Tandem Mass Spectrometry of Higher Molecular Weight Compounds

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Informative mass spectra of much higher molecular weight compounds have been obtained from microgram samples recently with special ionization techniques such as plasma desorption[1] and fast atom bombardment (FAB).[2-7] However, these spectra contain misleading adduct and matrix peaks that could be mistaken for peaks of other sample components, and some spectra of larger compounds (>2000 daltons) provide no fragment ion information.[1-3] We report that secondary mass spectra of such high mass ions, obtained by collisionally activated dissociation (CAD) using a tandem mass spectrometer of improved mass range,[8] can help resolve such problems.

To offset the decrease in sensitivity with increasing mass,[9,10] these measurements were made with wide slits[11] and high (10 kV) ion-acceleration potential, using an electrostatic second mass analyzer (MS-II).[8] The 60-cm, 2.3-T MS-I magnet separates 10-keV ions of mass 10000; $Cs_{35}I_{34}^+$ ions (m/z 8966) from FAB of CsI[3,12] yield the CAD spectrum of Figure 1. This CAD cross section is unusually high,[8,9] with ~80% of precursors lost by collision, yielding collectible product ions.

Fortunately, higher mass organic ions exhibit usefully large CAD cross sections; m/z 412 from FAB of the tripeptide Met-Asp-Phe-NH_2 yields 2.3% collectible CAD products, while m/z 2096 from the heptadecapeptide 15-Met-gastrin yields 1.6% (CH_4^+ yields 6.2%).[8] Such values for metastable ion (MI) formation are large (2.7% and 12%; CH_4^+ <0.1%), but most MI peaks represent side-chain losses. In contrast to the FAB mass spectrum of 15-Met-gastrin (Figure 2) and other larger peptides,[3,4] CAD of the resulting $(M + H)^+$ ions provides useful sequence information (Figure 2). Similar CAD behavior was found for ions from angiotensin, small cholecystokinins, and bradykinins (Figure 3), paralleling that reported for smaller peptides.[13] As in MI[6b] and

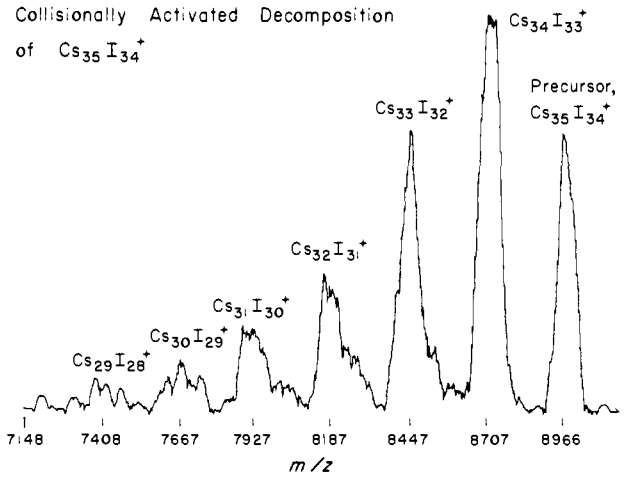

Figure 1. CAD spectrum of 10-KeV $Cs_{35}I_{34}^+$ ions, using helium collision gas pressure yielding 40% precursor transmittance. The unusual proclivity for metastable ion dissociation apparently causes the signals between lower mass peaks.

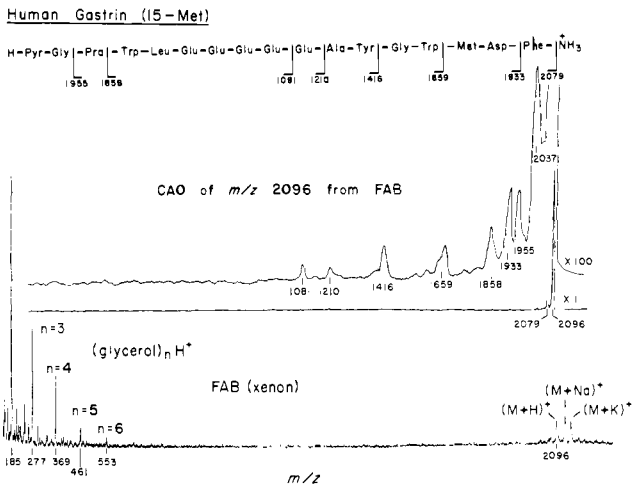

Figure 2. FAB mass spectrum of 15-Met-gastrin and the CAD spectrum of its $(M + H)^+$ ions (50% transmittance). The latter is the average of three spectra using ~10-μg samples.

