample, put it in an oven, treat it at ambient, 250 degrees, and finally 600 degrees (Celsius) to eventually pyrolyze what didn't come off before at the lower temperatures. In that respect, it was similar to our Viking experiment.

What comes off is introduced into a mass spectrometer, which was flying on the mission to analyze the atmosphere and what happens on the surface. By the way, the surface of Titan at one time was supposed to be an ocean of liquid ethane and methane, which was later reduced to just lakes, and oceans. There were people that worried that the probe could sink when it gets to the surface.

Great emphasis was therefore on what happens before it touches down. That experiment just collected those particles in the atmosphere then heated them up and what they found in the mass spectrum was ammonia and HCN. The title of the paper published in *Nature* on 8 December of last year was “Complex Organic Matter in Titan’s Atmospheric Aerosols by *in situ* Pyrolysis and Analysis.”

Basically they found complex organic matter making up those aerosols; but only by detecting ammonia and HCN. When you read that paper, it doesn’t really say what they mean by complex organic matter. But then since it’s a “Letter” in *Nature* they can’t put everything in the printed form because they have to be short, so there is supplemental material that you can get only online.

I read the paper, and I said, “That’s funny, just from ammonia and HCN, how can you get to complex organic matter?” It was in January when I got to my office -- I went to the library and looked at the hard copy, and I noticed that there was supplemental material and there were six figures in it and the last figure is the structure of that molecule. It is a very detailed structure which looks almost like a large steroid; it has an aromatic ring and a cyclohexane ring and is connected with aliphatic side chains which have various methyl and ethyl groups on them -- and then, hanging off, an amino group, two imino groups and two nitril groups. So that’s where the ammonia and the HCN comes from when you heat it and you pyrolyze it. I was so flabbergasted to see that somebody can draw a structure of that detail which, on Earth would take you at the minimum two months of detailed analysis but probably a year or so, that one can conclude that from ammonia and HCN. But it was published. So I read it in detail and it shows three figures of mass spectra collected from two samples. They showed the 600 degree pyrolyzate for sample 1 at 130 kilometers altitude; sample 2 at 25 to 20 kilometers altitude and then another figure that shows the mass 17 and mass 16 signal for 18 spectra, which they collected while they flushed the pyrolyzate into the mass spectrometer.

The mass spectrometer had measured the carbon isotope ratio in the methane of the atmosphere and calculated that ratio. So I measured on the paper, enlarged on the Xerox machine, the peak heights and it turned out that the entire mass 17 signal with the exception of one scan is accounted for by the required ^{13}C methane signal, so there is no ammonia. They said in the legend that for sample 1 the results are similar. In other words, there is no ammonia. And then they say that in sample 1 mass 27 (for HCN) is the same as in the background and in the other sample it’s a little bit. But you really don’t know whether that is from all HCN or whether it’s a fragment from ethylene or ethane, which you can’t tell because there’s so much nitrogen at mass 28 and its isotope at 29. There is a big peak at mass 30 because it’s from the flush gas -- they used pure $^{15}\text{N}_2$ to differentiate it from Titan’s atmospheric component. So, there’s no evidence for ammonia and for HCN but they say *in the title* they have discovered

complex organics; and to show what they mean by complex organics they show that structure of a “tholin”.

As another proof they show the pyrolysis gas chromatogram of the pyrolyzate, which they had produced in their own laboratory. That’s a sixty-minute chromatogram, but they cut it off at 10 minutes to only show from two minutes to ten minutes to show that ammonia and HCN is produced when they pyrolyzed their terrestrial junk (tholin). So that’s the other proof of that finding. There are two U.S. authors on it; the one who did the GC-MS and the atmospheric experiment, and the other one is a good friend of mine -- an astronomer who was on the Viking project doing the atmospheric analysis and isotope distribution of the noble gases.

I finally called him up and asked how he came to that information, and the mass spectrometer person said, “I just gave them the data and that’s their interpretation and so they told me they found ammonia and HCN, therefore there are complex organics there.” He was worried about it, and how they got to that, and I said (there are 22 authors on it), “Why did you leave your name on it?” He said, “I tried to take my name off, but it would have caused an international incident because it was a collaboration of the European Space Agency and NASA in the U.S.” Of course, my astronomer friend had even less to do with that interpretation.

But I’m now publishing a rebuttal of that in “Nature.” They agreed to do that but I haven’t cut it down to seven hundred words yet. Nature said they won’t publish it unless I can do that. It turns out that those people for many years didn’t do anything but work on their ideas that those compounds must be there because we can make it in the laboratory. Then on top of it is the fact that they say that the ambient temperature and the 250 degrees didn’t get any data because the gaskets which they used to close the oven up weren’t soft enough to close at those temperatures because they were very cold through that interplanetary flight. Therefore it leaked, there are no data for those temperatures — that part didn’t work. The GC connection didn’t work either. So they only have those two 600 degree pieces of data.

I wrote to them first. But they are so convinced that since you can make it on earth it has to be there, that they just believe it. None of them understand any organic chemistry apparently. In France it’s all government laboratories and they either didn’t realize that it didn’t work or can’t admit it or aren’t allowed to admit it because the marching orders of the European Space Agency was if we give you money—

GRAYSON: It works.

BIEMANN: -- for that experiment, you better publish some data. Not that I want to say NASA tells people to publish or perish a terrible death even if it’s wrong. But it’s kind of the same idea; because the people have been in that business for the last thirty years, so they are very entrenched and are close to retirement. Therefore, they think more about their retirement pay than the experiments that they do; and that is somewhat unfortunate. It’s very different from the situation thirty years ago when there were no preconceived notions of what was on Mars

because we didn't have twenty-five years to dream about what *could* be on Mars and therefore *had* to be on Mars.

GRAYSON: Careers didn't depend on the results.

BIEMANN: It would be good if something could be done to inject fresh independent blood and even more fresh and independent thinking into that system than to do things that way now.

GRAYSON: So you sound a little bit like the editor of *JACS* [*Journal of the American Chemical Society*] who said where is the melting point data? But you have better proof in your case where you had real hard information in front of you. But now these gentlemen are trying to say that there's something there because they see—I mean it's really hard to believe that they only saw those two compounds and they're going to say that represents a complex organic—

BIEMANN: On top of it, if what they say is not so.

GRAYSON: Yes, well I remember you sent an email asking for the relative abundance of the peaks in the methane spectrum. I'm thinking, "What does Professor Biemann want to do with methane?" That's an interesting commentary on the state of science.

BIEMANN: Of course one has to say that in that field you can't do one experiment today and another one tomorrow, and a third the day after and decide which one of the two were right, you wait another twenty years until you can do it.

GRAYSON: They need another career.

BIEMANN: You have to do it step wise and at each step just add the real fact. Not . . .

GRAYSON: . . . imagination.

BIEMANN: One of the reviewers of my rebuttal said it is important to point out that the original paper is incorrect because if it stands, then for the next twenty years people will base their terrestrial experiments on that. It wouldn't take long until one of the new brands of astrobiologists would say that we just have to move fifteen bonds around and we come up with something like ergosterol. Therefore there must be life on Titan, even at 98 degrees Kelvin.

GRAYSON: I think that is very good—

BIEMANN: Enough of philosophy.

GRAYSON: Yes, well that's a problem because there's this gentleman in Germany who got caught publishing something about organic semiconductors about five or six years ago, and he was able to show that he could create organic compounds that would behave as semiconductors. Obviously, this would be something pretty tremendous but then people tried to reproduce the work but it wasn't reproducible and eventually all his papers on the subject were withdrawn. But it's unfortunate that people get involved in these situations where they feel that their interpretation of something is greater than it really is.

BIEMANN: People can easily get caught in an idea and follow that and don't see the red lights flashing left and right because they only see the green light ahead of them, and don't realize that it's a reflection from the back. I always say that the horse is an animal with four legs, but not every animal that has four legs is a horse.

GRAYSON: Yes. I just wanted to make you aware of one comment that I read recently in the "Scientist". A gentleman at the University of Pennsylvania by the name of Blair who's involved in their proteomics effort, and there's a quote in this article in the "Scientist" where he says that "Mass spectrometers are like handbags, you can never have too many." So, that's a pretty interesting attitude about mass spectrometers that you just want to have more and more and more of them.

But I understand -- Dr. Gross consults with some of these pharmaceutical companies—they try to have one high performance mass spec per two investigators in the pharmaceutical research lab. So you can imagine that there are a lot of mass spectrometers in the pharmaceutical business. That whole idea of never having too many comes from that kind of attitude or approach. It definitely is a technique that has burgeoned in the last fifty years and to a large degree, your work has been an important part of that actually happening and being what it is today.

Thank you very much sir. I'm glad we were able to do this, and I hope that your wife is feeling better. Can I take a couple of quick pictures before I leave?

BIEMANN: Let me comb my hair.

GRAYSON: Okay. Sure. You have a word list for me?

BIEMANN: Yes, but now it's outdated, but it's probably only a quarter of the names we mentioned and I can expand it from my memory.

GRAYSON: Okay. You'll have an opportunity to see the transcript and we'll be able to read through it, and then it should prompt you also for the names that we didn't get.

BIEMANN: But I can send you that expanded list by email.

GRAYSON: Okay, that'd be great. Very good. You want to grab a seat there and I'll just take some informal pictures while we're chatting a little bit. And so you're going to a conference in, this October is it.

BIEMANN: Yes, end of October.

GRAYSON: And that one's going to be on the—

BIEMANN: I think I sent you the copy of that manuscript about laying the groundwork of proteomics.

GRAYSON: Yes, that's going to be kind of a retrospective—

BIEMANN: Retrospective.

GRAYSON: Is this a conference of protein chemists?

BIEMANN: Proteomics.

GRAYSON: Oh, that's the HUPO conference. That's kind of an awkward sounding name; HUPO. I believe that Judith Sjoberg is actually doing that conference now.

BIEMANN: Yes, I just got her email with the layout for the program. It mentions about the organization and gives an address in New Mexico, and I thought it's her.

GRAYSON: Yes, it is the same organization. She apparently has just starting doing that.

BIEMANN: Of course, it's logical branching out of mass spectrometry.

GRAYSON: Yes, she's been very effective in getting these things going real well, and then she's really been great I think for the society (American Society for Mass Spectrometry). I think she's done a really good job and helped in many ways to expand and make it a better conference.

BIEMANN: And I understand her son now works with her.

GRAYSON: Yes, Brent is working with her in that business. I think her daughter worked for a while with her but I guess all the details didn't work out. Her daughter is a pretty energetic person.

BIEMANN: Two energetic persons together sometimes doesn't work so well.

GRAYSON: I should actually take some pictures out your back window if I could. So you moved out here in '98?

BIEMANN: 1998

GRAYSON: That looks so wonderful. Let me step out on the porch here.

[END OF AUDIO, FILE 1.3]

[END OF INTERVIEW]

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Date 1/29/2009

Signed 

Klaus Biemann

The Massachusetts Institute of Technology Mass Spectrometry School

Klaus Biemann

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

The events that led to the establishment of organic and biochemical mass spectrometry at MIT by the author in 1958, and its growth over the past three and one-half decades are briefly chronicled. A major emphasis is placed on the work with graduate students and postdoctoral researchers who were educated in the field and in turn further contributed to the training of others. An attempt is made at the construction of a genealogy encompassing -7 to +2 generations (the author representing 0). (*J Am Soc Mass Spectrom* 1994, 5, 332-338)

The Special Editors of this issue have asked me to contribute a brief account of the origin and early development of the mass spectrometry group in the department of chemistry at the Massachusetts Institute of Technology (MIT). Many who are currently active in the field were members of that group at one time or another. It is a pleasure to accommodate this request, particularly as it provides an opportunity to recognize the many individuals who contributed to this laboratory's important earlier endeavors.

The phrase "to be in the right place at the right time" is appropriate for more than one instance that led to the origin and growth of the "MIT Mass Spectrometry School." The first of these was the author's attendance at a Conference on Food Flavors, held in Chicago in late 1956. Trained in synthetic organic chemistry (with I. Bretschneider at the University of Innsbruck, Austria) and later working on the synthesis and structure of natural products as a postdoc with George Büchi at MIT, I had no (scientific) interest in food flavors. However, Firmenich & Cie. (Geneva, Switzerland), who supported my work with Büchi, wanted a report covering this conference and I was happy to go to Chicago (my first airplane trip!) and take notes during the lectures.

One of these talks was by W. H. Stahl, from the U.S. Quartermaster Research and Engineering Center (in nearby Natick, Massachusetts!), who described the use of a mass spectrometer (a fancy instrument I had never heard of) to identify fruit flavor components, such as ethyl butyrate and butyl acetate, by comparing their mass spectra with data published in the collection of the American Petroleum Institute (API). At that time, I, like everyone else in organic chemistry, routinely used infrared and ultraviolet spectra for checking

products of syntheses or structures of natural products, and it occurred to me that mass spectrometry might be useful for the same purpose. Thus, after sending off my report to Firmenich, I began to look into the literature for papers describing the use of mass spectrometry with organic compounds. Most of the publications dealt with the qualitative and quantitative analysis of complex hydrocarbons, an application that had made the commercial production of mass spectrometers economically attractive. The Consolidated Electrodynamics Corporation's (CEC, Pasadena, CA) models 21-101 through 103 dominated the market from the 1940s to the 1960s. However, there were publications that dealt with the correlation of the mass spectra of small heteroatom-containing compounds with their known structure in an effort to develop "fragmentation rules." For example, to name a few, there were papers on aliphatic amines by Collin [1], lactones by Friedman [2], alcohols and ketones by Friedel [3] and Sharkey [4], and a brief review by McLafferty [5]. The then beginning pioneering studies of Ryhage and Stenhagen [6] on long-chain fatty acids utilizing a similar mass spectrometer designed and built by Ryhage [7] were not yet known.

The Beginnings

About that time, A. C. Cope, the Head of the Department of Chemistry at MIT and one of the most prominent organic chemists of his day in the country, decided that because, in his mind, organic chemistry comprised 80% of all chemistry, it should also be represented in the analytical division of the department. Having taught a course in qualitative, organic analysis at the University of Innsbruck prior to coming to MIT and as I was already there (right time, right place), I was appointed as of September 1, 1957, to the position of Instructor, then the first step of the academic ladder.

Address reprint requests to Professor Klaus Biemann, Department of Chemistry, Room 56-010, Massachusetts Institute of Technology, Cambridge, MA 02139-4307.

I now faced the dilemma that I could not base a research career in an analytical chemistry setting on what I had done previously, which was mainly organic synthesis and the determination of the structure of natural products in the conventional sense. However, it did not take long to realize that I could rearrange these fields around mass spectrometry, which would be a legitimate analytical centerpiece. The fact that I had no practical experience was an advantage, as it did not deter me, contrary to contemporary wisdom, from planning to put comparatively large and polar molecules, such as alkaloids and derivatives of amino acids and peptides, into the mass spectrometer.

The only obstacle was the lack of an instrument, which would cost \$50,000-60,000 and vastly exceeded the price of other commercial instrumentation that chemists used at the time. When I asked Cope why we did not have a mass spectrometer, he replied that it would take a full-time electrical engineer to keep it running. After some persuading to the contrary, he said "OK, I will find the money for the instrument, if you promise that it will not collect dust." History shows that we both kept our promises.

In the meantime, I had maintained contact with Max Stoll, Scientific Director of Firmenich & Cie. in Geneva, regarding their interest in applying mass spectrometry to flavor and fragrances research. As a consequence, this company supported the purchase of the CEC 21-103C mass spectrometer with a gift of \$10,000. In addition, Firmenich agreed to provide funds for a postdoctoral position for someone to work with me on my research projects and also measure the mass spectra of some compounds to evaluate the utility of this method in their work.

This was an important event because, by myself, I could not fully utilize the instrument, interpret the data, carry out the chemistry that needed to be done, and still fulfill my departmental duties in the analytical course cycle. Needless-to-say, the chance for an instructor working in an unknown field to get good graduate students right away was nil. Fortunately, I had kept in contact with the members of H. Bretschneider's research laboratory in Innsbruck. Therefore, I knew that Josef (Sepp) Seibl, who had worked with me in the same room in the chemistry building, was not happy with his job at a small Austrian company and that Fritz Gapp, who had been a graduate student when I left for MIT, had just obtained his Ph.D. degree and was looking for a position in the Austrian or German pharmaceutical industry. Since the instrument was scheduled to be installed in early May 1958, time was of the essence. In a flurry of correspondence, I offered the position to both, giving Seibl the preference. When he accepted, I had to send Fritz Gapp a "sorry, no" letter but a few days later I received word that my first National Institutes of Health (NIH) grant application had been approved, including funds for a postdoc. A telegram to Fritz (no fax machines in those days!) offered him that position, which he accepted at

once. Sepp Seibl arrived at MIT the day the installation of the instrument began (May 7, 1958) and Fritz Gapp came soon thereafter. During the first week of June, I attended my first E-14 meeting (now the American Society for Mass Spectrometry Conference) and, to date, I have only missed one of them. Seibl and Gapp began the string of postdocs from Innsbruck, the first six on the list (Figure 1) and later Willi and Ute Richter, and finally Heinz Nau.

With two full-time, enthusiastic co-workers, things got off to a quick start. My interest in peptide sequencing predated the involvement with mass spectrometry. The last paper I had published with H. Bretschneider [8] reported the synthesis of 1,2,4-triazoles involving a carboxylic acid hydrazide as one of the reactants. While developing the NIH proposal to use this reaction to mark the carboxyl end of a peptide obtained by partial hydrazinolysis of a protein to complement Sanger's [9] labeling of the amino terminal by reaction with dinitrofluorobenzene, I had become familiar with the problems in amino acid sequencing. From my training in organic chemistry, I was, of course, also acquainted with Karrer's [10] use of LiAlH_4 for, amongst other applications, the preparative reduction of amido groups to amines in derivatives of di- and tripeptides. As mentioned earlier, this grant was approved but I changed the approach proposed instead to the microscale reduction of the polyamide backbone of peptides to polyamino alcohols, which were sufficiently volatile to be introduced into the somewhat modified inlet system of the CEC 21-103. This work, describing the potential for peptide sequencing, was reported in our first publication [11] in the field of mass spectrometry.

Over the next two decades this methodology was improved and refined, first by Walter Vetter, then by Hans Förster, James Kelly, and Heinz Nau, and finally applied to the sequencing of the proteins monellin [12] (with Gail Hudson) and bacteriorhodopsin [13] (with Robert Andereg and Walter Herlihy, in collaboration with H. Gobind Khorana's research group). Soon thereafter, the invention of fast-atom bombardment (FAB) ionization [14] rendered the elaborate chemical derivatization of peptides obsolete and we switched to FAB, and later extended it to tandem mass spectrometry [15]. This work, exceeding three decades, was acknowledged by the Pehr Edman Award that was received in 1992. Also honored at the same time was Donald F. Hunt, whose career in mass spectrometry had begun in my laboratory a quarter of a century earlier.

