25 °C, 98% yield), and the product **28** was oxidized[26] to the corresponding ketone **29** (PCC, CH_2Cl_2, 25 °C, 95% yield).

The 25-hydroxy side chain with proper absolute stereochemistry at C-20 was then introduced by the use of the recently disclosed ene reaction.[27] Compound **30**, obtained by saponification of **29** (EtONa, EtOH, 25 °C), was subjected to Wittig reaction with ethylidenetriphenylphosphorane (THF, 25 °C) to give a 96:4 ratio (determined by GLC) of the desired 17*Z* olefin **31** and the corresponding 17*E* isomer. Since the two isomers could not be separated at this stage, they were acetylated (Ac_2O, pyridine, 25 °C, 96% yield) and the resulting acetates (mainly containing **32**) subjected to ene reaction with ethyl propiolate[27] ($EtAlCl_2$, CH_2Cl_2, 25 °C, 88% yield). We found that **32** reacted under these conditions at a considerably faster rate than the corresponding 17*E* isomer, allowing, therefore, dienoic ester **33** to be obtained virtually as single product. Catalytic hydrogenation of **33** proceeded stereospecifically to **34** (H_2, Pd/C, EtOH, 25 °C, 98% yield), and subsequent reduction with diisobutylaluminum hydride (CH_2Cl_2, toluene, −78 °C, 92% yield) gave the aldehyde **35**, which was converted to the olefin **36** with isopropylidenetriphenylphosphorane (THF, 25 °C, 89% yield). Oxymercuration and demercuration of **36** [$Hg(OAc)_2$, THF, H_2O, then $NaBH_4$, 25 °C] followed by oxidation[26] (PCC, CH_2Cl_2, 25 °C, 78% yield from **36**) afforded finally the desired hydroxylated Windaus and Grundmann ketone **5**,[28] identical in all respects with the compound prepared by ozonolysis of 25-hydroxycholecalciferol;[29] $[\alpha]^{25}_D$ +17.9 (*c* 0.5, EtOH); NMR ($CDCl_3$) δ 0.64 (3 H, s), 0.97 (3 H, d, J = 6.0 Hz), 1.22 (6 H, s).

With **5** and **6** in hand, the stage was set for the final convergent formation of 1α,25-dihydroxycholecalciferol (**1**). Wittig–Horner reaction at low temperature of **5** with excess of the lithium phosphinoxy carbanion prepared from **6** and butyllithium at −78 °C[5] in tetrahydrofuran proceeded exceedingly slow. At higher temperature, epimerization of **5** at C-14 began to occur. Much better results were obtained after protection of the hydroxy group of **5** (TMSI, THF, 25 °C, 98% yield). The trimethylsilyl ether obtained underwent Wittig–Horner reaction very smoothly (THF, −78 °C, 1 h) to give, after removal of the silyl groups[30] [$(Bu)_4NF$, THF, 25 °C], the desired 1α,25-dihydroxycholecalciferol **1**[31] in 87% yield from **5**: mp 118–119 °C; $[\alpha]^{25}_D$ +47.9 (*c* 0.3, EtOH); NMR[32] (CD_3OD) δ 0.57 (3 H, s), 0.96 (3 H, d, J = 6.0 Hz), 1.16 (6 H, s), 4.87 (1 H, br s), 5.28 (1 H, br s), 6.08 (1 H, d, J = 11.6 Hz), 6.32 (1 H, d, J = 11.6 Hz).

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Registry No. 1, 32222-06-3; **5**, 70550-73-1; **6**, 81522-68-1; **7**, 2244-16-8; **8**, 39903-97-4; (*E*)-**9**, 81570-18-5; (*Z*)-**9**, 81570-19-6; **10**, 81506-17-4; **11**, 81506-18-5; **12**, 81506-19-6; **13**, 81506-20-9; **14**, 81506-21-0; **15**, 81506-22-1; **16**, 81506-23-2; **17**, 81570-20-9; **18**, 81506-24-3; **19**, 81506-25-4; **21**, 31944-51-1; **22**, 81506-26-5; **23**, 81506-27-6; **24**, 81506-28-7; **25**, 81506-29-8; **26**, 81506-30-1; **27**, 81506-31-2; **28**, 81506-32-3; **29**, 81506-33-4; **30**, 81506-34-5; (*E*)-**31**, 81506-35-6; **32**, 81506-36-7; **33**, 81506-37-8; **34**, 81506-38-9; **35**, 81506-39-0; **36**, 81506-40-3; **5** TMS ether, 81506-41-4; (*Z*)-**31**, 81506-42-5.