FAB spectra,[2-7] the intensities of the possible sequence peaks[14] depend significantly on amino acid identities. Three bradykinins produce $(M + H - 157)^+$ by an unusual rearrangement loss of C-terminal arginine not noted in the FAB study.[2b]

In the FAB spectrum of cyanocobalamin (vitamin B-12) m/z 1329 is the most abundant high-mass peak,[6a] with peak groups centered at m/z 1341, 1355, 1368, 1386, 1404, and higher of much lower (10-30%) abundance. From their CAD spectra[11] (Figure 4) only m/z 1355 produces m/z 1329, indicating 1355 as $(M + H)^+$ yielding 1329 by cyano loss; CAD fragmentations of m/z 1355 and 1329 are also similar. The FAB m/z 1270 peak was assigned[6a] as loss of either cobalt or CH_3CONH_2; loss of the latter peripheral group is demonstrated by the similarity of the m/z 1270

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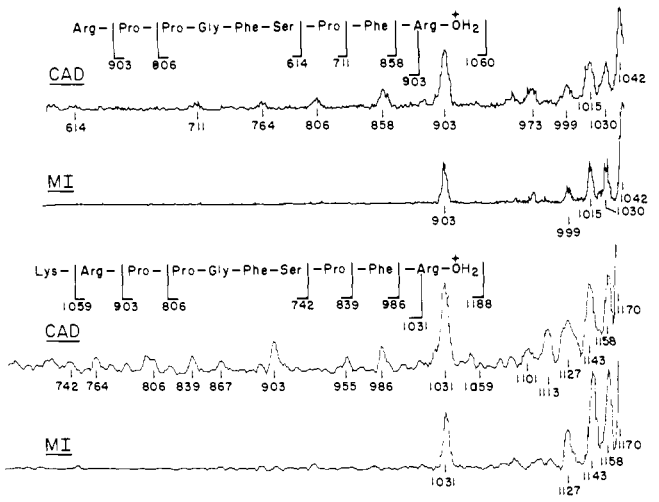

Figure 3. CAD and MI spectra of bradykinin and Lys-bradykinin (~5-μg samples); those of Met-Lys-bradykinin are similar (m/z 1162 is abundant).

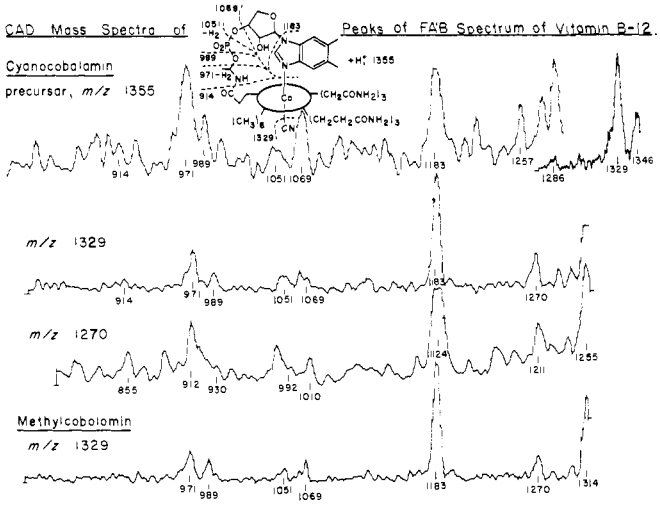

Figure 4. CAD spectra (top three) from m/z 1355, 1329, and 1270 of cyanocobalamin; (bottom) m/z 1329 of methylcobalamin (~5-μg samples).