With Seibl and Gapp fully occupied with amino acids and peptides, I had to carry out the early work in the structure determination of indole alkaloids by myself. Towards the end of my postdoc period with Büchi, I was to determine the structure of sarpagine. Since there was no one in his group to carry out this task by conventional means, I took a stab at it by mass spectrometry and succeeded [16] by correlating one of

its degradation products with that obtained from ajmaline, an alkaloid of known structure [17]. This approach became known as the "mass spectrometric shift technique." That work was presented at the IUPAC (International Union of Pure and Applied Chemistry) Conference on the Structure and Chemistry of Natural Products held in Australia in July 1960. Following the talk, Carl Djerassi asked me if I would help him in setting up his mass spectrometry laboratory at Stanford. I agreed to do so and spent part of the first quarter of 1961 teaching Herbert Budzikiewicz how to operate their newly acquired CEC 21-103C and to interpret the data. In his own account of history, Djerassi [18] states that "it was the elegant rationalization of Biemann at MIT of the mass spectral fragmentation behavior of alkaloids of the aspidospermine class that stimulated a serious effort at Stanford on organic chemical applications of mass spectrometry." Thus, one might indirectly include Carl Djerassi and all his mass spectrometry students and trainees in the extended MIT school.

The mass spectrometric determination of the structure of indole alkaloids became the research project of Gerhard Spiteller, another of the Bretschneider Ph.D.s who joined me as postdocs, along with Margot Friedmann (who later became his wife). Their work [19] resulted in the determination of the structures of a number of aspidosperma alkaloids.

The First Students

At that point in time, my research activities had reached the level of visibility that graduate students took notice. The first of these was James A. McCloskey, who had been admitted to MIT's Graduate School in 1957. After absolving his two-year duty with the U.S. Army, Jim returned in February 1961, and chose to work with me on the applications of mass spectrometry to new areas, namely, free amino acids, carbohydrates, and nucleosides. The low volatility of these compounds required designing a technique that would permit the vaporization of very polar molecules directly into the ion source of the mass spectrometer. Jim, therefore, developed and constructed direct introduction probes for both the CEC 21-103C and the Bendix time-of-flight (TOF) mass spectrometers. The latter instrument had been acquired with funds from NASA, which was interested in obtaining any information on organic compounds in an inorganic matrix. This was more easily done with the open construction of the TOF ion source. Alma L. Burlingame had entered MIT as a graduate student in 1959 with L. B. Rogers, who resigned from MIT at the end of the 1960-1961 academic year. Al then switched to my group to join the alkaloid activities in early 1961. He finished his Ph.D. slightly ahead of Jim McCloskey in 1963.

These two were followed by a number of graduate students, who sooner or later were able to pursue

academic careers where the growth of organic mass spectrometry in the early 1960s provided numerous opportunities. Two reviews [20, 21] and a book [22] had appeared in 1962. The interest of organic and biological chemists was further heightened when we improved the utility of exact mass measurements developed by John Beynon's "peak matching" [23] of certain ions in the mass spectra of organic compounds. In May of 1961, we had taken delivery of a double-focusing mass spectrometer (CEC 21-110) to make use of the Mattauch-Herzog geometry of this instrument to record a complete high-resolution mass spectrum on photographic plates. The lines on the plate were then read, first by a manually operated and later by a computer-controlled densitometer. This work was briefly reported at the 1963 E-14 Conference and expanded upon a year later in Montreal, demonstrating that complete high-resolution mass spectra (i.e., elemental composition data) could be obtained from alkaloids eluting from a gas chromatography column coupled to the mass spectrometer. This set of four consecutive presentations generated a flurry of activity by other manufacturers to match this performance by using various computer-based recording techniques, the forefathers of today's data systems.

Federal Grant Support

The purchase of the CEC 21-110 mass spectrometer had been jointly funded by the National Science Foundation and NIH. By the early 1960s, the latter agency had established a new funding category, the "Training Grants," which were awarded to Principal Investigators to train graduate and postdoctoral students in areas the agency thought to be new and important. Fortunately, NIH placed high-resolution mass spectrometry in this category and asked me to apply. Upon indicating the need for an additional instrument and my own computer, it was pointed out that the data-generating capacity of that equipment and manpower would exceed the needs of my own research projects, as well as the funding usually provided by training grants. But there was yet another new category, termed "Research Facilities," for the purpose of making novel but expertise- and capital-intensive methodologies available to the "biomedical community." Thus, NIH suggested that I also apply for one of these grants. Clearly, the early 1960s was the time when NIH support was on a steeply rising incline, another instance of being in the right place at the right time. Because of this early and significant financial support, one-half of all my graduate students obtained their Ph.D. degrees between 1963 and 1975, and they include all those who presently hold senior and distinguished academic positions. A similar statement can also be made for the postdoctoral category. This training grant, thus, has paid off in an exponential fashion, even beyond its termination in 1975.

With the decline of federal funding for basic research, which hit junior people particularly hard, and the end of university faculty expansion, academic positions were more difficult to come by, not the least because of the also increasing capital cost of high-performance mass spectrometers. Fortunately, the advent of novel ionization methods and the coming-of-age of the biotechnology industry opened up a new source of challenging employment opportunities. As a consequence, a good portion of graduate students and postdocs took up leading positions in this research-minded industry. The first was Walter Herlihy, who joined the Repligen Corp. in its formative state and soon became its vice-president of research.

The "Family Tree"

Having discussed the development of my own research group, it is worthwhile to look back at our academic ancestry (Figure 1). This idea was initiated years ago by the request of William T. Cooper at Florida State (a student of John Hayes), whose department wanted to establish the academic ancestry of their faculty. It was relatively easy to go back a few steps based on my own memory and with the help of colleagues in Austria, as long as there was a documented, conventional mentor-student relationship. But before the 1850s, this relationship was not as straightforward as it is today, because then it was more of an apprenticeship. Thus, while Hlasiwetz was quite clearly a pupil of Redtenbacher, the latter's relationship to von Liebig is more diffuse. Having obtained an *M.D.* under Mohs in *botany*, which in Vienna at that time was combined with chemistry, Redtenbacher was appointed professor of *chemistry* at the University of Prague (then part of the Austrian-Hungarian Empire under Leopold II). He had felt the need to learn more about chemistry and joined von Liebig's laboratory in Giessen. Interestingly enough, the only but very extensive paper they published together [24] concerned the "exact" atomic weight of carbon by combustion of silver acetate. The result of 75.854 seems to be quite different from the present value (12.011), but is fairly accurate considering that the atomic weight of hydrogen was thought to be about 6.24 at that time. Unfortunately, unlike their descendants 120 years later, von Liebig and Redtenbacher did not have access to a mass spectrometer. According to Ernest Campaigne, Professor Emeritus in Chemistry at Indiana University, von Liebig in turn goes back to Lavoisier via Gay-Lussac and Berthollet.

Figure 1 is an attempt to put the "Family Tree" together, listing the entire first generation of students and postdocs, etc., with a numerical indication for those of the second generation. To extend further downward becomes more and more difficult for me but I hope that this article will encourage those who studied with and/or were trained by those listed in the first and third columns of Figure 1 to let me know

their relationship. Since my graduate students are my (and therefore von Liebig's or even Lavoisier's) descendants, their students are part of this genealogy, regardless of whether they are mass spectrometrists or not. For example, W. T. Cooper, a student of John Hayes, Distinguished Professor of Biogeochemistry at Indiana University, already has his own crop of graduate students who, therefore, represent the third generation. On the other hand, postdoctoral associates have their own academic lineage but may be included in the MIT School of Mass Spectrometry. For their students and postdoctoral or other associates, only those trained in mass spectrometry and with continuous substantial activity in this field should be counted. To use John Hayes again as an example, only those 21 of his total of 36 postdoctoral associates whom he trained in mass spectrometry, rather than other areas of biogeochemistry, are considered members of the extended MIT School. In Figure 1, this distinction is made by listing the number of students first, followed by the number of other associates trained in mass spectrometry, separated by a slash. At last count, I had (or have) 54 students. They in turn had 180 students, for a total of 234 (counting only the first and second generations) who thus go back to von Liebig.

The 83 postdoctoral associates, visiting scientists (more than three months), and those technical assistants who pursued a permanent career in mass spectrometry (Figure 1) trained a total of 231 graduate students and 98 postdoctoral associates, etc., in mass spectrometry, while my own graduate students trained 289 in the latter category. Thus, at present, the MIT School of Mass Spectrometry numbers 937 members, not including third and further generations which would put it well over one thousand. Of course, there are a few duplications, as some of my postdoctoral associates (e.g., Mathews, Pang, Kassel, Annan, and Ghosh) were Ph.D. students of my own graduate students (Murphy, McCloskey, Watson, Vouros, and Anderegg), but their number is probably compensated by inadvertent omissions in the second generation.

Finally, it is noteworthy that a number of my students and associates have been active in various elective positions of the American Society for Mass Spectrometry. Three of these (McCloskey, Hites, and Murphy) then served as Presidents of the Society. Under the leadership of one of them (R. A. H.) this Journal was established.

Conclusion

This account reveals some of the ingredients important for the success of an academic career: (1) the advantage of entering a new, largely unexplored field, which in this case was triggered by hearing (accidentally) about mass spectrometry from the lecture of W. H. Stahl, and combining that information with one's own, quite different scientific background; (2) not to be deterred by difficulties that may be preconceived by

STUDENTS*

- 12/39 James Augustus McCloskey, Ph.D. '63
 12/66 Alma Lyman Burlingame, Ph.D. '63
 Dennis Howard Smith, B.S. '64
 13/8 Heinrich Konstantin Schnoes, Ph.D. '65
 24/8 Jack Throck Watson, Ph.D. '65
 29/9 Paul Vouros, Ph.D. '65
 Julieta Moran - Barron, S.M. '65
 3/17 Dominic Morse Desiderio, Ph.D. '66
 20/21 John Michael Hayes, Ph.D. '66
 David William Thomas, Ph.D. '67
 Robert Berj Gagosian, B.S. '67
 0/21 Sanford Philip Markey, Ph.D. '68
 2/5 Paul Vincent Fennessey, Ph.D. '68
 36/20 Ronald Atlee Hites, Ph.D. '68
 Charles Ewell Hignite, Ph.D. '69; P.D. '69-74
 Bonnie March King, B.S. '69
 James Robert Althaus, Ph.D. '70
 9/18 Robert Carl Murphy, Ph.D. '70
 0/2 George Preti, Ph.D. '71
 2/5 Harry Steven Hertz, Ph.D. '71
 0/5 James Anthony Kelly, Ph.D. '72
 James Edward Biller, Ph.D. '72; P.D. '72-75; R.S. '75-
 John Joseph Dolhun, Ph.D. '73
 Arthur Lucien Lafleur, Ph.D. '73
 Brian Dean Andresen, Ph.D. '74
 Tyrone Ralph Smith, Ph.D. '75
 5/23 Lan Kan Wong, Ph.D. '77
 10/1 Robert James Anderegg, Ph.D. '77; P.D. '77-79
 Gail Ann Hudson, Ph.D. '77
 Olga Nakamine de Wong, S.M. '77
 John Milan Lavoie, Ph.D. '79
 Walter Curtin Herlihy, Ph.D. '79; P.D. '79-80; R.S. '80-81
 Linda Jean Anthony, Ph.D. '80
 0/2 Steven Alfred Carr, Ph.D. '81; P.D. '81-82
 Agnes Marie Van Langenhove, Ph.D. '81
 David Allan Kidwell, Ph.D. '82
 Nancy Jean LeGendre, Ph.D. '82
 Kin Sing Chiu, Ph.D. '83
 Hwa-Fu Chen, S.M. '83
 5/4 Bradford Wayne Gibson, Ph.D. '84
 John Charles Karris, S.M. '84
 0/10 Julie Ann Leary, Ph.D. '84
 0/3 Stephen Alfred Martin, Ph.D. '85; P.D. '85-86; R.S. '88-89
 Ioannis A. Papayannopoulos, Ph.D. '87; P.D. '87-90; R.S. '90-93
 John Joseph Gagel, Ph.D. '88
 Richard Scott Johnson, Ph.D. '88
 Eric Henry Block, S.M. '88
 James Edward Vath, Ph.D. '90
 James Amherst Hill, Ph.D. '92; P.D. '92-
 Joseph Zaia, Ph.D. '93; P.D. '93-
 Chenhui Zeng, G.S. '90
 Heinrich Josef Köchling, G.S. '90-
 Vladimir V. Papov, G.S. '90-
 Andrew Rhombert, G.S. '93-

Justus von Liebig
 (1803 - 1873)
 Ph.D. Chemistry
 Univ. of Erlangen, 1822
 Prof. of Chemistry, Univ. of Giessen

↓

Josef Redtenbacher
 (1810 - 1870)
 M.D. (Botany, under Mohs)
 Univ. of Vienna, 1834
 Prof. of Chemistry
 Univ. of Prague, 1840
 Trained with von Liebig in Chemistry
 Univ. of Giessen, 1839-1841

↓

Heinrich H.C. Hlasiwetz
 (1825 - 1875)
 Ph.D. Chemistry
 Univ. of Prague, 1849
 (moved to Univ. of Innsbruck, 1851)

↓

Ludwig Barth von Barthenau
 (1839 - 1890)
 Ph.D. Organic Chemistry
 Univ. of Innsbruck, 1860
 (moved to Univ. of Vienna, 1876)

↓

Rudolf F. Wegscheider
 (1859 - 1935)
 Ph.D. Organic Chemistry
 Univ. of Vienna, 1882

↓

Ernst Späth
 (1886 - 1946)
 Ph.D. Organic Chemistry
 Univ. of Vienna, 1910

↓

Hermann Bretschneider
 (1905 - 1985)
 Ph.D. Organic Chemistry
 Univ. of Vienna, 1928

↓

Klaus Biemann
 (1926 -)
 Ph.D. Organic Chemistry
 Univ. of Innsbruck, 1951

Figure 1. Academic genealogy of the author and the names and years of graduation of his students and the names of postdoctoral and other trainees with the period of their stay at MIT. The numbers preceding some of the names are that individual's own students and postdoctoral associates, respectively (see text). *G.S. = Graduate Student; P.D. = Postdoctoral Associate/Fellow; R.S. = Research Scientist/Staff; T.A. = Technical Assistant; V.S. = Visiting Scientist.

POSTDOCTORAL ASSOCIATES, etc.*

- 22/11 Josef Seibl, P.D. '58-60
Fritz Gapp, P.D. '58-59
Walter Vetter, P.D. '59-62
- 77/3 Gerhard Spittler, P.D. '60-61
Margo Friedmann-Spittler, P.D. '60-61
Helga Diechtl-Vetter, P.D. '60-62
Gottfried G.J. Definer, P.D. '61-62
Wolfgang Benz, P.D. '62-63
- 19/10 Bhupesh Das, P.D. '62-64
- 10/3 Don C DeJongh, P.D. '62-63
David B. MacLean, V.S. '62-63
- 2/5 Wilhelm J. Richter, P.D. '62-64
Ute S. Richter, P.D. '62-64
Georg E. Albers-Schönberg, P.D. '63-65
Peter Rommer, P.D. '63-65
- 0/2 John L. Occolowitz, V.S. '63-64
0/2 Walter J. McMurray, P.D. '63-64
Chris Falshaw, P.D. '64
William Hargrove, V.S. '64
Hans Achenbach, P.D. '64-65
Sukenari Tsunakawa, V.S. '64-66
Brian R. Webster, P.D. '64-65
Pierre Witz, P.D. '64-65
Shigenobu Okuda, V.S. '65
Guy P. Arsenault, V.S. '65-66; R.S. '67-77
Norman Mancuso, P.D. '65-79
Conrad Cone, P.D. '65-68
Istvan Lengyel, P.D. '65-67
Robert Lovins, P.D. '65-69
Helmut Seidl, P.D. '65-67
- 6/1 Fabrizio Bruner, P.D. '66-68
John Edgar, P.D. '66-67
Agnes Jacquesy, P.D. '66-67
- 19/3 Asher Mandelbaum, P.D. '66-68
0/4 Lubomir Baczynskyj, P.D. '67-68
David A. Evans, P.D. '67-69
- 45/10 Donald F. Hunt, P.D. '67-68
Ray Salamone, P.D. '67-69
August Curley, V.S. '68
Steven Hecht, P.D. '68-69
- 3/16 Vernon N. Reinhold, P.D. '68-71
Shang-Wai Tam, P.D. '68-69
Paul Donaghue, P.D. '69-70
- 0/3 Hans-Joachim Förster, P.D. '69-72
M.-Mehdi Nafissi-Varchei, P.D. '69-70
Milica Djuricic, P.D. '70-72
- 22/21 Heinz Nau, P.D. '70-74
Toshio Sakai, V.S. '70-72
Catherine E. Costello, P.D. '70-75; R.S. '75-
Ferdinand Wirtz-Peitz, P.D. '71-72
Leonard C.-C. Wan, P.D. '72-74
Abdul M. Choudhury, P.D. '75-76
Bary W. Wilson, P.D. '76-78
- 1/0 Pierre Borgeat, P.D. '77
Andrew Schkuta, T.A. '78-80
Robert A. Bethem, T.A. '78-79, '80-82
Linda (Li) Ng, P.D. '79-80
Michel A. Lhermitte, V.S. '79
Koka Jayasimhulu, P.D. '79-80
W. Rodney Mathews, P.D. '81-84; R.S. '84-85
Asao Murai, V.S. '82
Thomas F. Dorsey, T.A. '82-85
Henrianna Y. Pang, P.D. '82-84
Hubert A. Scoble, P.D. '82-85; R.S. '85-86
Markus Zollinger, P.D. '83-85
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Bruno Domon, P.D. '86-87
- 3/3 Achille Capiello, P.D. '87-89
Pierangela Palma, P.D. '87-89
Daniel B. Kassel, P.D. '88-89
Adam S. Plaziak, P.D. '89-90
Wade J. Adams, V.S. '89-90
Roland S. Annan, P.D. '89-92
Amit Ghosh, P.D. '89-91
Alain Jaquier, V.S. '90-91
Peter Juhasz, P.D. '90-93; R.S. '93-
Kevin M. Downard, P.D. '91-
Peter M. Gehrig, V.S. '92-
Hélène Perreault, P.D. '92-
Binghuang Wang, P.D. '92-93
Yoon-Seok Chang, P.D. '92
Stephan Apfalter, V.S. '92-93

the practitioners of that time; (3) to have associates and students who are enthusiastically following that new direction; and last, but not least, having some luck at being in the right places at the right times.

Apologies

The author takes full responsibility for any inaccuracies, omissions, and false claims, but would like to be made aware of them.

Pleas

As mentioned earlier, it would be appreciated if those who obtained their academic or mass spectrometric training from descendants of my graduate students or postdoctoral associates, etc., would inform me of their names, present affiliation, and relationship. More importantly, I would be grateful for copies of photographs taken of individuals or equipment in my laboratory, because I myself never took any and John Hayes and Shirley Sze (a masterful operator of the computer-controlled densitometer) were the only camera-bugs around. Fortunately, I kept all my correspondence, which permitted me to reconstruct the exact dates for some of the early events.

Acknowledgment

Aside from the earlier financial support discussed in detail, funding from NIH (grant nos. GM05472 and RR00317, now in their 36th and 27th consecutive years, respectively) continues to the present. As mentioned at the outset, the seminal, early contributions of Firmenich & Cie. were particularly important and led to a professional relationship that has continued to the present time. Later, there was a long period of support by NASA from about 1961 to 1980, in connection with the APOLLO and VIKING projects, activities that will be briefly discussed elsewhere. Most important to the educational and training activities, which represent the major thrust of this article, are those individuals who had increasing responsibilities in connection with the NIH Mass Spectrometry Facility grant (no. RR00317). On the one hand, there were the Associate Directors of the Facility, starting with Bob Lovins (1966-1969), Chuck Hignite (1969-1974), and for the last two decades, Cathy Costello, who now holds the rank of Senior Scientist. Then there is Jim Biller, who inherited the IBM 1800 computer as a graduate student and over time successfully DECed-it-out to our present computer system. Without the help and cooperation of these senior associates and the continuous cross-fertilization among the members of the group, the growth of the MIT School of Mass Spectrometry would not have been possible. Finally, I thank Professors E. Schmid (Vienna) and G. Bonn (Innsbruck, Linz) and particularly Andrew Rhomberg for providing me with extensive information about Barth von Barthenau and H. Hlasiwetz, as well as the obituary of H. Bretschneider. K. Wundsdorfer of the Austrian Academy of Sciences made the obituary of Redtenbacher available, which led to von Liebig. E. Campaigne (Indiana University) supplied further information.