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^{127}I-Plasma Desorption Mass Spectrometry of Insulin

P. Håkansson, I. Kamensky, and B. Sundqvist*

Tandem Accelerator Laboratory, Uppsala, Sweden

J. Fohlman and P. Peterson

Institute for Cell Research, The Wallenberg Laboratory University of Uppsala, Uppsala, Sweden

C. J. McNeal and R. D. Macfarlane*

Department of Chemistry, Texas A&M University College Station, Texas 77843

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We report the first observation of the molecular ion of insulin in a mass spectrum. Using a beam of 90-MeV ^{127}I ions directed on the surface of a thin film of bovine insulin, we have been able to desorb and detect the molecular ion plus prominent fragment ions relating to the α and β chain of insulin. To our knowledge, this is the largest naturally occurring peptide for which it has been possible to detect the molecular ion by a mass spectrometric method.

Since the introduction of ^{252}Cf-plasma desorption mass spectrometry (^{252}Cf-PDMS), which utilizes the 80–100-MeV fission fragment ions (M = 100–140) from a ^{252}Cf source to induce ion desorption from thin films,[1] it has been suggested that heavy ions from a nuclear accelerator in the same mass–energy domain could also produce the short-lived, high-temperature tracks in thin dielectrics that are responsible for ion desorption. The efficacy of ^{252}Cf-PDMS in desorbing large biomolecules such as β-endorphin[2] and synthetic protected oligonucleotides[3] has already been demonstrated. The most important properties of the incident ion for enhanced desorption are mass, energy, and atomic charge state.[4] The 90-MeV ^{127}I (+20 charge state) beam from the Uppsala Tandem Accelerator was chosen for this study. Since the mechanism for desorption and ionization is the same as for ^{252}Cf fission fragments, we shall refer to this method as ^{127}I-plasma desorption mass spectrometry (^{127}I-PDMS).

A thin film of bovine insulin (Sigma) was prepared by electrospraying[5] a solution in trifluoroacetic acid onto a thin aluminized Mylar film (1.5 μm thick, Steiner Film Corp.). The deposit weighed 25 μg spread over an area of 80 mm^2. The sample film was mounted in the ion source of a specially designed time-of-flight (TOF) mass spectrometer having a field-free length of 35 cm.[4] The detection of each transmitted ion initiated a mass scan covering a range of m/z 0–12 000. The sample foil was maintained at a 20-kV potential, and ions desorbed from the surface of the sample with the same polarity as the target voltage were accelerated to ground potential through a 90% transmission Ni grid. These were transmitted to the end of the flight tube where they were detected by using microchannel plate electron multipliers (Galileo Electro-Optics). The time intervals between the detection of a ^{127}I ion passing through the target and ions arriving at the end of the flight tube were measured by using a time-to-amplitude converter (TAC). The ouput of the TAC was fed directly into

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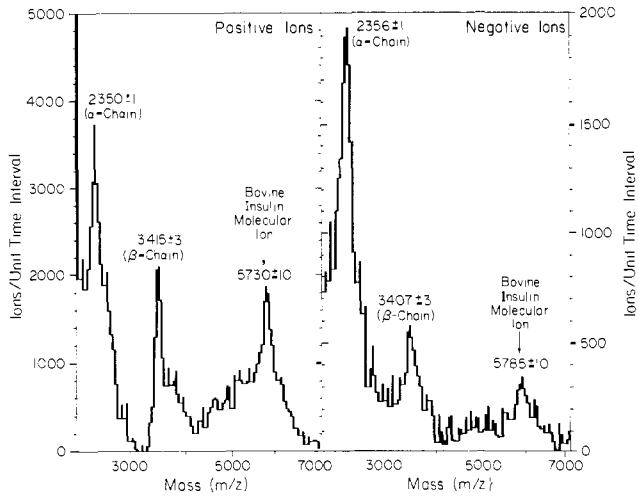

Figure 1. ^{127}I-PDMS spectra of bovine insulin recorded over a 1.5-h period with a 90-MeV ^{127}I (+20) beam current of 2000 s^{-1}.

a PDP-15 computer operating as a multichannel analyzer. Mass calibrations were made by using $(Cs_2I)^+$ cluster ions in the positive ion spectrum and $(CsI_2)^-$ cluster ions in the negative ion spectrum.