and 1329 CAD spectra. (Even the CAD spectrum m/z 1386 is strikingly similar to these; its structure could be that of m/z 1329 with CH_3 replaced by $CH_2CH_2CONH_2$ or H by CH_2CONH_2.)[15] The sequential losses from the axial chain of 146 (dimethylbenzimidazole + H), 114 and 132 (sugar), 80 (HPO_3), and 57 mass units ($CH(CH_3)CH_2NH$) produce the significant peak groups at m/z 1183, 1069, 1051, 989, 971, and 914 in the FAB spectrum.[6a] However, if this were a real unknown this structural assignment would be compromised by the problem of distinguishing peaks due to such sequential loss from the equal number in this region of the FAB spectrum resulting from fragmentations in other parts of the molecule. The CAD spectra of these FAB peaks can resolve this problem by delineating the fragmentation pathways;[16] for example, that of m/z 1270 shows the axial chain peaks shifted by 59 mass units vs. the mass 1329 CAD spectrum, demonstrating that the 59 mass units comes from a different part of the molecule. However, the CAD spectra of the FAB peaks centered on m/z 1183, 1069, 1051, 989, and 971 show lower mass peaks at these same values; for example, m/z 914 is the most abundant high-mass peak in the CAD spectrum of the FAB mass 971 peak. The cobalamin assignement for m/z 1329 can be confirmed with reference CAD spectra;[9,16] those from methylcobalamin (Figure 4), hydroxycobalamin, and coenzyme B-12 (not shown) are closely similar. Thus for an unknown these CAD data should provide significant additional evidence of the structure.

We are investigating sensitivity and resolution improvements using ion-counting detection, increased number of collisions and collision energies (post-MS-I acceleration),[2] other collision gases, double-focusing MS-II,[2] alternative ionization methods, and further computer automation.

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Registry No. Cesium iodide, 7789-17-5; gastrin, 9002-76-0; bradykinin, 58-82-2; cyanocobalamin, 68-19-9.

(15) FAB of cyanocobalamin gives m/z 1329, 1355, and 1386 peak abundances of 10:3:1 initially but 10:1.5:2.5 after 10 min, consistent with at least part of the 1386^+ precursor being formed by the FAB process.

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An Unsymmetrically Substituted Diphosphene: NMR Spectroscopic Data Pertinent to the Phosphorus–Phosphorus Double Bond[1]

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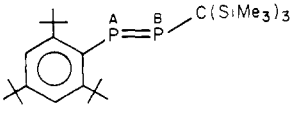
The use of appropirate steric blockades has permitted the recent isolation of symmetrically substituted compounds featuring silicon–silicon,[2] germanium–germanium,[3] and phosphorus–phosphorus[4] double bonds. We now report the first unsymmetrically substituted compound featuring multiple bonding between heavier main-group elements. The isolation of this unsymmetrical diphosphene (RP=PR′) has led to new NMR spectroscopic data pertinent to the phosphorus–phosphorus double bond.

A mixture of 0.97 g (2.9 mmol) of $(Me_3Si)_3CPCl_2$ and 0.5 g (1.45 mmol) of $(2,4,6\text{-}(t\text{-}Bu)_3C_6H_2)PCl_2$ in 50 mL of THF was treated with 176 mL of 0.49 M sodium naphthelenide in 50 mL of THF at −78 °C. After evacuation of the solvent and naphthalene, the resulting red-brown solid was dissolved in hexane and separated by column chromatography on silica gel. The first and third fractions were identified as the symmetrical diphosphenes $(Me_3Si)_3CP{=}PC(SiMe_3)_3$ (**1**)[4b,f] and $(2,4,6\text{-}(t\text{-}Bu)_3C_6H_2)P{=}P\text{-}(2,4,6\text{-}(t\text{-}Bu)_3C_6H_2)$ (**2**),[4b,c,e] respectively, on the basis of NMR data.[4] Evaporation of solvent from the second fraction resulted in the unsymmetrical diphosphene **3**. The high-resolution mass



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spectrum (HRMS) for the parent peak of **3** showed the following:

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