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Four Decades of Structure Determination by Mass Spectrometry: From Alkaloids to Heparin

Klaus Biemann

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

The early (1950's and 1960's) use of mass spectrometry in natural products chemistry and its evolution to the present significance in biochemistry is recounted. This methodology allowed the facile and speedy determination of the structure of a number of indole alkaloids, such as sarpagine, quebrachamine, and two groups isolated from the roots of *Aspidosperma quebracho blanco*. At the same time, the first strategy for the sequencing of small peptides by mass spectrometry was demonstrated. It slowly advanced, over a period of two decades, to an important alternative of the ubiquitous automated Edman degradation. Further advances in methodology and instrumentation established mass spectrometry as today's indispensable tool for the characterization of proteins in biochemistry and biology. A new concept of the ionization of highly acidic compounds as the protonated complexes with basic peptides, which allows the accurate determination of the molecular weights of the former, a highly sensitive method for the sequencing of heparin fragments and related sulfated glycosaminoglycans was developed more recently. (J Am Soc Mass Spectrom 2002, 13, 1254-1272) © 2002 American Society for Mass Spectrometry

In the 1950's the most vigorously pursued research efforts in organic chemistry concerned the study of the structure and chemistry of natural products and the elucidation of the detailed mechanism of chemical reactions. Both were fostered by developments before, during, and after World War II. At that time rational drug design was still a relatively unknown concept. Instead, a search for pharmacologically and medically useful plant products was on all over the world, especially in the tropics. Many of these were alkaloids, because they could be most easily isolated by aqueous acid from an otherwise complex plant extract.

An early success was an alkaloid isolated in 1932 from the root of *Rauwolfia serpentina* and named reserpine [1]. Its structure was not determined until 12 years later [2]. Reserpine was one of the first antihypertensive drugs and became a huge financial success for CIBA (Basel, Switzerland) in the early 1950's. Research laboratories in the pharmaceutical industry and academia raced to duplicate this achievement and shelves began to fill with alkaloids, which had names and melting points, but unfortunately, mostly lacked useful pharmacological activity.

The determination of the structure of these complex molecules (aromatic-alicyclic, about 20 carbon atoms, two nitrogens and a few oxygens) was tedious, time and material-consuming. At that time (1950's) it generally required degradation or conversion to known com-

pounds and establishing their identity using elemental analysis (by combustion) and "mixed melting point" [the melting point of a mixture of two identical compounds is the same as that of each of the components, while it is depressed if two different compounds of same melting point are mixed], the gold standard of the time. The understanding of reaction mechanisms in vitro had led to the formulation of biosynthetic pathways in vivo, which aided in the postulation of structures that then could be proven by conventional means. Ultraviolet spectra were used routinely for the detection of chromophores, particularly aromatic substitution patterns and infrared spectra came slowly into use.

Alkaloids

The circumstances that led me to choose mass spectrometry—a field at that time well established in physics, the petroleum industry, and then analytical chemistry, but virtually unknown among organic chemists—as my area of research have been described earlier [3]. In 1957, when I made the transition from a synthetic organic chemist to instructor (in those days the bottom rung of the tenure-track at MIT) in analytical chemistry, I had been considering the determination of the structure of sarpagine, an indole-alkaloid also isolated from *R. serpentina*.

However, before I could design a proper strategy, three research groups independently suggested [to limit the number of references listed in this historical paper, the interested reader is referred to further details quoted in those cited here] structure **1**. The aromatic

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Address reprint requests to Dr. K. Biemann, Department of Chemistry, MIT, Cambridge, MA 02139-4307, USA. E-mail: kbiemann@mit.edu

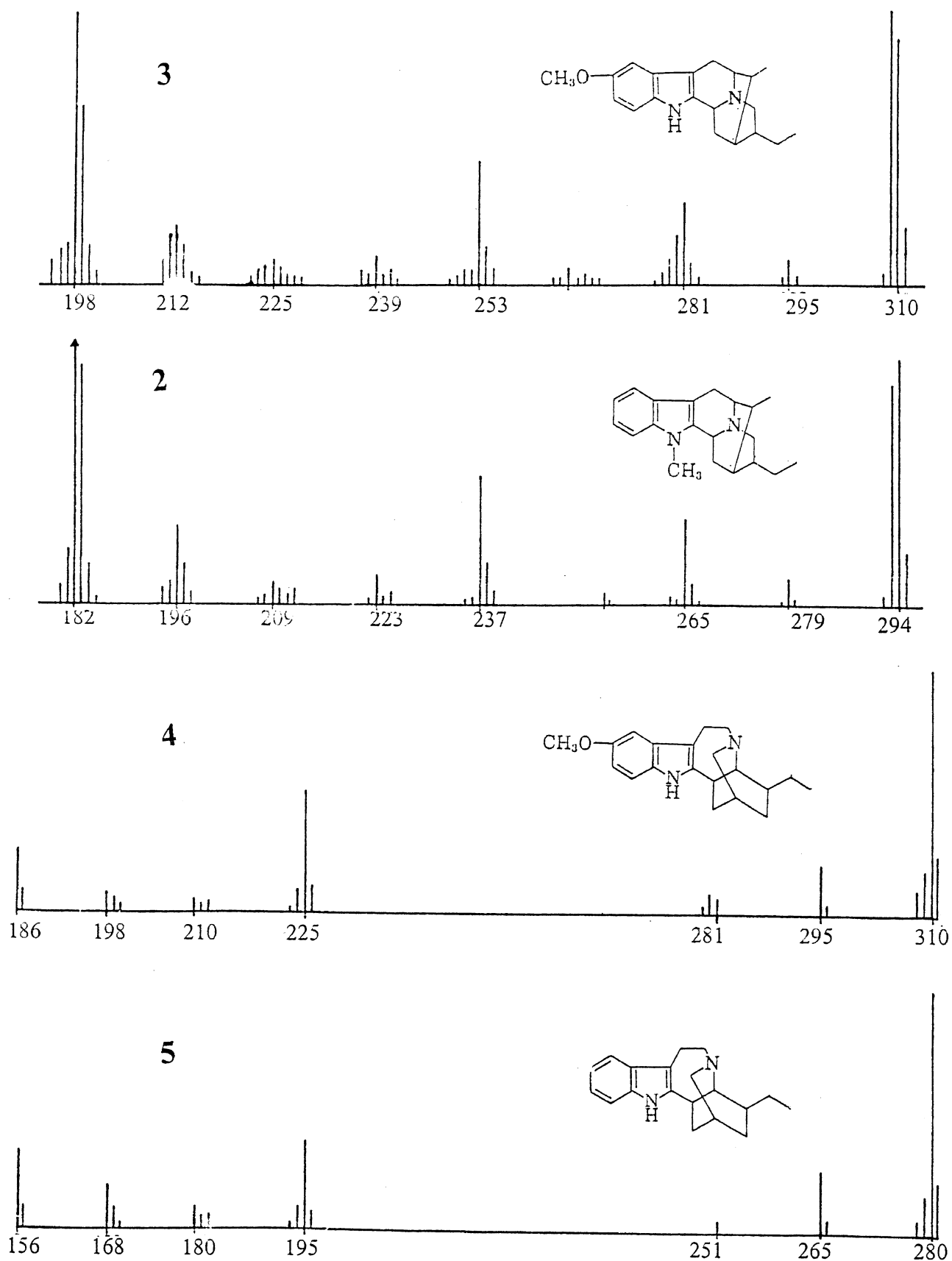
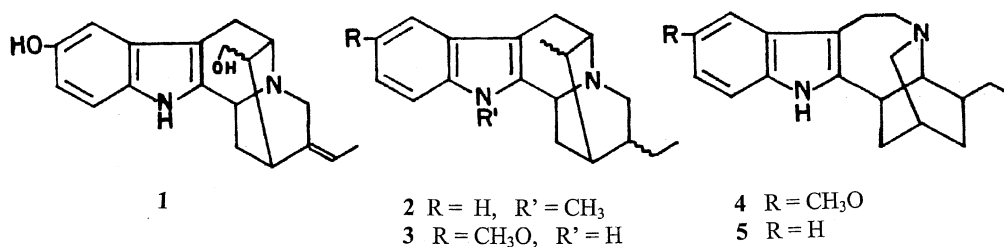


Figure 1. Mass spectra of 3 from sarpagine, 2 from ajmaline, and of ibogaine (4) and ibogamine (5) (reproduced from Reference [6], copyright 1960, with permission of Elsevier Science).



portion was supported by UV-spectroscopy, but the alicyclic system was chiefly based upon biogenetic considerations. One of the papers [4] promised to provide final proof of the structure by conversion of sarpagine to **2**, which can be made from another alkaloid, ajmaline, of known structure [5]. No such report appeared, probably because the conversion of **1** to **2** would be quite tedious, requiring the removal of an aromatic and an aliphatic hydroxyl, introduction of a methyl group at the indole-nitrogen, and reduction of a double bond. The product would have to be purified to constant melting point, followed by the proof of identity with **2** by mixed melting point.

It occurred to me that this correlation could be accomplished much easier and requiring much less material by comparison of the mass spectra of two analogous, but not necessarily identical compounds. Rather than removing the aromatic hydroxyl group, which is very difficult, it was simply methylated; the aliphatic hydroxyl group was removed by tosylation and reduction, and the double bond was catalytically hydrogenated to yield **3**. Fortunately, the comparison Compound **2** was easier to come by: It required only a quick trip up the river to Professor Robert B. Woodward's laboratory at Harvard where it was sitting on the shelf, left over from earlier work [5].

The mass spectra of **2** and **3** (Figure 1) exhibited a remarkably similar pattern, shifted of course, in the m/z values of the peaks due to the different substituents at the aromatic system (methoxyl versus methyl = 16). Thus, what a sizeable Swiss research group could not do in a couple of years, was accomplished [6] in a few weeks by the use of mass spectrometry and a few milligrams of sarpagine kindly provided by Dr. A. Hofman.

It should be noted that these spectra were obtained by electron ionization. In contrast to today's widely used "soft" ionization (CI, FAB, ESI, and MALDI) this "hard" ionization generates molecular radical cations of high excess energy which fragment extensively and very reproducibly.

Because this proof of structure was novel and unprecedented, it had to be shown that another isomer of different carbon skeleton exhibits a different mass spectrum which is, however, specific for that type. Fortunately, Dr. William I. Taylor (CIBA, Summit, NJ) had such a pair at hand: Ibogaine (**4**) and ibogamine (**5**), the structures of which he had determined shortly before by conventional means [7]. The mass spectra of these

two alkaloids (Figure 1) indeed showed an identical pattern (with a shift of 30 u, CH₃O versus H), which is, however, very different from that exhibited by **2** and **3**.

Having thus demonstrated the validity of the new concept, which later became known as the "mass spectrometric shift technique", it was enticing to look for other examples, preferably indole alkaloids, for which structures had been proposed but final proof was difficult by conventional methods. One such case was quebrachamine isolated long ago [8] from *Aspidosperma quebracho blanco*. It was known to contain an indole-system unsubstituted on its benzene ring and nitrogen, and a tertiary amino group. However, there was no functionality which could provide a site suitable for selective degradation. The more drastic "zinc dust distillation", another procedure in the tool chest of the classical organic chemist, led to a complex mixture of alkyl indoles and alkyl pyridines [9]. They were tediously purified by crystallization as picrates and identified by elemental analysis, melting points, and mixed melting points when authentic samples were available. As one of the earliest examples of a proton-NMR spectrum used in this field, the presence of an unsubstituted indole was corroborated, but a previously suggested N-methyl group could be ruled out [10]. The lack of resolution and extensive chemical shift data at that time did not allow the unraveling of the alicyclic system. On the basis of all these rather meager data, Witkop et al. suggested structures **6a** or **b** for quebrachamine [11]. A complete qualitative and quantitative analysis of the pyridine mixture was required to differentiate these two possibilities and would have needed a much larger amount of the alkaloid than was available.

Therefore, we repeated the Zn-dust distillation of quebrachamine on a very small scale (13 mg). The mass spectra of the pyridine fraction, after separation on a packed GC column and collection of each peak, revealed that the most abundant product by far was 3-ethylpyridine, while 3-methyl-5-ethylpyridine, which had supported Structure **6b** was a very minor component. It could well have arisen by thermal rearrangement from **6a** during the harsh conditions of the experiment. This structure is closely related to aspidospermine, the major alkaloid of *Aspidosperma quebracho blanco*. Its structure (**7a**) had just been determined by x-ray crystallography [12]. It was quite clear that cleavage of bond *a* in **7b** (desacetylaspidospermine) would generate **9**, a me-

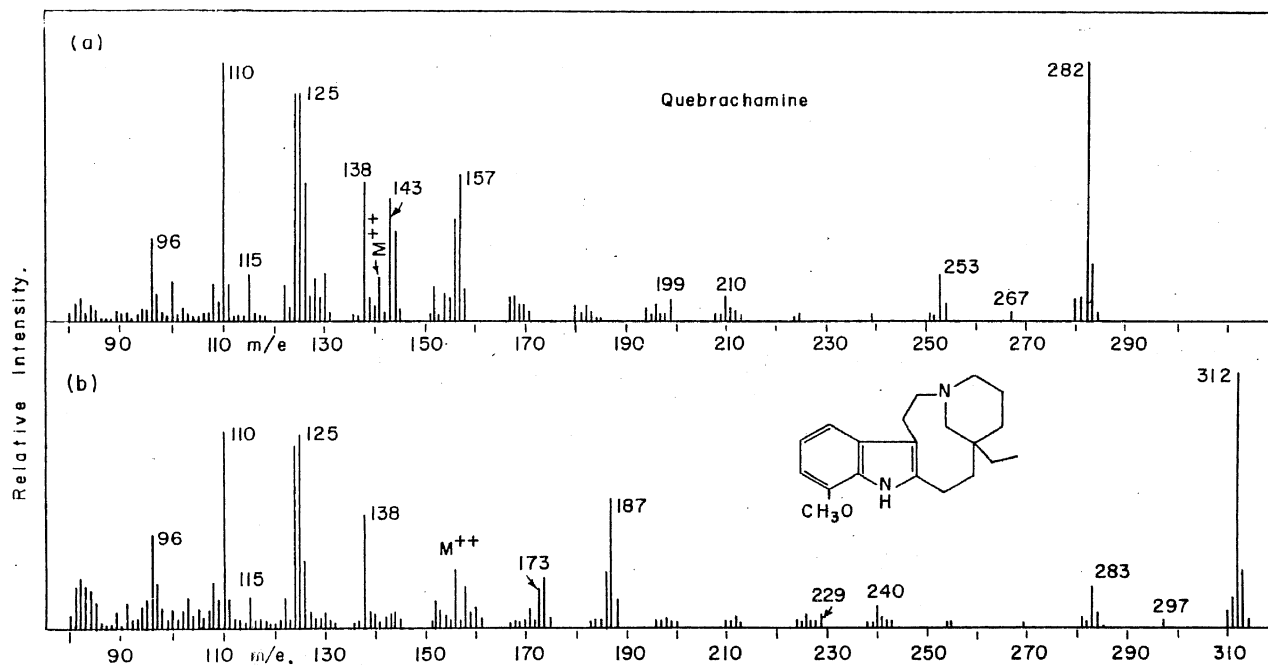


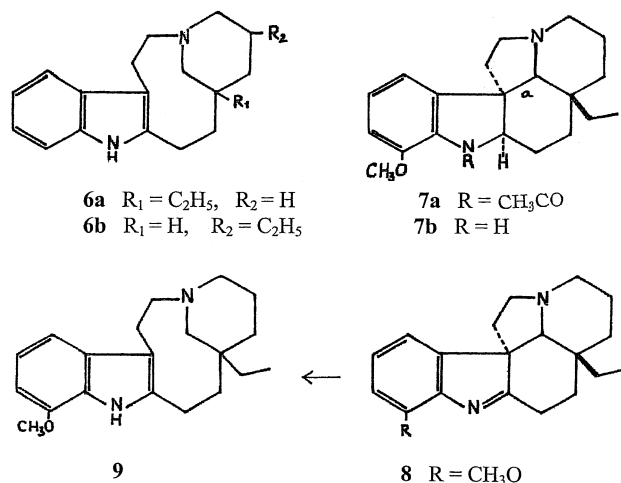
Figure 2. Mass spectra of (a) quebrachamine and (b) its methoxy analog derived from desacetylaspidospermine (reproduced with permission from Reference [13], copyright 1962, American Chemical Society).

thoxy analog of **6a** enabling correlation of the two structures by the mass spectrometric shift technique.

This conversion was easily accomplished by dehydrogenation of **6b** to the indolenine **8** which could be reduced with sodium borohydride to the ring-opened form **9** [13]. The mass spectra (Figure 2) of quebrachamine and **9** clearly exhibited the same pattern, considering the identity of the fragments representing the alicyclic system and the shift by 30 u for the aromatic ones. The relative abundance of these fragments would have been significantly altered for the less highly branched isomer **6b**.

The work described above produced not only analogs varying in aromatic substituents ("substituent labeling") but also isotope labeling by the use of catalytic deuteration, LiAlD_4 and NaBH_4 . Their mass spectra made it possible to delineate the fragmentation pathways of these rather complex radical molecular ions [13–15, 18].

When this work was presented at the IUPAC Conference on Natural Products held in Melbourne, Australia, in August 1960 it created much attention among the leaders of this field and put mass spectrometry on the map of organic chemistry. Indeed, right after the lecture, Professor Carl Djerassi came up to invite me to Stanford University to help with the installation of a mass spectrometer in his laboratory and to train a postdoc (who turned out to be Herbert Budzikiewicz) in its operation and the interpretation of the spectra. He recounted his impression 32 years later: "... It was the elegant rationalization by Biemann et al. of the mass spectral fragmentation behavior of alkaloids of the



aspidospermine class that stimulated a serious effort at Stanford on organic chemical applications of mass spectrometry." [16].