The experiment was performed so that the mass spectrum from each scan was added to the previous scans at the rate of 2000 scans s^{-1}. The "growing" mass spectrum was monitored on a graphics terminal linked to the computer. We first set the acceleration voltage for positive ion analysis and commenced the ^{127}I beam irradiation. Only a few moments of data collection were needed before it became apparent that the insulin molecular ion was indeed present in the mass spectrum as well as lower mass ions in the region of the α- and β-chain molecular weights. The polarity of the acceleration voltage was then reversed, and a search was made for negative ions of insulin. The mass spectrum was weaker but a mass pattern similar to that for the positive ions was observed. Careful measurements of the positive and negative ion spectra were made for a 1.5-h period involving 10^7 separate mass scans (or 10^7 incident I ions). A smooth exponentially decaying background (characteristic of ion-induced desorption using TOF) was subtracted from the gross spectra, and masses for the prominent peaks were calculated from the positions of the centroids of the mass peaks in the insulin spectrum and the cesium iodide cluster calibration ions. These results are summarized in Figure 1, which shows the positive and negative ion spectra in the mass region m/z 2000–7000. Several mass peaks were observed below m/z 2000 but these were not analyzed in this experiment. In addition, there was evidence for a peak at $m/z \sim 12000$, which presumably is a dimer ion of insulin.

The ion in the vicinity of the molecular weight of insulin has m/z 5730 ± 10 and a yield of 3×10^{-4} per incident ^{127}I. The isotopically averaged molecular weight (C = 12.011) of bovine insulin is 5733. Thus, this molecular ion appears to contain all the pieces of the highly complex structure of insulin. The lower mass ions are at m/z 3415 ± 3 and 2350 ± 1. The molecular weights of the α and β chain of bovine insulin are 3398 and 2335, respectively. There is little doubt that the fragment ions are associated with these two pieces of insulin, but it is clear that something else is attached to them—perhaps a water molecule. The negative ion spectrum shows additional evidence for small molecule or ion attachment: the highest mass ion is at m/z 5785 ± 10 and the apparent α and β chain related negative fragment ions are at m/z 3407 ± 3 and 2356 ± 1. This has been the first opportunity to examine gas-phase ion formation of such a complex species large enough to adopt a well-developed tertiary structure. Association of small neutral molecules or counterions into the complex structure may be important for the formation of a singly charged gas-phase ion, particularly when there are so many charge centers in the molecule.

An important milestone in mass spectrometry development has been realized with the detection of gas-phase molecular ions of insulin. This is not only because it is an important molecule for biomedical applications but also because it is near that semantic boundary in mass space beyond which a peptide becomes a protein.

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Book Reviews

Nuclear Chemical Engineering. By M. Benedict (Massachusetts Institute of Technology), T. H. Pigford (University of California, Berkeley), and H. W. Levi (Hahn-Meitner-Institut für Kernforschung, Berlin). McGraw-Hill Book Company, New York. 1981. xv + 1008 pp. $37.95.

The first edition of this book was published in 1957 and has been widely recognized as the reference work on chemical engineering practice in the nuclear industry. Some 24 years later, and after a long period in preparation, the second edition is finally available. Although the general outline and style generally conform to those of the original book, this second edition may be regarded as an entirely new book: the amount of material presented has approximately doubled and a third author, Hans Levi, now shares the credit with Manson Benedict and Thomas Pigford.

Chapters 1–3 set the stage for the remainder of the book and introduce the concepts required to fully appreciate the substance of the developments presented in Chapters 4–14. It is to be noted that Chapter 3 contains a particularly helpful introduction to the area of nuclear fuel management, a topic generally poorly covered in the literature.

Solvent extraction, uranium, thorium, zirconium, and hafnium are covered in Chapters 4–7 while the processing and properties of irradiated fuel, actinide elements, and other reactor materials are dealt with in Chapters 8–10 as well as in a disappointing new chapter entitled Radioactive Waste Management (Chapter 11). Isotopic separation principles and processes are discussed in Chapters 12–14.

The large amount of new material is a reflection of the vast changes which have occurred over a period of nearly 25 years and a result of a somewhat greater emphasis on process description. The main asset of the book remains the same however: the authors' extraordinary ability to combine scientific and engineering principles in an area to which they have given an identity and contributed extensively—nuclear chemical engineering.

Albert J. Machiels, *University of Illinois*

Synthetic Aspects of Biologically Active Cyclic Peptides—Gramicidins and Tyrocidines. By N. Izumiya, T. Kato, H. Aoyagi, M. Waki, and M. Kondo. Halsted Press (Wiley), New York. 1979. xii + 166 pp. $29.95.

The purification, characterization, synthesis, and study of biologically active peptides isolated from microorganisms have long been areas of intensive scientific investigation. Of particular interest have been those peptides which possess antibiotic activities. Because they contain uncommon and D-amino acids and are usually cyclic, peptide antibiotics have presented unusual challenges to structural and synthetic peptide chemists. These properties have however made them very valuable as models for the development of methods for the chemical synthesis of cyclic peptides and for biosynthetic and conformational studies. Over 300 different peptide antibiotics have been discovered to-date. Of these,