That elucidation of the fragmentation (Scheme 1) of desacetylaspidospermine (**7b**), which leads to the mass spectrum shown in Figure 3, prompted us to search for other alkaloids produced by *Aspidosperma quebracho blanco*. Upon injection of an extract of the bark of this plant [because of the demand by pharmaceutical research laboratories at that time, tropical plant materials could be obtained commercially (in this case from S. P. Penick and Co.)] into a packed GC column held at 265 °C, a chromatogram (Figure 4) was obtained, which

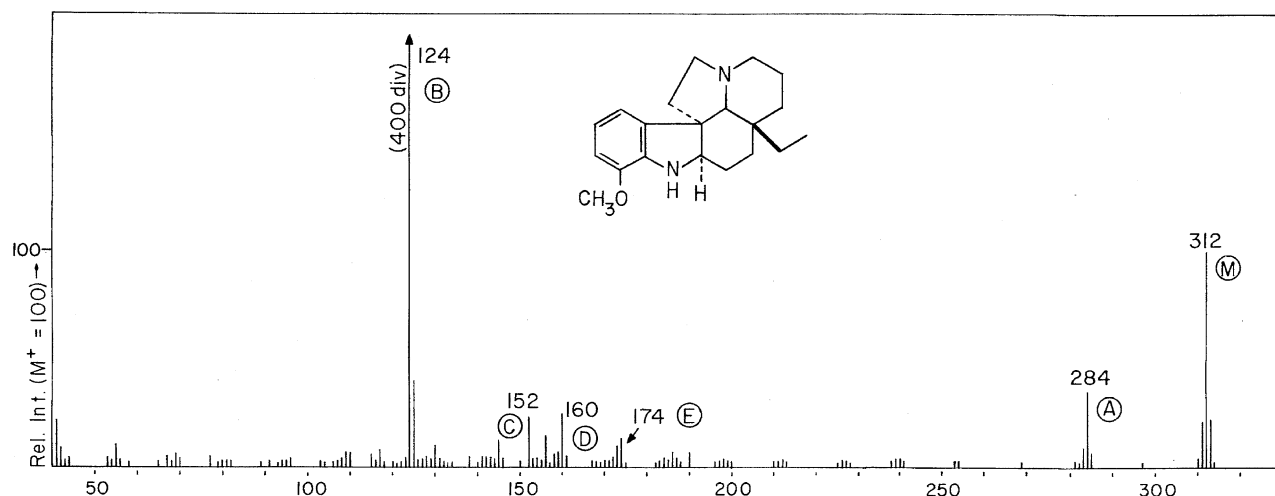


Figure 3. Mass spectrum of desacetylaspidospermine (**7b**). (Reproduced with permission from Reference [18], copyright 1963, American Chemical Society.)

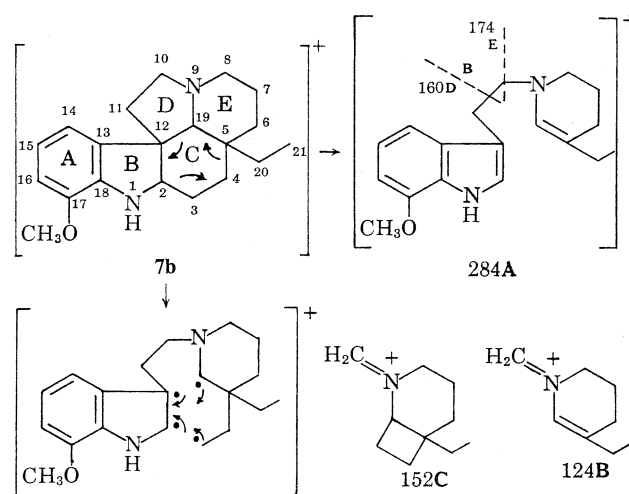
looks awful by today's standards, but at that time it was terrific. Fractions were collected individually by sticking a melting point capillary over the exit of the column and cooling it with a piece of dry ice. The capillary was then placed into the heated inlet system of the CEC 21-103C mass spectrometer. The spectra revealed the presence of at least 17 different alkaloids. Judging from the easily discerned molecular weights, most of them were new. It was clear from the spectra that there were two types of carbon skeletons present. One (Group A), recognized by the strong signal at m/z 124, corresponded to the aspidoamine type (see Figure 3), but the other one (group B), dominated by a peak at m/z 136 had to represent a different structural type.

When a larger portion of the extract was separated on an alumina column using gravity chromatography, sufficient pure material was obtained to record good, individual mass spectra. Based on the mass and UV spectra it was possible to assign structures to all the components belonging to the aspidoamine class (Group A, Figure 5). From what had been learned about the spectra of several indole alkaloids and using the mass shifts of the various components (Figure 6), it was possible to assign Structure **10** to alkaloid 266B (Scheme 2) and its five congeners (Figure 7). The positions of substituents on the benzene ring of the indole moiety were again deduced from UV data. Quite a few of the components were sufficiently abundant to crystallize so that their melting points could also be determined. The value for 338B ($157-9^\circ\text{C}$) compared well with 162°C of a substance isolated 80 years earlier [8] from the same plant and named aspidoamine. The identity of the two compounds was further corroborated by elemental analysis and optical rotation, the only other data reported by Hesse who had assigned it a molecular formula $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_2$, i.e., a CH_2 too much.

In the past it was common practice to report newly discovered natural products after characterizing them by their elemental composition (determined by com-

bustion) and melting point. A name was assigned, generally based on a part of the botanical name of the plant from which the product had been isolated. Since we determined the structure of the new compounds right after their isolation, we did not need to invent a trivial name for each one of them but simply characterized them first by their molecular weight and structural type. After the individual structures were determined, we could derive a chemical terminology based on the historical names aspidoamine and aspidoamine and using the common insert -idi- for the unsubstituted analog.

When submitting the full paper for publication in JACS, we ran into a problem with the Assistant Editor, A. L. Autrey, who remarked in a letter dated October 9, 1962: "... More important is the need for pointing out the restricted usefulness of the work in its present form ... the samples you have applied it [mass spectrometry] to are open to question as to their homogeneity. You have detected 20 compounds, but we question that you



Scheme 1

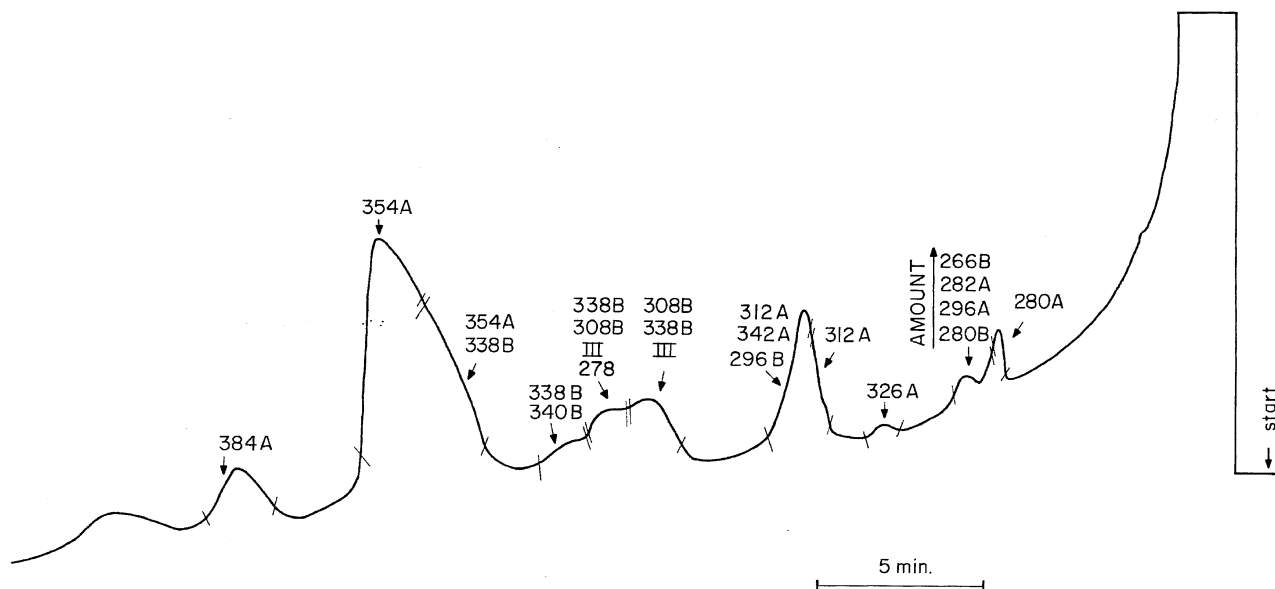
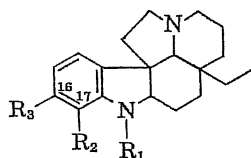


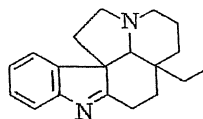
Figure 4. Gas chromatogram of the extract from the bark of *Aspidosperma quebracho blanco*. Slanted lines bracket fraction collection. Code numbers indicate molecular weight and pattern type (reproduced with permission from Reference [18], copyright 1963, American Chemical Society).

have "isolated" 16 of them. There is no demonstration, other than by gas chromatography, of their purity. None of the usual criteria of purity have been applied, none have been characterized by classical means: No melting points, no [combustion] analyses, few UV and no IR spectra . . . It is quite possible that in the future natural product chemists will take the turn your work has indicated, and that investigators will obtain materials, name them, and deduce their structures without characterizing them in presently accepted ways. However, until this is more common, a clear indication of the extent of your departure from past methods is necessary."

In his ardent defense of the status quo Dr. Autrey



- 282A ($R_1 = R_2 = R_3 = H$): aspidospermidine
 296A ($R_1 = CH_3, R_2 = R_3 = H$): 1-methylaspidospermidine
 312A ($R_2 = OCH_3, R_1 = R_3 = H$): deacetylaspidospermine
 326A ($R_1 = CH_3, R_2 = OCH_3, R_3 = H$): 1-methyldeacetylaspidospermine
 342A ($R_1 = H, R_2 = R_3 = OCH_3$): deacetylpyrifolidine
 354A (= II; $R_1 = CH_3CO, R_2 = OCH_3, R_3 = H$): aspidospermine
 384A ($R_1 = CH_3CO, R_2 = R_3 = OCH_3$): (-)-pyrifolidine



280A: 1,2-Dehydroaspidospermidine

Figure 5. Structures of alkaloids belonging to group A (aspidospermine type).

had apparently also overlooked the fact that we had determined the melting points of the more abundant new compounds, which crystallized, and even burned a few milligrams of 338B to compare the elemental analysis with Hesse's data of 1882. It took a three-page rebuttal and a copy of my book [17], which had just appeared, to get the paper finally published [18]. Fortunately, natural products chemists quickly accepted and adopted our "departure from past methods".

The need to establish elemental compositions by combustion analysis, which required a few milligrams, much more than needed for a mass spectrum and other non-destructive spectral data (UV, IR, and later NMR) was soon eliminated by the advent of high resolution mass spectrometry. Inspired by J. Beynon's use of the "peak matching" method [19] for accurate mass measurements with a Nier-Johnson double-focusing mass spectrometer (MS-8, which became the commercial MS-9 of AEI), we recorded complete high resolution spectra on photographic plates exposed in a Mattauch-Herzog instrument [17]. The ability to deduce the elemental composition not only of the intact molecule, but also of each one of the fragments in a single experiment greatly facilitated the determination of structures from that time on.

An example where high resolution data became crucial was the determination of the structure of Vinblastine, a "dimeric" alkaloid of oncolytic activity. It had been established previously that it consists of two indole alkaloids, Velbanamine and Vindoline, but their connectivity was unknown. Because of the size of this molecule, elemental analyses were inconclusive, but the exact mass of 810.4219 (a world record at the time) established a composition of $C_{46}H_{58}N_4O_9$ [20]. The composition of a number of key fragments allowed the

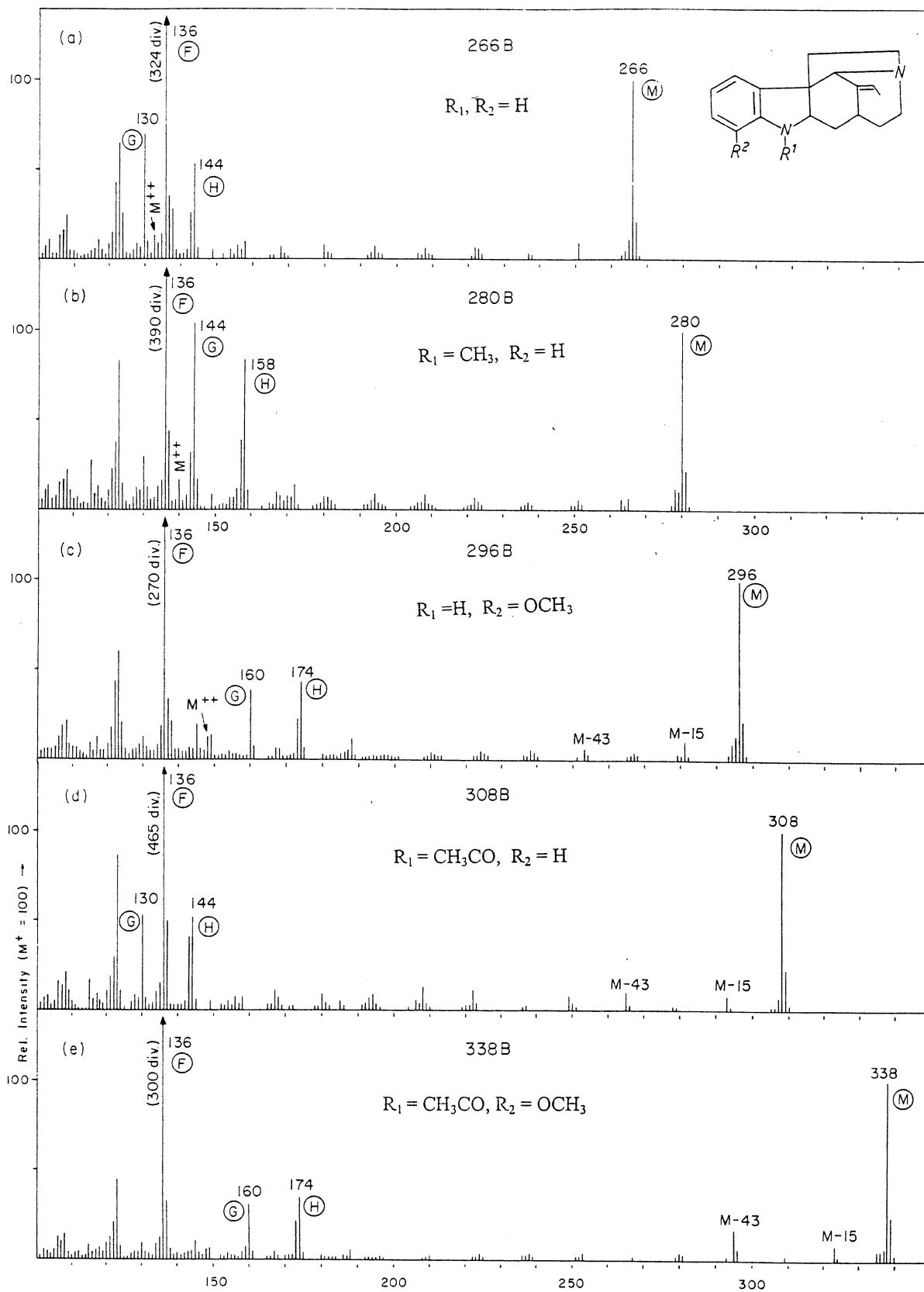
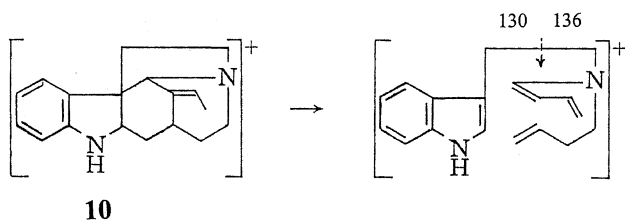
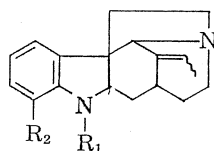


Figure 6. Mass spectra of alkaloid type B (reproduced with permission from Reference [18], copyright 1963, American Chemical Society).

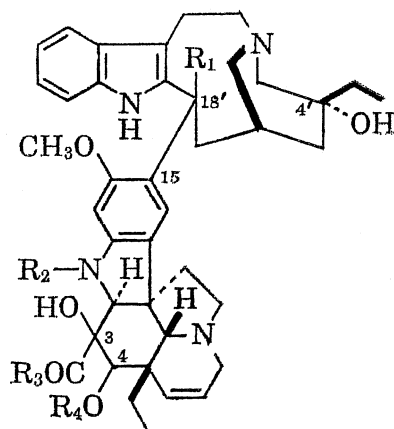


Scheme 2



- 266B ($R_1 = R_2 = H$): aspidospermatidine
 280B ($R_1 = CH_3, R_2 = H$): 1-methylaspidospermatidine
 296B ($R_1 = H, R_2 = OCH_3$): deacetylaspidospermatine
 308B ($R_1 = CH_3CO, R_2 = H$): 1-acetylaspidospermatidine
 338B ($R_1 = CH_3CO, R_2 = OCH_3$): aspidospermatine
 340B: 14,19-dihydroaspidospermatine

Figure 7. Structures of alkaloids belonging to group B (aspidospermatine type).



- 11** $R_1 = COOCH_3; R_2 = CH_3;$
 $R_3 = OCH_3; R_4 = COCH_3$
12 $R_1 = COOCH_3; R_2 = CHO;$
 $R_3 = OCH_3; R_4 = COCH_3$

determination of the complete structure of Vinblastine (**11**) as well as that of the related Vincristine (**12**) [21].

This work continued until the end of the 1960's when we at MIT and Djerassi's group at Stanford, as well as others, had established the structures of close to hundred alkaloids [22], in part or entirely by using mass spectrometry in a way first demonstrated only a decade earlier [6]. Now the shelves were bare and the quest for new natural products of that category had subsided, chiefly because none of them had become a block-buster drug. A minor exception was vinblastine, for quite some time the most useful medication for a relatively rare type of leukemia, Hodgkin's disease.

Early Instrumentation

In the light of today's compact, microprocessor controlled mass spectrometers incorporating sophisticated data systems and sometimes even robotic sampling devices, it may be educational, at least entertaining, to look back almost half a century. Much of what is described briefly in the following is outlined in detail in Reference [17].

The Mass Spectrometer

At the time I planned to use mass spectrometry for the determination of the structure of natural products, the choice of commercially available instruments was practically limited to a single one: The Consolidated Electro-dynamics Corporation (CEC) model 21-103C (Figure 8). This was because of its dominance in the petroleum industry and patent protection, which kept the fledgling British AEI (Associated Electrical Industries, now split into Kratos and Micromass, Manchester, UK) MS-2 and German ATLAS Werke (now Finnigan MAT, Bremen, Germany) CH-4 out of the United States. A further selling point was the American Petroleum Institute's (API) collection of mass spectra, mainly of hydrocarbons and simple monofunctional organic compounds, most of which had been recorded on instruments of the 21-103 series. The dominance of quantitative, analytical applications of MS made this database very valuable and provided good public relations for CEC.

Obtaining a mass spectrum was quite different from the highly automated process of today. The sample had to be vaporized into an all-glass inlet system held at up to 200 °C. Enough sample (0.1–1 mg) was needed to generate 10^{-2} mm Hg pressure in the reservoir from which it leaked through a multi-hole molecular leak (CEC's dominating patent!) into the ion source. That

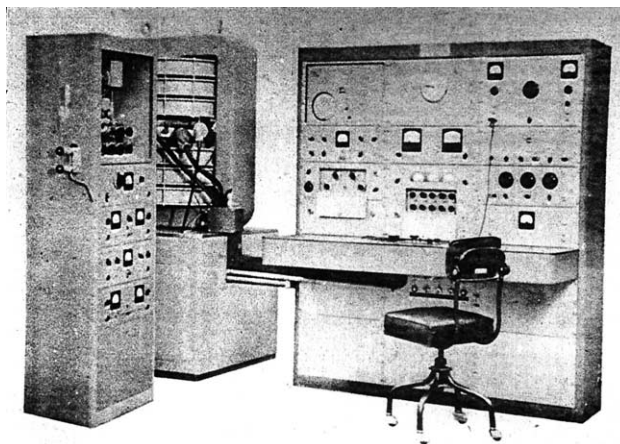


Figure 8. The Consolidated Electro-dynamics Corp. Model 21-103C mass spectrometer. Left: Inlet system cabinet (in the author's laboratory, the top portion was enclosed and heatable to 250 °C); center: 3-Coil electromagnet, the flight tube in the middle, main pumping system housed below; right: Operator console (note the round door in the upper left for retrieval of the photographic recording paper).

large amount was needed so that the pressure remained relatively constant during the ca. 20 min. required to record the spectrum. Scanning was by decreasing the accelerating voltage from 3 kV to 300 V at constant magnetic field, because that was easier and much more reproducible than scanning the latter. The penalty was a limited (1:10) mass range, and one thus had to record a spectrum in two pieces (e.g., m/z 10–100 and say, 50–500) at two different magnetic field settings. Each scan took about 5–10 min., limited by the recording device, a 5-mirror galvanometer that deflected a light beam onto a roll of photographic paper. At the end of the scan, the paper had to be retrieved using a black silk sleeve, taken to the darkroom, developed, fixed, and dried. A quick inspection of the 1–2 m long record showed whether the spectrum was any good and the sample could be pumped out from the inlet system. While the wet processing of the record was cumbersome, it had the advantage of producing a permanent record with a black-on-white trace. In that respect, the self-developing UV-sensitive paper that came into use somewhat later was a regression because the trace was of low contrast and faded quickly in daylight. Attempts to use a copy machine turned the record completely black.

While there was a "mass marker" (triggered by the electric field), which put a dot on the bottom of the record for every 10 mass units, it was not reliable enough to establish the mass scale. Therefore, one had to recognize the ever-present peaks at m/z 18, 28, 32, and 44, due to unavoidable air leaks, and then manually count up from there. The high dynamic range of the instrument made this quite easy, because the five galvanometers had deflection ratios of 1:3:10:30:100, providing a range of better than 1:100,000 from full-scale on the least sensitive trace. Once the mass scale was established on the low mass scan, the bottom end of the high-mass scan had to be matched and the counting continued to the upper mass end (Figure 9). A good checkpoint was always the characteristic pattern of the mercury isotopes at m/z 196–204 (a free benefit provided by residual back-streaming from the Hg-diffusion pumps used).

After establishing the m/z value of each peak, its height had to be measured with a ruler and converted to its absolute value by multiplication with the appropriate factor. Needless to say that it took quite some time and effort until one could draw the type of "bar-graph" nowadays instantly provided by the instrument's data system. The tediousness of this process had, however, two great advantages: First, it assured that only very few but significant mass spectra were recorded; and second, that the investigator had a lot of time to think about each spectrum, and what it may mean. I personally did almost all my thinking and preliminary interpretations during these otherwise boring tasks.

The requirement to achieve a sample pressure of about 10^{-2} mm Hg in a relative large sample reservoir was dictated by the use of the mass spectrometer for the

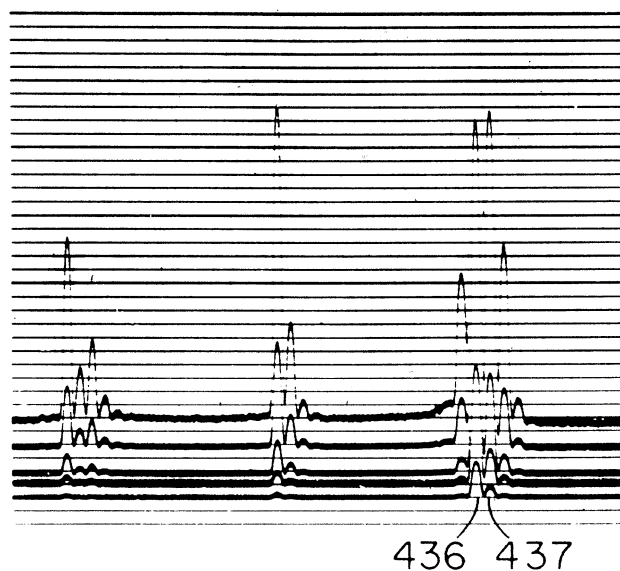


Figure 9. Part of the oscillograph record at high mass. Most sensitive trace on top, least sensitive on bottom.

quantitative analysis of hydrocarbon mixtures in the petroleum industry. The procedure was, however, wasteful as far as the required amount of sample was concerned, and limited the methodology to compounds that can be vaporized at <250 °C to achieve such a pressure without decomposition. For our purposes the precise reproducibility of relative intensities was not important and we therefore tried to circumvent the entire inlet system by vaporizing the sample directly into the ion source, which required only a vapor pressure of 10^{-6} mm Hg and to be maintained only in the small volume of the ion source housing.

Preliminary experiments were carried out with a Bendix time-of-flight (TOF) mass spectrometer. It was equipped with a pyrolysis probe consisting of a resistance-heated filament that could be placed below the relatively open ion source through a vacuum lock. By replacing that filament with a heating coil holding a short piece of melting point capillary containing the sample close to the ionizing electron beam, we were able to obtain the mass spectra of intact, underivatized nucleosides and amino acids [23]. Because of the inferior resolution of the TOF spectrometer, we then constructed similar direct introduction probes for the CEC 21-103C and finally for the high-resolution mass spectrometer briefly outlined in the following.

As mentioned above in the section on dimeric indole alkaloids, the determination of the elemental composition of the intact molecule and all the fragments thereof via the measurement of exact mass became an important tool in the beginning of the 1960's. We chose the CEC 21-110 model, because it was based on the Mat-tauch-Herzog geometry, which allowed for the simultaneous focusing of the entire spectrum. By placing a 340×50 mm photographic plate into the focal plane, one could record a complete high-resolution spectrum during an exposure time of less than one minute. With



Figure 10. The author (standing left) discussing a photographic plate with Walter McMurray and Peter Bommer (seated) in front of the operator panel of the CEC 21-110 high-resolution mass spectrometer (photo ca. 1982/1983).

a 1 mm high slot in a mask ahead of the focal plane, one could accumulate up to 30 spectra onto one plate before it had to be replaced through a vacuum lock (Figure 10).

Processing of the large amount of data represented by each exposure required a number of steps: Measuring the exact position of each of the often more than hundred narrow lines along the plate by using a microdensitometer (also called "comparator"); conversion of position to mass, based on the positions of lines from perfluorokerosene always added as an internal mass standard; and calculation of all possible elemental compositions that would fit within a specified limit (e.g., ± 0.003 u). These principles were well known from spark-source mass spectrometry, used for qualitative and quantitative analysis of metals, alloys etc. In those applications only a few lines were present and even fewer needed to be measured, which could easily be accomplished manually. In our case, hand measurements and calculations sufficed to provide a feel for the accuracy attainable, but not for general use.

Computers

Fortunately, MIT had begun to make its computer center's IBM 709 available to research in other departments, as long as one provided a FORTRAN program for the task and the data to be processed, everything punched into IBM cards. The results could be picked up the next day, printed on reams of paper as well as punched on cards for any further use. Punching the measured distances onto the cards without mistakes soon became too tedious and we began to automate the entire process. A variable speed motor replaced the

hand-crank of the comparator, a numerical position encoder mounted on its precision screw and another one on the photomultiplier output. These two signals were fed into a card punch operated in the continuous mode. To avoid recording the useless data (>95%) of the baseline between the lines from one nominal mass to the next, the operator slowed down the scanning speed when a line (or multiplet of lines) approached and pushed a button to activate the card punch. It was part of the data processing algorithm to find the center(s) of the line(s) from the profile recorded.

This system worked well for a while, but the number of boxes of IBM cards that had to be moved back and forth steadily increased and MIT's computer center started to charge ever higher user fees. When, in 1964, NIH asked me (yes, at that time they sometimes *asked*) to apply for one of the newly created National Research Facility grants (now NCRR) to make high-resolution mass spectrometry available to the biomedical community, and also for a training grant in the same area, the opportunity had come to set up my own computer system. Again, the choice was very limited: The only system then on the market and capable of recording continuous data "on the fly" was the IBM 1800. Its original purpose was to serve as a process monitor for industrial plants. To put it into perspective with today's MS data processors, which in addition also control much of the mass spectrometer's operation and are hidden within the instrument cabinet, the 1800 stood about 6 feet high. The central processing unit (CPU) operated with a core memory of 32,767 16-bit words, from which data could be dumped on one of three magnetic disks (12,000 words each) housed in a separate cabinet (Figure 11). Thus, the total space to work with was 135,534 bytes, less than one-tenth the capacity of one of today's 3 1/2 inch floppy disks! Final storage was on digital tape. The entire system was first leased for \$50k annually, purchased for \$250k two years later, when its usefulness had been established.

Interfacing a new, specially designed comparator (D. Mann, Burlington, MA) to the IBM 1800 system took care of all the data generated by the high-resolution mass spectrometers, of which we had two by 1965. It also provided us with the opportunity to develop methodologies to explore and utilize the vast amount of information generated by continuously scanning low-resolution mass spectrometers, especially when interfaced with a gas chromatograph (GCMS) (see next section). We had already begun this work by recording data on analog and digital tapes, but the installation of the IBM 1800 made it possible to accomplish this for the first time "on-line". The process began with digitizing the electron multiplier output of the computer-controlled, continuously scanning (3 s/scan, m/z 40–600) Hitachi RMU-6D mass spectrometer, defining all peak centers, assigning its m/z value and storing all resulting mass spectra on disk. For further processing other concepts, most notably "mass chromatograms", automated identification by "library searching", etc. were

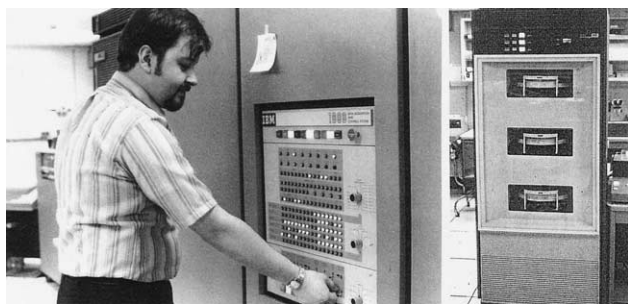


Figure 11. The IBM 1800 computer. Left: The CPU cabinet; the banks of lights tell the operator (here Ed Ruiz) the status of processing. Right: The cabinet holding three disks.

developed [24]. To facilitate inspection and interpretation of the entire data set “off-line” (“multi-tasking” of the CPU was not possible at that time) each GCMS data set (all spectra and all mass chromatograms) was automatically put on a roll of microfilm by using a 16 mm Bolex movie camera focused on a CRT screen, which automatically displayed one spectrum and one mass chromatogram after the other. The investigator then could look at the data leisurely on any one of the film readers we had around our laboratory.

While this system was never duplicated elsewhere, instrument manufacturers began to incorporate the concepts into their products as computer technology rapidly advanced. Size and cost of processors decreased while memory, speed and storage capacity increased almost exponentially. In retrospect, NIH’s money handsomely paid its dividends for the benefit of the biomedical (and chemical) community.

Gas Chromatography

In the mid 1950’s Professor E. R. H. Jones, University of Manchester, UK, spent a summer at MIT and, almost accidentally, told us about the usefulness of gas chromatography, which had just been invented by James and Martin [25]. As an analytical method, it had not immediately attracted the attention of organic chemists, but Professor Jones’ examples of its use in the organic synthesis laboratories of Imperial Chemical Industries (ICI) in the UK made us aware of the unique significance of this methodology. As a consequence, our machine shop cranked out the necessary parts so that each laboratory could put together its own “preparative” GC suited not only for the quantitative analysis of reaction mixtures, but also for the isolation of their components.

Compared to today’s gas chromatographs, the contraption was very simple, one may say primitive: It employed a U-shaped glass tube packed with finely ground firebrick (capillary columns were invented much later) coated with Apiezon (a commonly available purified stopcock grease). Helium (at about 30 mL/min) passed first over a thermistor, then through the column and again over another thermistor to exit. Both

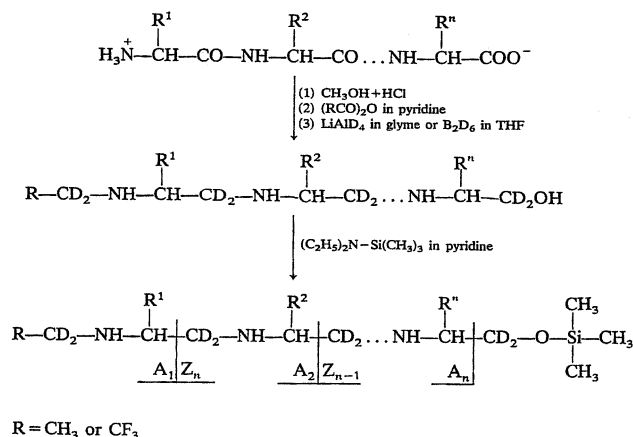
were part of a Wheatstone bridge circuit and the difference in current (representing differences in thermal conductivity of the gas mixture) was displayed on a strip-chart recorder, the only expensive part of the contraption. The column was kept at constant temperature (easy to implement, compared to the temperature programming used much later).

Packed columns required, but also could accommodate, large (milligram) samples, an advantage for the organic chemist. Constant temperature meant that volatile components eluted fast at the beginning and less volatile ones came off as broad peaks (see Figure 4). But, if necessary, the mixture could be injected at two different column temperatures to optimize resolution. Most importantly, the detector was non-destructive, i.e., the effluent could be collected and used for further experiments (record a mass spectrum or other data) or re-injected for better resolution.

Collecting fractions was tedious and we, as well as others, thought to overcome that step by interfacing the exit port of the gas chromatograph directly to the ion source of the mass spectrometer. This problem was finally solved in a practical way simultaneously by Ragnar Ryhage [26] and in our laboratory [27]. The former used the diffusion principle, a jet orifice in front of an aperture (modeled after processes previously used for the separation of uranium isotopes as their hexafluorides), while we used the faster effusion of small molecules (helium) over large molecules through the wall of a tube of fritted glass. Because the “Ryhage separator” was patented, it could only be purchased along with an entire LKB GCMS system, while the “Watson-Biemann” separator (not patented) was freely used by the other instrument manufacturers and without royalties. These “carrier gas separators” were needed because of the high flow rates required by packed columns and the limited pumping capacity of the mercury diffusion pumps of earlier mass spectrometers. They became obsolete with the advent of capillary columns, the commercial use of which was delayed in the United States (compared to Europe) by a patent held by the Perkin Elmer Corp. Efficient oil diffusion pumps also could easily handle the lower gas flow rate of these columns.

Peptides and Proteins

It took a little longer to convince the biochemists and biologist of the usefulness of mass spectrometry although my very first paper published in this field in 1959 outlined a strategy for the determination of the amino acid sequence of small peptides [28]. Methodologies for peptide sequencing were still in their infancies. Fred Sanger’s N-terminal labeling of di- and tripeptides with a dinitrophenyl group, followed by total hydrolysis and identification of the labeled and unlabeled amino acid(s) by paper chromatography, enabled him to establish the first structure of a protein, insulin in 1953 [29]. At about the same time Pehr Edman began to

R = CH₃ or CF₃

Scheme 3

develop the stepwise degradation of proteins from their N-terminus [30].

I had planned to develop a chemical approach for the labeling of the C-terminus of small peptides, to complement Sanger's method, but as outlined earlier [3] abandoned that idea in favor of mass spectrometry. I realized that peptides should be ideally suited for this methodology because they represent linear molecules of repeating backbone units substituted with a limited set of side chains, all of which differ in mass with the exception of the isomeric pair leucine and isoleucine, and the isobaric glutamine and lysine. The main obstacle was, of course, the utter involatility of these zwitterionic molecules, which prevented their vaporization so necessary for electron ionization. Here again, training in organic chemistry came in handy, as I knew that this could be overcome by acylation of the amino group(s) and esterification of the carboxyl group(s). Furthermore, the polar amido groups could be converted to the much less polar secondary amines (first step of Scheme

3). These reactions also caused the modified side chains of glutamine and lysine to differ in mass. The use of lithium aluminum deuteride instead of the hydride avoided the side chain of aspartic acid to become identical in mass to that of threonine. As I had predicted—or at least hoped—the mass spectra of the products, ethyl-oligoethylenediamino alcohols were extremely simple due to their specific cleavage at the ... NH-CHR ... bonds, resulting in sequence specific fragments (Figure 12).

It was clear from the outset that any viable sequencing method had to be applicable to the complex peptide mixtures resulting from the chemical or enzymatic degradation of the protein of which the structure is to be determined. Gas chromatography seemed to be the method of choice. Compared to paper chromatography, it had superior resolving power and loading capacity, but made it even more important to use derivatives that could be vaporized at atmospheric pressure, not just in the vacuum of the mass spectrometer. The earliest experiments already demonstrated [31] that the sequences of five peptides could be determined by mass spectrometry after conversion of the mixture to amino-alcohols, separation by GC, and collection of the components as they eluted (similar to our alkaloid studies described above) (Figure 13).

The development of mass spectrometric methods for peptide and protein chemistry has been chronicled in more detail elsewhere [32]. It took more than 15 years from the original experiments until the first sequencing of a small protein, subunit I (44 amino acids long) of monellin, was accomplished by mass spectrometry [33]. For that work, the protein had to be subjected to partial acid hydrolysis to generate the complex mixture of many overlapping di- to hexapeptides. By that time we had continuously improved the methodology through the replacement of acetylation with trifluoroacetylation

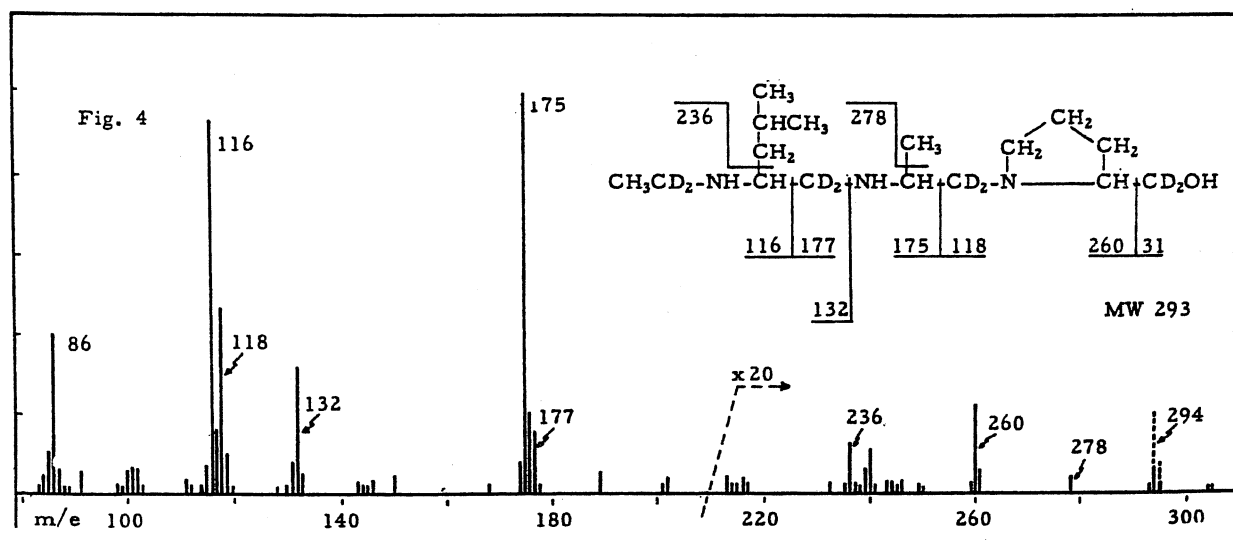


Figure 12. The mass spectrum of the reduction product of N-acetyl-isoleucyl-alanyl-proline methyl ester (from Reference [31], copyright 1960, Elsevier Science [U.S.A.], reproduced with permission).

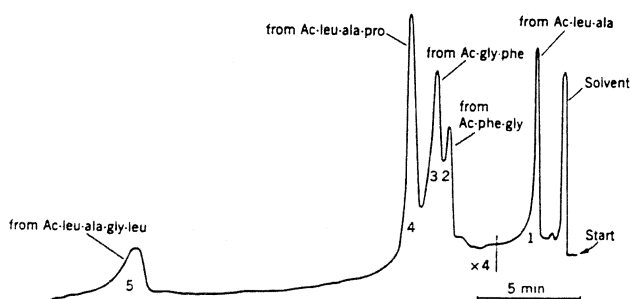


Figure 13. Gas chromatogram of the reduction product of a mixture of five peptides (from Reference [31], copyright 1960, Elsevier Science [U.S.A.], reproduced with permission).

[34] (second step of Scheme 3) and O-trimethylsilylation [35] to increase volatility, which extended applicability to these larger peptides. Direct coupling of the gas chromatograph with the mass spectrometer [27] made it possible to identify 61 peptides in the hydrolyzate of monellin-I in a single experiment.

During the 1960's the stepwise Edman degradation had been essentially automated and had become the mainstay of protein sequencing. Its major deficiency, the inability to work on blocked N-terminal amino acids, provided a niche for mass spectrometry which on individual small peptides could not only deduce their sequence but also identify the blocking group. Post-translationally modified amino acids which were not amenable to the Edman procedure, such as γ -carboxyglutamic acid, could be identified by mass spectrometry. Osteocalcin, a calcium-binding protein rich in this component, could thus be successfully sequenced by GCMS [36].

Furthermore, stretches of hydrophobic amino acids, particularly toward the C-terminus, led to high losses of material from the reaction vessel of the Edman apparatus because of "wash-out". This had been one of the difficulties in the Edman sequencing of monellin [37], which has a very hydrophobic C-terminal sequence -Gly-Pro-Val-Pro-Pro.

Extreme hydrophobicity is also a common characteristic of trans-membrane proteins such as bacteriorhodopsin, the amino acid sequence of which could only be determined correctly by a symbiosis of Edman degradation and GCMS [38a, 38b]. This protein, which turned out to be 248 amino acids long, was very resistant to enzymatic cleavage because of its insolubility in water. In aqueous suspension it was slowly attacked at a single bond by chymotrypsin, producing two large peptides, C-1 and C-2. These in turn could only be dissolved in 70% formic acid, the medium in which cyanogen bromide (CNBr) cleaves at methionine, which is converted to homoserine in the process. C-2, which contained five methionines resulted in six peptides, CNBr-1 through CNBr-5a and b. About two-thirds of the sequences of CNBr-1 to CNBr-4 could be determined by the automated Edman degradation, but excessive washout precluded reaching the C-termini. CNBr-5a and b were too short for this method.

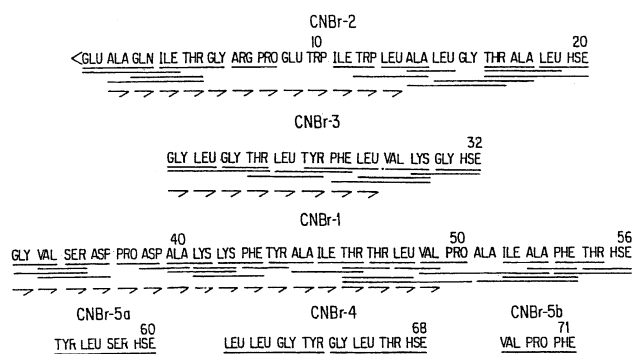


Figure 14. The amino acid sequence of cyanogen bromide peptides of C-2 of bacteriorhodopsin (CNBr-1 through 5a, b correspond to elution sequence from liquid chromatograph). Arrows indicate Edman data, underlining denotes sequences determined by GCMS.

The GCMS experiments carried out in parallel not only confirmed the Edman data (Figure 14), but also provided the information necessary to assemble the complete sequence of C-2: (1) Establish the missing C-terminal sequences; (2) sequence the short peptides CNBr-5a and b; (3) identify the blocked N-terminus of CNBr-2; and (4), most importantly, permit the proper alignment of the six peptides to a single sequence. For this, the amino acids adjacent to each of the five methionines of C-2 had to be determined. This was accomplished by partial acid hydrolysis of C-2, converting the complex mixture of peptides to the O-TMS polyamino alcohol derivatives and injecting it into the GCMS. The entire set of 250 scans was then searched for ions specific for a sequence A-Met . . . (where A is any amino acid), the minimum sequence for a peptide defining an overlap. Using the principle of coinciding mass chromatograms [24], the mass spectra of all methionine-containing derivatives could be extracted from the mass of data and their sequence determined [38a]. The same strategy was then successfully applied to the larger C-1, thus completing the structure of bacteriorhodopsin [38b].

By 1980 it had become possible to sequence the gene coding for a protein. However, the "reading" of the gels was still beset with errors. Combination with an entirely different approach employed simultaneously seemed to be the most efficient strategy. Because of its high sensitivity, our GCMS methodology for peptide sequencing was used to aid in the determination of the amino acid sequence of very large proteins (some close to 1000 amino acids long), such as aminoacyl-tRNA synthetases [39]. By matching random, short amino acid sequences obtained by GCMS continuously to the DNA data as they were acquired, it was instantly possible to see whether the latter were correct. A missing or erroneously inserted nucleotide causes a "frame shift", which then translates into a fictitious amino acid sequence that would not appear in any of the GCMS data produced from the real gene product, the protein. That

information made it possible to detect the error and to correct it.

During these two decades since our first publication [28] there were very few laboratories, in addition to ours, which explored or used mass spectrometry for peptide sequencing. Foremost among them was the research group of Howard Morris (Imperial College, London), who used N, O-methylated N-acetyl methyl esters of small peptides, which could be crudely fractionated directly into the ion source of the mass spectrometer [40]. Somewhat later D. F. Hunt used similar derivatives for chemical ionization and a triple-quadrupole mass spectrometer in conjunction with a high-performance liquid chromatograph to sequence mixtures of peptides [41]. All these strategies required considerable expertise to be used at the sensitivity required and thus were practiced almost exclusively in the laboratories where they had been developed. But, as a consequence, biochemists and biologists had become aware of the potential of mass spectrometry in protein chemistry.

The situation started to change dramatically in the early 1980's. The late Michael Barber had discovered the ionization of comparatively large, polar molecules by "fast atom bombardment" (FAB) [42]. The spectrum of an undecapeptide ([Met-Lys]-bradykinin) of mol. wt. 1318 opened the way to new strategies in peptide and protein characterization. It now became possible to employ proteolytic enzymes of very high structural specificity, which by necessity produced relatively large peptides. While our GCMS methodology became almost instantly obsolete, we could quickly adapt the field desorption ion source of our MAT 731 Mattauch-Herzog type high resolution mass spectrometer to FAB by installing an argon gun [43].

As a "chemical" ionization method FAB generated abundant protonated molecule ions of low internal energy and thus low tendency to fragment. While not providing much sequence information, it allowed the reliable measurement of molecular weights of all components of a mixture of relatively large peptides without prior separation and at the nanomole level. Thus FAB-MS made it now possible to solve many problems quickly, such as the identification of mutant proteins, detection and corrections of errors in previously suggested sequences, or conversely, the identification of a protein the sequence of which is known (reviewed in [44]).

The lack of sequence information, caused by the low tendency of the protonated molecules generated by FAB to fragment, was overcome by their collisionally induced dissociation (CID) in a four-sector magnetic or triple-quadrupole tandem mass spectrometer. The former permitted high-energy collisions producing simple, sequence-specific fragmentation [45]. We used this approach for the first time in the sequencing of a number of glutaredoxins [46] and thioredoxins [47], proteins about 100–110 amino acids in length. In the course of this work, a computer algorithm for the

interpretation of high energy CID spectra of peptides (SEQPEP) was developed [48].

Novel ionization techniques continued to revolutionize mass spectrometry in general and protein chemistry in particular. John Fenn had demonstrated that exposure of fine droplets of a solution to a high electric field generates highly charged ions of even very large molecules. Not much attention was paid to his work until he reported the successful ionization of intact proteins at the 1988 ASMS conference in San Francisco [49]. Then people took notice and the measurement of molecular weights by mass spectrometry became commonplace. One of the major advantages of this method was its compatibility with the then ubiquitous quadrupole mass spectrometers, because the high charge state of the multiprotonated molecules made limited mass range not an issue. As the analyte had to be in aqueous solution, direct interfacing with a liquid chromatograph was ideal for the analysis of complex mixtures by this method, termed electro-spray ionization (ESI).

In the same year, Hillenkamp and Karas developed matrix assisted laser desorption ionization (MALDI), also capable of ionizing intact proteins [50]. This was even simpler and required less material, but at least at that time, could only be implemented on a time-of-flight (TOF) mass spectrometer. As mentioned in the Instrumentation section above, that instrument flourished briefly in the early 1960's, but had not been able to keep up with the ever improving performance of magnetic mass spectrometers and the quadrupoles that followed.

The simplicity of MALDI and its potential got us interested in its application to the determination of protein structure. Hillenkamp and Karas had carried out their experiments by modifying a TOF instrument designed for laser ionization of inorganic materials. Rather than duplicating that, we contracted with Vestec Corporation (Houston, TX) to build a MALDI-TOF mass spectrometer (following a design of Brian Chait at Rockefeller University) more suited for peptides and proteins. The instrument was delivered in October of 1990. This prototype (designated Model 2000) evolved, after the acquisition of Vestec by PerSeptive Biosystems (then by Perkin-Elmer which is now Apelera Corp.) into the Voyager Elite.

The commercial availability of such a powerful instrument and methodology at a reasonable price enabled many other laboratories to enter this field of research. The measurement of the molecular weight of a protein, such as myoglobin, to better than 1 Da [51] or the unraveling of the (inhomogeneous) pattern of glycosylation at 13 sites in invertase from yeast [52] are only a few examples from our own work besides that of many others. The development of powerful computer techniques for the identification of known or DNA derived protein sequences originally conceived by Henzel et al. at Genentech [53] and expanded by others a few years later is particularly noteworthy.

Because of its simplicity, high sensitivity, and ease of

over-all operation MALDI-MS became the sine qua non of protein chemistry. The enormous amount of highly specific and accurate data that can be produced in a single experiment, requiring only minute amounts of protein (such as that present in a spot on a 2-D gel) opened up new dimensions in biological research. The coincidence of these developments with two others—the explosion in computer technology that permits the use of large data bases on a desktop computer and the unraveling of the sequence of the human genome—led to a new field, “proteomics”. The rest of it is now history. This could not have been imagined over forty years ago, when we first demonstrated the feasibility of sequencing small peptides by mass spectrometry!

Heparin

To minimize ambiguities when using MALDI-derived molecular weights of peptides generated by enzymatic or chemical digestion as well as those of proteins, it was important to make these measurements as accurate as possible. In an effort to optimize the experimental parameters using a series of well defined peptides as internal standards, Peter Juhasz in our laboratory one day picked the oxidized A-chain of bovine insulin (A_{ox}) as the standard and bovine insulin as the “unknown”. Surprisingly, there was no signal at all at m/z 2552.7 for the $(M + H)^+$ ion of the standard, but a very large peak at m/z 8264.9 in addition to the expected one at m/z 5734.5 for the $(M + H)^+$ ion of insulin. Puzzled at first we soon realized that the signal at high mass represented the protonated complex of the two components of the mixture, $(M_{A_{ox}} + M_{ins} + H)^+$, calculated 8266.3, caused by the high acidity of A_{ox} , which contains four sulfonic acid groups in the form of cysteic acid. This complexing phenomenon, observed by chance, turned out to be rather general. When a number of highly acidic compounds we had at hand were mixed with peptides, they also ionized as protonated complexes. Not unexpectedly, more basic peptides worked best.

The most highly acidic biologically significant materials are heparin and other related sulfated glycosaminoglycans (GAG), like heparan sulfate, chondroitin, etc. From earlier, unsuccessful efforts to obtain mass spectra of short heparin fragments we still had left over a sample of a hexasaccharide with eight sulfuric acid

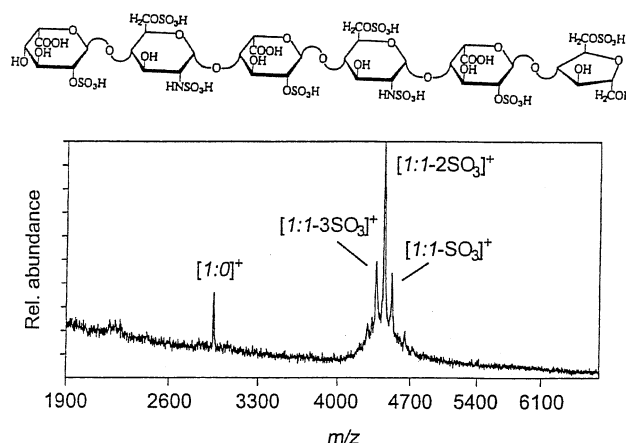
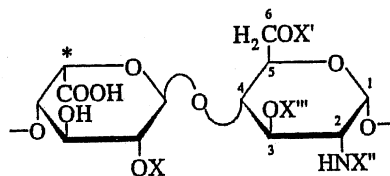


Figure 15. MALDI mass spectrum of the complex of the hexasaccharide shown and a peptide containing seven arginines and two lysines (reproduced from Reference [54], copyright 1994, National Academy of Sciences, U.S.A.).

half-ester groups. An equimolar mixture of 3 pmol thereof with a tetradecapeptide of mol. wt. 2942.41 containing seven arginines and two lysines gave an excellent MALDI spectrum (Figure 15) [54].

The implications of this experiment were very exciting as it opened a possibility for developing a strategy for the determination of the sequence of fragments of heparin—and ultimately perhaps of heparin itself—analogous to that we had developed for peptide and protein sequencing outlined in the previous section. The fact that there are no efficient, sensitive, and fast methods existing for the analysis of these highly acidic substances made such an effort even more important.

From a procedural point of view, the analogies with protein sequencing are striking: (1) While proteins are linear “polymers” of a set of α -amino acids, heparin and its congeners are linear polymers of disaccharides, consisting of one uronic (glucuronic or iduronic) acid and one glucosamine (Structure 13); (2) while there are 20 well defined amino acids commonly found in proteins, there are 32 different known modifications of the repeating disaccharide 13 (depending on the number and position of sulfate groups or N-acetylation), all of them differing in mass by at least 4 Da [55]; and (3) like proteins, which can be degraded specifically by proteolytic enzymes or chemical reactions, heparin can be



13

Repeating unit of GAG heparin. X, X', and X''' –H or SO₃H, X'' –SO₃H or COCH₃. Both epimers can occur at * (L-iduronic or D-glucuronic acid).

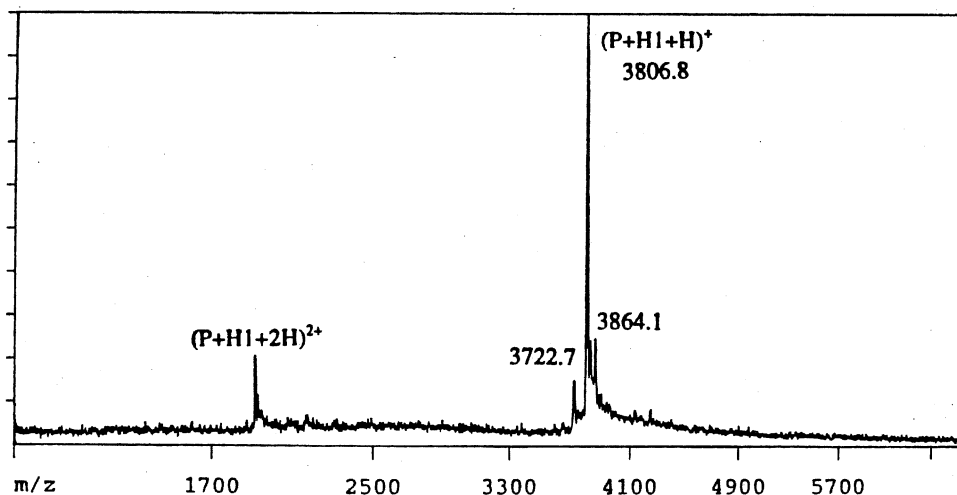


Figure 16. MALDI mass spectrum of the same hexasaccharide as in Figure 15, but complexed with $(\text{Arg-Gly})_{10}$ (reproduced from Reference [56], copyright 1960, with permission of Elsevier Science).

cleaved enzymatically with heparinases, at specific glycosidic bonds; there are also some oxidative reactions which split the carbohydrate backbone at certain bonds.

The first step in the design of such a sequencing strategy was to develop an experimental procedure for the reliable determination of the true molecular weights of heparin fragments of the size expected from such enzymatic or chemical cleavage reactions. Clearly, the elimination of one to three SO_3 groups from $-\text{OSO}_3\text{H}$ to $-\text{OH}$ (see Figure 15) must be avoided, because it simulates the presence of less sulfated molecules. Because of the quite regular spacing of the acidic groups along the linear disaccharide backbone it was expected that complexes with peptides of similar length and equally spaced arginines (the most basic amino acid) would be most stable and might thus not fragment. This turned out to be correct when we began using a properly designed synthetic peptide $(\text{Arg-Gly})_n$, where $n = 10$. It was extended to $n = 15$ when it was found

that the number of strongly basic groups in the peptide must exceed the number of sulfates in the oligosaccharide. The result of these optimization experiments, which also included the evaluation of various matrices and wavelengths, is shown in Figure 16. As expected from these data, the molecular weights of compounds of different sizes present in a mixture could be measured reliably. It was also observed that the relative signal decreases with decreasing number of sulfate groups. Thus the method is more sensitive for the larger molecules, the direction desirable for its practical application [56].

Thus, the stage was set to explore the sequencing of heparin fragments by their enzymatic cleavage followed by identification of the products by MALDI. Heparinase I was chosen because the specificity of this enzyme was best known: It preferentially cleaves at the glycosidic bond between glucosamine sulfated at the amino group and the 4-hydroxyl of an iduronic acid

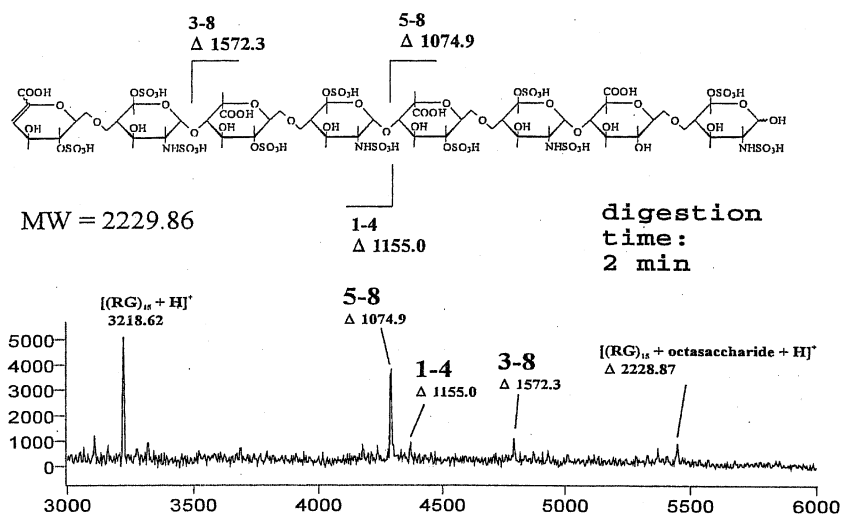


Figure 17. MALDI mass spectrum of the complex of $(\text{Arg-Gly})_{15}$ with the heparinase I digest of the octasaccharide shown (for details see text).

moiety sulfated at the 2-hydroxyl. This is an elimination reaction, leaving two products, one with the glucosamine at the "reducing end", the other with a Δ -4,5-dehydro uronic acid at the "non-reducing end".

An octasaccharide was digested with heparinase I and aliquots analyzed at various time points by MALDI after addition of the peptide (Arg-Gly)₁₅. The spectrum obtained after only 2 min of digestion (Figure 17) shows that the octasaccharide has been almost completely degraded. The tetrasaccharide 5–8 which can be formed from the octasaccharide as well as from the first product (3–8) is most abundant because heparinase I cleaves tetrasaccharides only slowly. At this time point the signals for fragments 3–8 and 1–4 are small but clearly observable [57].

These results demonstrated the feasibility of sequencing heparin and heparin like sulfated polysaccharides by mass spectrometry. In the course of an extensive study [55, 58] the experimental conditions were optimized using heparinases I and II. To simplify the sometimes complex mixture of products, capillary electrophoresis was explored successfully. Because sometimes identical subunits can be formed during digestion, it was found useful to mass-label the reducing end by conversion to a semicarbazone.

During that time we began an extensive collaboration with Professor Ram Sasisekharan and his research group at MIT, who were interested in exploring the detailed mechanism of the cleavage of heparin by heparinases. For that purpose, our method of characterization and sequencing of sulfated GAGs was extremely well suited because it was fast, required little material, and allowed the following of the time-course of cleavage [59–61]. With my official retirement in 1996, graduation of my last student (Andrew Rhomberg) in 1998, and closing of my research laboratory, I was happy to transfer the technology, including the Vestec 2000 mass spectrometer, to that group. It is gratifying to see our new methodology being applied so quickly to the study of these important biological processes.

Conclusions

The successful determination of the structure of a number of indole alkaloids starting in 1958 brought mass spectrometry, until that time confined to physics, the petroleum industry, and then analytical chemistry, to the attention of organic chemists. Academic and pharmaceutical research laboratories quickly embraced this methodology and added it to the arsenal of physical methods now so widely accepted.

At the same time we began to develop a strategy and procedure to determine the sequences of small peptides in the complex mixtures produced by the chemical or enzymatic hydrolysis of proteins. Over about two decades, mass spectrometry emerged as an alternative to the automated Edman degradation, which it complemented by its ability to determine the end-group and sequence of N-terminally blocked peptides, long

stretches of hydrophobic amino acid sequences, as well as the characterization and location of post-translational modifications. The explosion of new and efficient ionization techniques and developments in instrumentation, beginning in the late 1980's, made mass spectrometry the preeminent technology in protein research.

The observation that highly acidic compounds, normally not amenable to mass spectrometry, ionized efficiently as protonated complexes with well-defined basic peptides made it possible to determine the molecular weight of heparin fragments and similar highly sulfated glycosaminoglycans with very high sensitivity. Based on these experiments, a strategy for the sequencing of such compounds by their specific cleavage with enzymes (heparinases) was developed, in analogy to that which we had so successfully employed for peptides and proteins. This work opens the way to study the structure, chemistry, and biological function of heparin and heparin-like glycosaminoglycans, a field so hampered in the past by the lack of suitable methodologies.

Acknowledgments

It is not possible to give proper credit to all those who contributed to the work discussed in this article, but all my graduate students and postdoctoral associates are named in Reference [3]. The National Institutes of Health funded the work described over the entire period, beginning with grant GM 05472 awarded in 1958 and running continuously through 1998, and later through grant RR00317, 1966, which ran from through 1995. The early alkaloid work and high resolution mass spectrometer was also supported by a grant from the National Science Foundation.

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Laying the groundwork for proteomics Mass spectrometry from 1958 to 1988

Klaus Biemann*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, United States

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Dedicated to Professor Donald F. Hunt at the occasion of his 65th birthday; Don received his training in mass spectrometry in the author's laboratory as a postdoctoral fellow 1967–1968.

Abstract

The development of mass spectrometric methodologies for the sequencing of peptides and proteins are recounted. Early strategies for the determination of very large proteins based on a combination of nucleotide sequencing and mass spectrometric amino acid sequencing are described and their historical significance to the new field of proteomics is outlined.

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Keywords: Mass spectrometry; Proteomics; Peptide sequencing; Protein sequencing; Aminoacyl-tRNA synthetases

1. Introduction

Today a protein can be identified, including its post-translational modifications, from a spot on a 2D gel. This became possible in the late 1990s due to the confluence of developments in DNA sequencing, computer technology and mass spectrometry. But it had been only in 1953 that Sanger completed the first determination of the primary structure of a protein, insulin [1]. It was in the same year that Watson and Crick proposed the double helix structure for DNA and the genetic code had not yet been deciphered. In the 1950s chemists used mass spectrometry mainly for quantitative analysis in the petroleum industry and for the identification of relatively small and volatile organic compounds by matching their spectra with those of known samples.

2. Peptide sequencing by mass spectrometry

In 1958 we began to develop a method for peptide sequencing by mass spectrometry (MS) suitable for the determination of the primary structure of proteins. The major obstacles were the non-volatility of peptides due to their zwitter-ionic character, and the

fact that the large number of possible sequences (400 dipeptides, 8000 tripeptides, . . .) made identification by matching impossible. Therefore, we had to convert the peptide to a more volatile derivative that retains sequence information and produces a mass spectrum from which the sequence can be deduced directly. This was achieved by converting the carboxylate anion to a methyl ester and the ammonium ion to an amido group. These reactions eliminated the zwitter ion. Reduction of the carbonyl group of the peptide bonds and methyl esters by lithium aluminum deuteride resulted in an amino alcohol [2] (Fig. 1). The newly formed $-NH-CHR-CD_2-NH-$ group not only retains the sequence information but also cleaves preferentially due to the stabilizing effect of the adjacent imino groups. The result is a very simple mass spectrum (Fig. 2) from which the sequence of the parent peptide can be easily deduced directly, without the availability of an authentic sample [3]. The incorporation of deuterium was required to differentiate the side chains of serine and threonine from those formed by the reduction of aspartic acid and glutamic acid. The only ambiguities among the 20 natural protein amino acids were leucine and isoleucine due to their isomeric side chains, and the fact that asparagine and glutamine were partially converted to aspartic and glutamic methyl esters by methanolysis before reduction.

The ultimate aim of this strategy, the sequencing of a protein, required its applicability to the complex mixture of small

* Tel.: +1 617 253 7221.

E-mail address: kbiemann@mit.edu.

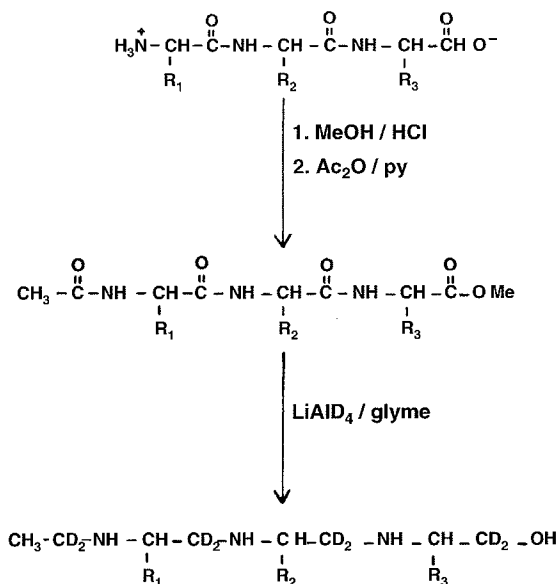


Fig. 1. Reaction scheme for the reduction of peptides to polyamino alcohols.

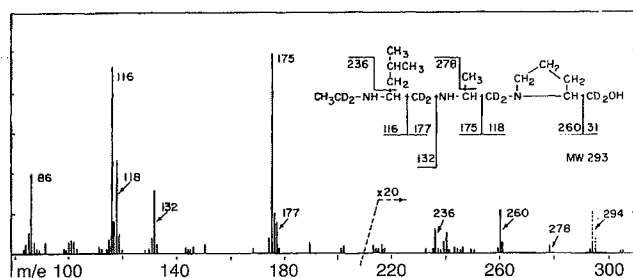


Fig. 2. Mass spectrum of the derivative of a tripeptide (Leu-Ala-Pro) (Reprinted from Ref. [3] with permission from Elsevier).

peptides produced by partial acid or enzymatic hydrolysis. It required efficient separation on a micro-scale after the derivatization of that mixture. The relatively high volatility of the polyamino alcohols allowed their separation by gas chromatography (GC) (Fig. 3), a method developed a few years earlier by James and Martin in the UK. Each fraction could be collected manually and placed into the inlet system of the mass spectrometer (CEC model 21–103C) to produce the spectrum. Thus, the feasibility of this new approach to peptide, and ultimately protein sequencing, had been demonstrated. However,

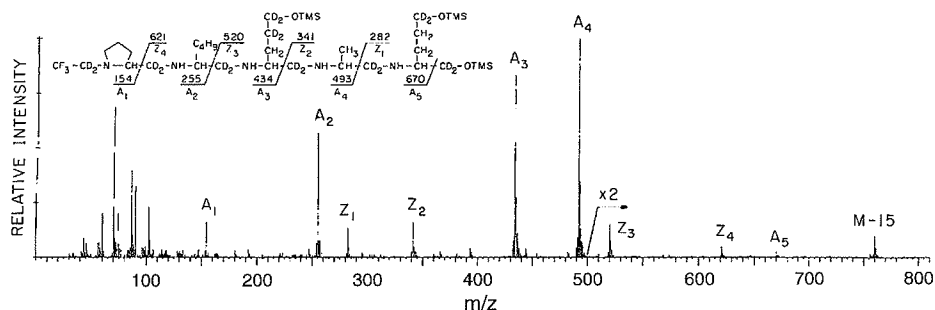


Fig. 4. Mass spectrum of the derivative obtained from a pentapeptide after trifluoroacetylation and trimethylsilylation of the corresponding amino alcohol.

GAS CHROMATOGRAM OF POLYAMINOALCOHOLS

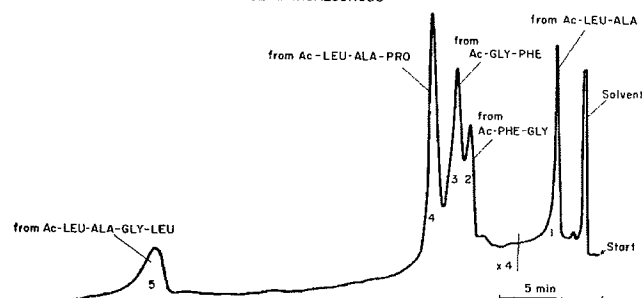


Fig. 3. Gas chromatogram of the polyamino alcohols derived from a mixture of di-, tri- and tetrapeptides (Reprinted from Ref. [3] with permission from Elsevier).

further improvements were necessary to apply it successfully to the very complex mixtures of small peptides consisting of any of the 20 protein amino acids. Trimethylsilylation of the free hydroxyl groups and trifluoroacetylation not only extended the gas chromatographic separability to larger and more polar polyamino alcohols, but also further improved the specificity of the mass spectra (Fig. 4) [4,5]. The development of the direct interface of the GC with the MS [6] eliminated the tedious and time consuming collection of individual fractions; novel computer algorithms facilitated the interpretation of the resulting vast amount of data [5,7].

Another type of peptide derivative suitable for sequencing, acetyl-N,O-permethylated methyl esters, were developed by Morris and Williams at Cambridge (UK). In these derivatives the hydrogen bonding NH-groups had been replaced by $-\text{NCH}_3$ but the carbonyl groups were left intact. Therefore, the volatility of these compounds was too low to be amenable to gas chromatography and thus were sublimed into the ion source, which provided some degree of fractionation [8]. These “permethylated” peptide derivatives were also extensively used by Hunt et al. [9].

3. Sequencing proteins

When the final version of our GCMS methodology [4,5] was applied to the partial acid hydrolyzate of subunit 1 of monellin, a small, sweet-tasting protein of unknown structure, 55 di- to hexapeptides could be identified in a single chromatogram (Fig. 5). Because of the extensive overlap, these could be assembled to one unique sequence (Fig. 6) [10].

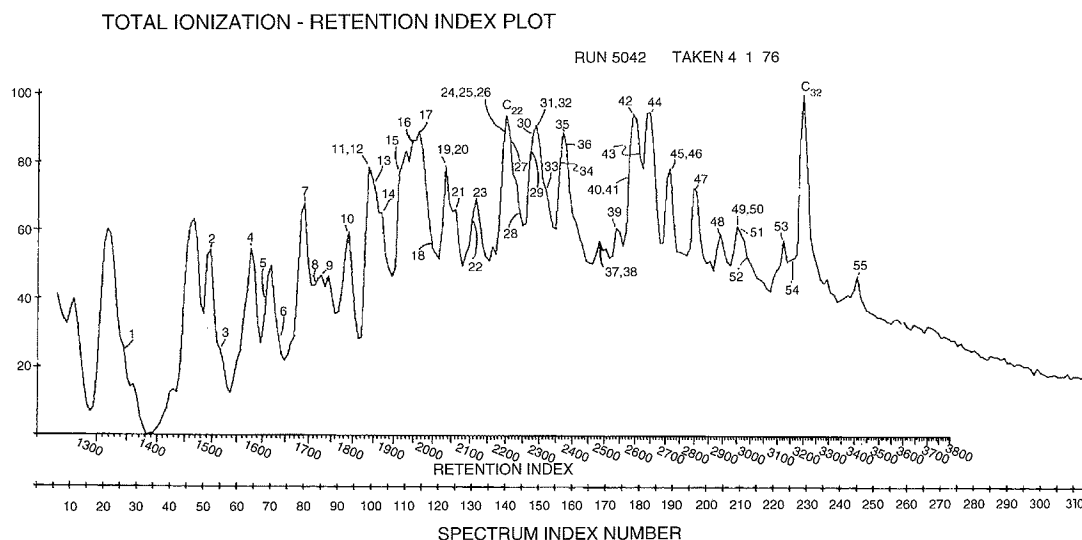


Fig. 5. Total ionization plot (gas chromatogram) of the derivatized partial acid hydrolyzate of subunit 1 of monellin. Numbers indicate elution of peptides identified by their mass spectra. C-22 and C-32 refer to the hydrocarbons added as retention time standards (Reprinted from Ref. [10] with permission from Elsevier).

In the meantime Edman had developed the stepwise degradation and sequencing of peptides and proteins [11]. This methodology became widely used after its automation [12] and commercialization. However, it had some limitations, such as N-terminally blocked and cyclic peptides, highly hydrophobic peptides, peptides containing certain chemically or post-translationally modified amino acids, etc. In such cases our mass spectrometric method provided solutions: the first instance of a N-myristylated peptide [13]; the above mentioned monellin, which has a very hydrophobic C-terminus that caused “wash-out” in the Edman sequencer [14]; peptides containing γ -carboxy-glutamic acid [15], etc.

3.1. Hydrophobic proteins

Because of the reciprocal complementarities of these two very different methods, they were often applied together during the next few years. A good example is the determination of the primary structure of bacteriorhodopsin, the light-sensitive protein from *Halobacterium halobium*. This protein, which loops through the cell membrane seven times, is so hydrophobic that it

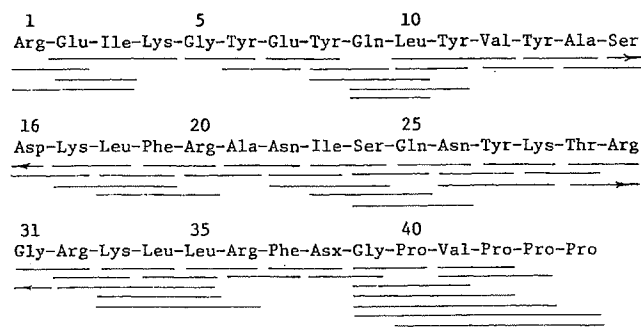


Fig. 6. Amino acid sequence of subunit 1 of monellin. Underlining indicates peptides identified by GCMS (Reprinted from Ref. [10] with permission from Elsevier).

is insoluble in the aqueous buffers used for enzymatic digestions. In suspension chymotrypsin cleaved at a single peptide bond, producing two polypeptides, C-1 and C-2, which could be separated by gel permeation chromatography. Both were still only soluble in 70% formic acid, the preferred solvent for cyanogen bromide. This reagent cleaves at the C-terminal side of methionine, producing peptides ending in homoserine. For C-2 these could be separated into five fractions, labeled CNBr-1 to 5a,b according to their elution from a reverse phase column, therefore their molecular size. These were sequenced by the Edman degradation as well as by GCMS. As is apparent from Fig. 7, the latter data revealed the sequence of all six peptides, with the exception of a few gaps and missing overlaps in CNBr-1 and CNBr-2. Fortunately, these were covered by the Edman data, which in turn could not reach the hydrophobic C-terminal region of any of these peptides; 5a and 5b were too short to be amenable to the Edman method. In order to assemble these six peptides in the correct order we had to find overlapping peptides containing methionine. This was accomplished by searching [7] the GCMS data set obtained from a partial acid hydrolyzate of intact C-2 for characteristic fragment ions predicted for all potential X-Met-Y sequences. The same strategy revealed the sequence of C-1. Since the N-terminus of CNBr-2 was pyroglutamine the sequence must be C-2-C-1 as shown in Fig. 8, consisting of a linear string of 248 amino acids [16].

4. The advent of DNA sequencing

In 1978 Fowler and Zabin published a series of six papers describing an 8 year effort at UCLA to determine the 1021 amino acids long sequence of β -galactosidase from *E. coli* using the Edman method. By the time the last [17] of these papers appeared, Gilbert's laboratory at Harvard had developed his DNA sequencing method [18] and had applied it to the gene coding for that protein. Their data confirmed the first 145 amino acids of β -galactosidase. With this development a new vista

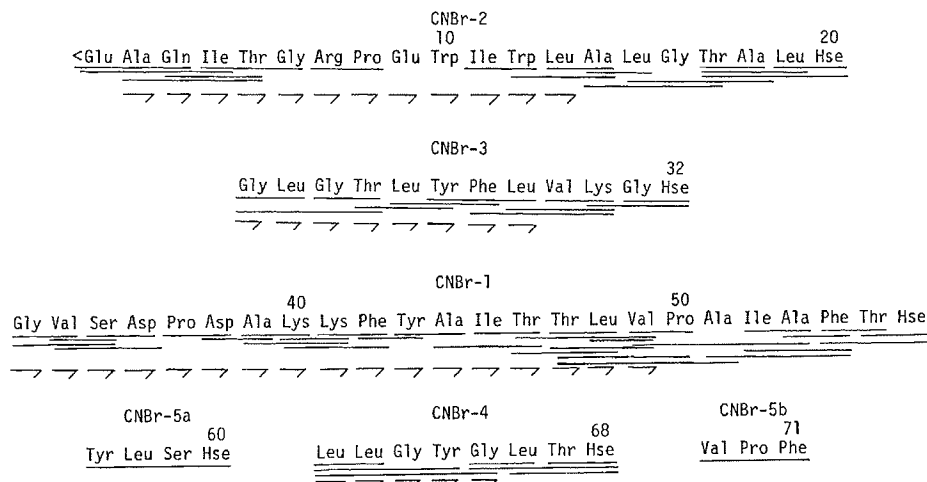


Fig. 7. Amino acid sequences of the peptides obtained by cyanogen bromide cleavage of the N-terminal segment of bacteriorhodopsin. Underlining: GCMS data; half-arrows: Edman data (adapted from Ref. [16a]).

for protein structure determination opened and DNA sequencing became an attractive alternative. However, it was still in its infancy and prone to errors: the four lanes on long one-dimensional electrophoresis gel strips had to be read manually and repeatedly, because only a short range (about 50 nucleotides) was sufficiently resolved and sharp enough in each experiment. These problems were exacerbated by the fact that a single missing or erroneously inserted nucleotide causes a phase shift and completely changes the derived amino acid sequence. Furthermore, even a correct nucleotide sequence represents three “reading frames”, each one leading to a different protein sequence and the correct one had to be identified. This could be done by identification of the N- and C-terminal amino acids of the protein, but two compensating errors in the interior of the DNA strand can still result in a long stretch of incorrect amino acid sequence. In the DNA sequence, the start could be identified by the initiating

ATG codon for methionine and the termination by one of the “stop” codons (ATG, TAA or TAG), but this was subject to the same phase-shift problem. In addition, the “coding strand” has to be identified, because the complementary “non-coding strand” gives rise to yet another three different amino acid sequences. All these problems become more and more severe the longer the protein chain and, therefore, the coding DNA strand is.

4.1. Combination of nucleotide sequencing with mass spectrometry

Paul Schimmel, at that time a professor of biology at MIT, was interested in the structure and mechanism of action of aminoacyl-tRNA synthetases. These are large (up to 1000 amino acids long), multifunctional enzymes which recognize and attach a specific amino acid to the corresponding transfer-

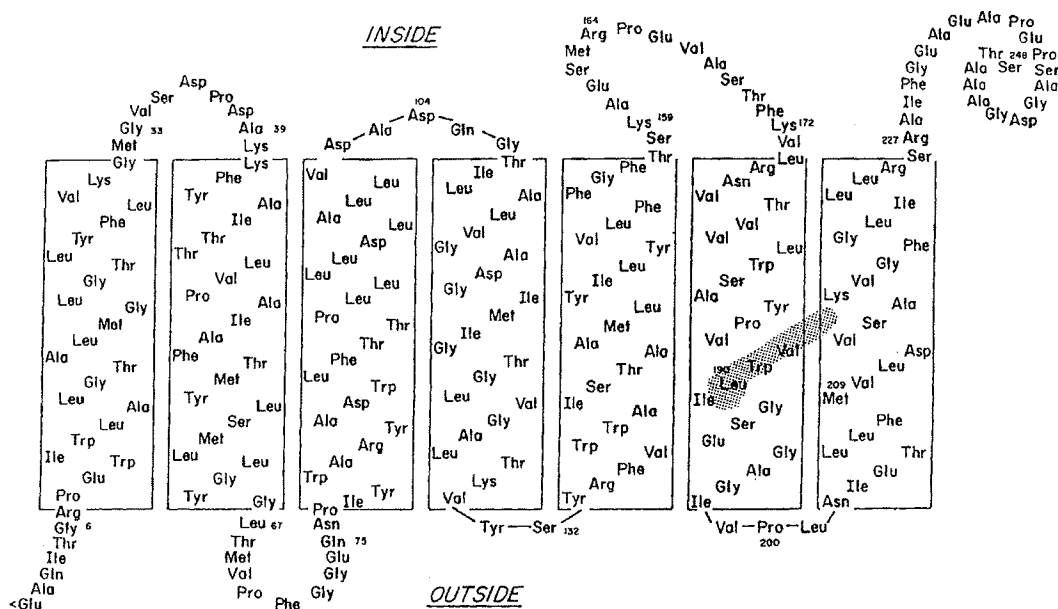


Fig. 8. Primary structure of bacteriorhodopsin from *H. halobium*. Boxes represent the regions where the protein loops through the cell.

RNA for the elongation of a growing polypeptide chain. Rather than embarking on the tedious Edman degradation of such large proteins, he decided to determine the about 3000 nucleotide long sequence of the structural gene coding for alanyl-tRNA synthetase (ARS) from *E. coli*. When discussing the difficulties mentioned above, I realized that all of these could easily and efficiently be overcome by our GCMS method. The identification (by sequence) of a relatively small number of peptides scattered over the entire protein would reveal and help to correct all these potential errors. Matching these peptides to the three protein sequences corresponding to the three reading frames not only allows bracketing the region where an error occurred, but also whether it is a deletion or an insertion of a nucleotide. Re-examination of that particular sequencing gel identifies the error and eliminates the unnecessary proofreading of all the others. As a consequence, we developed a strategy for multiple phase checks by mass spectrometry, using partial enzymatic digests of the corresponding protein [19].

In this collaboration Schimmel's group began to sequence the gene using the Maxam–Gilbert method, while we digested the about 400 amino acids long N-terminal segment (termed T-1) with thermolysin and pepsin, respectively. These enzymes were chosen to minimize the production of free amino acids and dipeptides, which would be useless. The resulting very complex digests were then derivatized and the GCMS data set processed using computer programs written for this purpose [5,7,20]. The sequences of tri- to pentapeptides so identified were then fed into our DEC PDP-11/45 computer along with the gradually accumulating nucleotide sequences to match them to the three reading frames.

The results for the first 89 codons are schematically depicted in Fig. 9. The N-terminus had been identified by a few Edman steps, thus defining reading frame 1. Three overlapping tri- and tetrapeptides matched amino acids 11 through 15 (nucleotides 31–45), but others fit amino acids 48–51 (nucleotides 142–153) and 81–84 (nucleotides 241–252) only in reading frame 3. This indicated that one nucleotide had been missed in the region between 46 and 141, but the remainder of the sequence up to nucleotide 252 was correct. Re-inspection of the gels covering this stretch of less than hundred nucleotides revealed and corrected the error. This process was continued until the entire sequence of T-1 and then also of the C-terminal section (T-2) had been defined, resulting in the complete primary structure of ARS, which turned out to be 875 amino acids long. The work

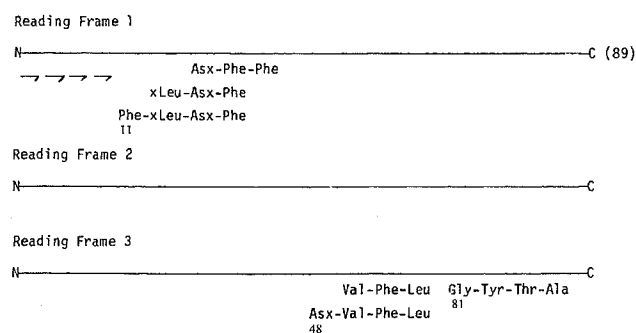


Fig. 9. Detection of an error in the DNA sequence (see text).

was published in 1981 in *Science* [21], which used the amino acid and DNA sequences to illustrate the cover of that issue. The significance of this complementary strategy was immediately recognized by others working on the determination of the structures of these and other large proteins. Even before the sequence of ARS was completed, we began a collaboration with Söll at Yale on glutaminyl-tRNA synthetase from *E. coli* [22].

A remarkable turning point in the application of mass spectrometry to peptide sequencing was reached in 1981 with Barber's (Manchester, UK) invention of "fast atom bombardment" (FAB) ionization. This novel technique made it possible to ionize an intact, underivatized peptide, such as Met-Lys-bradykinin (mw 1318) [23]. As a so-called "soft ionization" technique, it produced very stable $(M+H)^+$ ions, which have little tendency to fragment. This had the disadvantage of a lack of sequence specific fragment ions (unless a very large or very pure peptide sample was used), but the great advantage that it was now possible to measure the molecular weights of large peptides directly, even in mixtures. Furthermore, it was easy to use this method by simply fitting a commercially available argon atom gun to the ion source of almost any existing mass spectrometer. Now we could use trypsin to specifically cleave a protein at the C-terminal side of all arginines and lysines and produce a mixture of peptides of the size just right for FAB–MS. It was only necessary to separate the digest by liquid chromatography into a few simpler mixtures to obtain the molecular weight of most or all the peptides produced from the protein by cleavage with trypsin (Fig. 10).

This development, which almost over night rendered obsolete the derivatization chemistry we had developed and so successfully applied over more than two decades, greatly expanded the use of mass spectrometric peptide and protein sequencing. At that time, we were working on the sequencing of Gln-tRNA synthetase as mentioned above, but completed it using FAB–MS [22]. The sequences of Gly-, Met-, His- and Glu-tRNA synthetases were determined by the same collaborative DNA sequencing (some using the Sanger method [24])/FAB–MS strategy (Table 1). The basic difference between this and the earlier GCMS approach is demonstrated in Fig. 11. Rather than matching many short sequences, we calculated the molecular weights of all tryptic peptides predicted for the amino acid sequences corresponding to each of the three reading frames,

Table 1

Sequences of aminoacyl tRNA synthetases deduced by a combination of DNA sequencing and mass spectrometry

	Number of amino acids	References
Ala-tRNA synthetase ^{a,c}	875	[21]
Gln-tRNA synthetase ^{a-c}	550	[22]
Gly-tRNA synthetase ^{b,c}	990	[25]
Met-tRNA synthetase ^{b,d}	751	[26]
His-tRNA synthetase ^{b,c}	324	[27]
Glu-tRNA synthetase ^{b,c}	471	[28]

^a By GCMS.

^b By FAB–MS.

^c From *E. coli*.

^d From yeast.

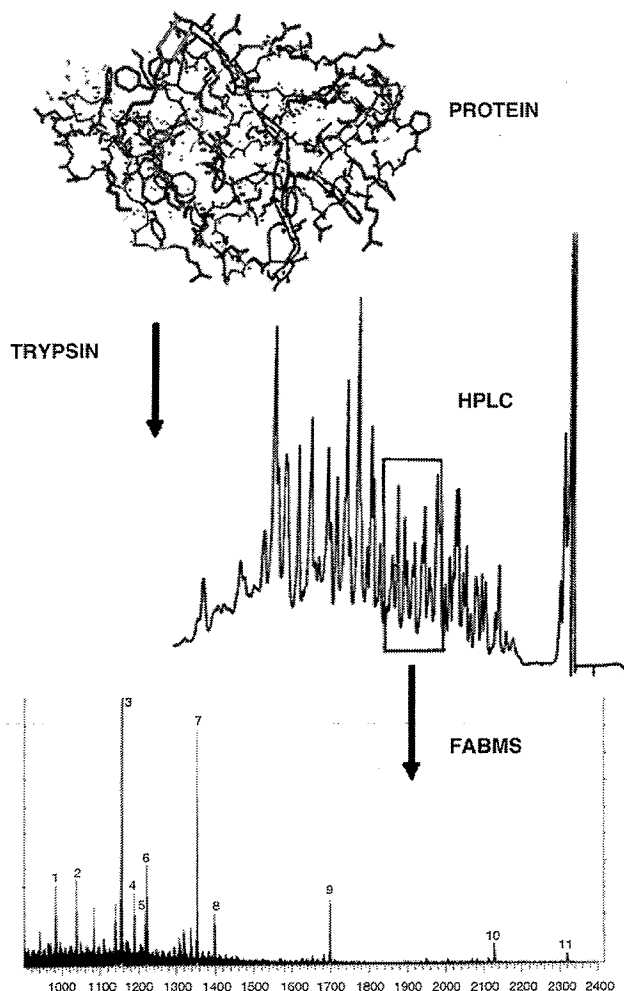


Fig. 10. Schematic illustration of the determination of the molecular weights of the components of a tryptic digest. The mass spectrum (bottom) indicates the molecular weights of 11 peptides in the range from 900 to 2400 in the HPLC fraction (box) collected.

and matched them with those experimentally determined from the tryptic digest of the protein.

In the example shown (Fig. 11), the values for two peptides matched those predicted for tryptic peptides from reading frame 1, but others matched reading frame 3. This indicated that one nucleotide had been missed in the region of the 41 in between. Insertion of one of the four nucleosides at each consecutive position would create 168 possibilities to be tested. However, the most frequent omission of a nucleotide occurs when it is preceded by the same one, which may cause the two consecutive bands in the same lane of the gel to be too close to be visually resolved. Thus, “doubling up” each one of the 41 nucleotides one at a time generated only 42 new potentially correct sequences from which 8 would produce one or more tryptic peptides. For only two of these (7 and 17) did the predicted molecular weight correspond to one (1444) actually present in the tryptic digest of the protein. These could be distinguished by three steps of “subtractive” Edman degradations, which involve measuring the molecular weight changes after each step. The N-terminus turned out to be Leu-Ala-Asp, indicating that sequence seven

is the correct one and the error was due to the omission of an additional guanidyllic acid between G and A [26].

A different problem arose in the case of Met-tRNA synthetase from yeast. When sequencing the gene coding for this large protein, three Met codons were found close together, but an attempt to identify the initiating codon by determination of the N-terminus of the protein using the Edman method failed. A FAB-MS experiment on a tryptic digest revealed the presence of three peptides, the $[M + H]^+$ ions of which fit only between the first and the second Met, indicating that the first one initiates transcription but is then removed and the serine that follows acetylated, a common feature of post-translational processes [26].

Such transformations of the nascent polypeptide chain to the various biologically active forms of a protein cannot be deduced or predicted from the nucleotide sequence of the gene. To pinpoint and identify these modifications, sequence specific fragmentation of the tryptic (or other enzymatically or chemically produced) peptides was necessary. This was achieved by tandem mass spectrometry (MS/MS) which involves the collision of an $(M + H)^+$ ion produced in the first mass spectrometer with a noble gas at low (a few eV) kinetic energy in a triple quadrupole MS [29] or at high (kV) energy in a four-sector magnetic MS [30]. These spectra, particularly those involving high energy collisions, provided clear, complete sequence information, including the differentiation of leucine and isoleucine. This approach not only revealed the type and position of post-translational modifications [31] but also permitted the sequencing of proteins entirely by FAB-MS/MS, as first demonstrated on a number of thioredoxins [32] and later glutaredoxins.

5. Mass spectrometry of proteins “going public”

The work described above had made the biochemistry community acutely aware of the value and unique significance of mass spectrometry to the field of gene/protein structure correlation. The potential of matching the molecular weights of tryptic (or other specific cleavage) peptides to known or predicted protein sequences was quickly recognized. In 1989, at the third Symposium of The Protein Society, Henzel from Stults’ group at Genentech presented an algorithm for using this principle to identify proteins by matching such mass spectral data to then already available digital databases. Four years later four papers, three of them from other laboratories, demonstrated the utility of this approach and elaborated on it [33–36].

The strategies described above soon became the basis of today’s proteomics, a new field further stimulated by two major advances in instrumentation. In 1988 matrix-assisted laser desorption ionization (MALDI) was developed by Hillenkamp at the University of Münster (Germany) [37] and soon thereafter electrospray ionization (ESI) by Fenn at Yale [38]. These techniques, particularly ESI, which lent itself well to interfacing with liquid chromatography, essentially replaced FAB-MS. Finally, “nanospray” ESI developed by Wilm and Mann in Denmark made it possible to obtain the molecular weights of the components of tryptic or other enzyme digests of a protein extracted from a spot on a two-dimensional electrophoresis gel [39]. Hunt

RF1	---	Glu	Lys		Val	Val	Arg	Pro	Arg		Leu	Ala	Met	Pro	Ser	Ser	Ser	Ser	Thr	Pro	Thr	Val	Lys	Asn	-----									
		GAG	AAA	GTC	GTT	CGT	CGC	CGT	CTG	GCC	ATG	CCG	AGT	TCT	TCT	TCA	ACA	CCG	ACC	GTA	AAA	AAC	GTC	TTG	AAG	ATA	ACC	TGC	CGC	GCC	TGC			
RF3	-----	Ser	Gly	Asp	Ala	Glu	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys		Arg		Leu	Gly	Asp	Asn	Leu	Pro	Arg		Leu							
Base Inserted at Position	1																																	
		TCT	GGC	GAT	GCC	GAG	TTC	TTC	TTC	AAC	ACC	GAC	CGT	AAA	AAA																			
		Ser	Gly	Asp	Ala	Glu	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys																			
		MW= 1404, 1532																																
	2																																	
		CCT	GGC	GAT	GCC	GAG	TTC	TTC	TTC	AAC	ACC	GAC	CGT	AAA	AAA																			
		Pro	Gly	Asp	Ala	Glu	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys																			
		MW= 1414, 1542																																
	.																																	
	.																																	
	6																																	
		CTG	GCC	GAT	GCC	GAG	TTC	TTC	TTC	AAC	ACC	GAC	CGT	AAA	AAA																			
		CTG	GCC	GAT	GCC	GAG	TTC	TTC	TTC	AAC	ACC	GAC	CGT	AAA	AAA																			
		Leu	Ala	Asp	Ala	Glu	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys																			
		MW= 1444, 1572																																
	.																																	
	.																																	
	15																																	
		CTG	GCC	ATG	CCG	AGC	TTC	TTC	TTC	AAC	ACC	GAC	CGT	AAA	AAA																			
		Leu	Ala	Met	Pro	Arg	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys																			
		MW= 586, 945, 1073																																
	16																																	
		CTG	GCC	ATG	CCG	ACT	TTC	TTC	TTC	AAC	ACC	GAC	CGT	AAA	AAA																			
		CTG	GCC	ATG	CCG	AGT	TTC	TTC	TTC	AAC	ACC	GAC	CGT	AAA	AAA																			
		Leu	Ala	Met	Pro	Ser	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys																			
		MW= 1444, 1572																																
	.																																	
	.																																	
	42																																	
		CTG	GCC	ATG	CCG	AGT	TCT	TCT	TCA	ACA	CCG	ACC	GTA	AAA	AAA																			
		Leu	Ala	Met	Pro	Ser	Ser	Ser	Ser	Thr	Pro	Thr	Val	Lys	Lys																			
		MW= 1304, 1432																																

Fig. 11. Detection and correction of an error in a DNA sequence by FAB–MS. Underlining: tryptic peptides detected and predicted; half-arrows: subtractive Edman data. For details see text (Reprinted from Ref. [26] with permission from NAS).

and co-workers achieved automated high-throughput analysis of enzymatic digests of mixtures of proteins [40]. In Yates' laboratory, the database matching algorithms mentioned above were expanded to include the use of predicted MS/MS spectra [41], and later to analyze entire protein complexes by multi-dimensional liquid chromatography and ESI–MS/MS methodology using the yeast genome sequence [42]. The completion of the sequencing of the entire human genome in the year 2000 finally eliminated the need to painstakingly determine the amino acid sequence of each of the about 20,000 proteins coded by the nucleotide sequence. But to follow the cascades of post-translational conversions to the biologically active structures, mass spectrometry is still the principal methodology available for this important, but daunting task [43].

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