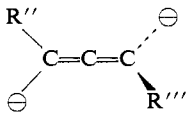
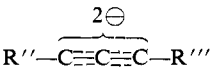
that IV is expected to have a stability not greater or even lower than that of an allyl anion, since there is a poor overlap between the orbitals containing the electron pairs and the π system. We favor structure V, which we submit to call an extended acetylene or a



IV



V

sesquiacetylene.[15] Here we have the unusual occurrence of two identical four-electron three-center systems delocalized symmetrically. The orbitals of each π system, being parallel, have better overlap and permit bond-length adjustment for higher stability.

Extended acetylenes can be metalated further, when a hydrogen is linked to the sp carbons. Thus metalation of propyne[16] yielded C_3Li_4 which would be the parent lithium sesquiacetylide, and prolonged metalation of 1-phenylpropyne[17] gave $C_6H_5C_3Li_3$ or lithium phenylsesquiacetylide. These compounds were identified by the formation of tetrakis(trimethylsilyl) and trideuterio derivatives, respectively.[16,17]

Low delocalization of electrons in the anions II and III is supported by the different chemical shifts of protons C and E in these compounds, revealing a high bond order and slow rotation, on the nmr scale, around the initially double bond. Pentadienylic anions[15] show a faster rotation around this bond at the temperatures of our measurements.

Acknowledgment. We are grateful to Professor J. Musher for an interesting discussion.

(15) These are extended acetylenes containing a carbon atom in addition to the ordinary two-carbon acetylene group. Other extended acetylenes, containing different atoms, *e.g.*, nitrogen, are conceivable. The dianion formed from a nitrile could be called a sesquiazacetylene

$$-^{2-}C-C\equiv N \longleftrightarrow -C\equiv C-N^{2-}$$

(16) R. West, P. A. Carney, and I. C. Mineo, *J. Am. Chem. Soc.*, **87**, 3788 (1965).

(17) J. E. Mulvaney, T. L. Folk, and D. J. Newton, *J. Org. Chem.*, **32**, 1674 (1967).

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Feline Gastrin. An Example of Peptide Sequence Analysis by Mass Spectrometry

Sir:

The potential of mass spectrometry in sequence analysis of peptides has been recognized for some time.[1] However, although considerable success has been achieved with cyclic and antibiotic peptides,[2] there seems to have been no practical use of the technique with more typical peptides derived from the normal protein biosynthetic pathway. This is doubtless because there is a severe limit to the size of peptides amenable to current techniques (of the order of ten residues depending on their composition), and hence mass spectrometry must be applied in conjunction with other methods of peptide analysis. We now report an example of sequence analysis of a mammalian peptide hormone.

The gastrins are heptadecapeptide amides with powerful stimulatory action on the secretion of gastric acid.[3] The structures of gastrins from five mammalian species have been reported,[4] and they display sequence variations corresponding to single-base substitutions in genetic codons. Professor R. A. Gregory recently isolated the gastrin (*ca.* 400 μg) from 200 cat antra and generously gave it to us for structure determination. Amino acid analysis (17 nmol) demonstrated its essential similarity to other members of the gastrin family. The composition $Ala_2Asp_1Gly_2Glu_5Leu_1Met_1$-$Phe_1Pro_1Trp_2Tyr_1$ was compatible with five structures (**1**) corresponding to single base changes in genetic codons, although other possibilities could not be excluded. With the remaining material (180 nmol) it was possible to assign a unique sequence to the hormone by mass spectrometry, without recourse to conventional chemical sequencing methods.

The feline gastrin was treated with 0.1 *N* HCl at 20° for 2 hr to hydrolyze the phenolic sulfate ester[5] and then with chymotrypsin (enzyme:substrate ratio 1:7.5) at pH 8 and 4° for 24 hr. The four chymotryptic peptides were isolated by gel filtration on Sephadex G-25 and then treated as follows. Chym-III was esterified (0.1 *N* methanolic HCl at 20° for 12 hr) and then acetylated (acetic anhydride–acetic acid (1:1) at 20° for 12 hr); direct mass spectrometry sufficed to establish the dipeptide structure (**4**). Chym-IV was treated similarly. Its mass spectrum showed the expected molecular ion, but it was dominated by side-chain fragmentation. The tripeptide structure (**5**) was confirmed by the identity of its spectrum with that of an authentic sample. Chym-I was permethylated[6] to give a product sufficiently volatile for easy obtention of the mass spectrum. Its spectrum contained ions attributable to the tetramethyl derivative **2** together with a pentamethyl derivative in which C-methylation had occurred at the glycine residue.[7] The spectrum

(1) *E.g.*, K. Biemann, *Chimia*, **14**, 393 (1960); E. Stenhagen, *Z. Anal. Chem.*, **181**, 462 (1961); F. Weygand, A. Prox, H. H. Fessel, and K. K. Sun, *Z. Naturforsch.*, **20b**, 1169 (1965); M. M. Shemyakin, Y. A. Ovchinnikov, A. A. Kiryushkin, E. I. Vinogrodova, A. I. Miroshnikov, Y. B. Alakhov, V. M. Lipkin, Y. B. Shvetsov, N. S. Wulfson, B. V. Rosinov, V. N. Bochkarev, and V. M. Burikov, *Nature*, **211**, 361 (1966).

(2) *E.g.*, M. Barber, P. Jolles, E. Vilkas, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **18**, 469 (1965); A. Prox and F. Weygand, "Peptides," Proceedings of the Eighth European Peptide Symposium, North-Holland Publishing Co., Amsterdam, 1967, p 158; T. Wieland with G. Lüben, H. Ottenheym, J. Faesel, J. X. de Vries, W. Konz, A. Prox, and J. Schmid, *Angew. Chem.*, **80**, 209 (1968).

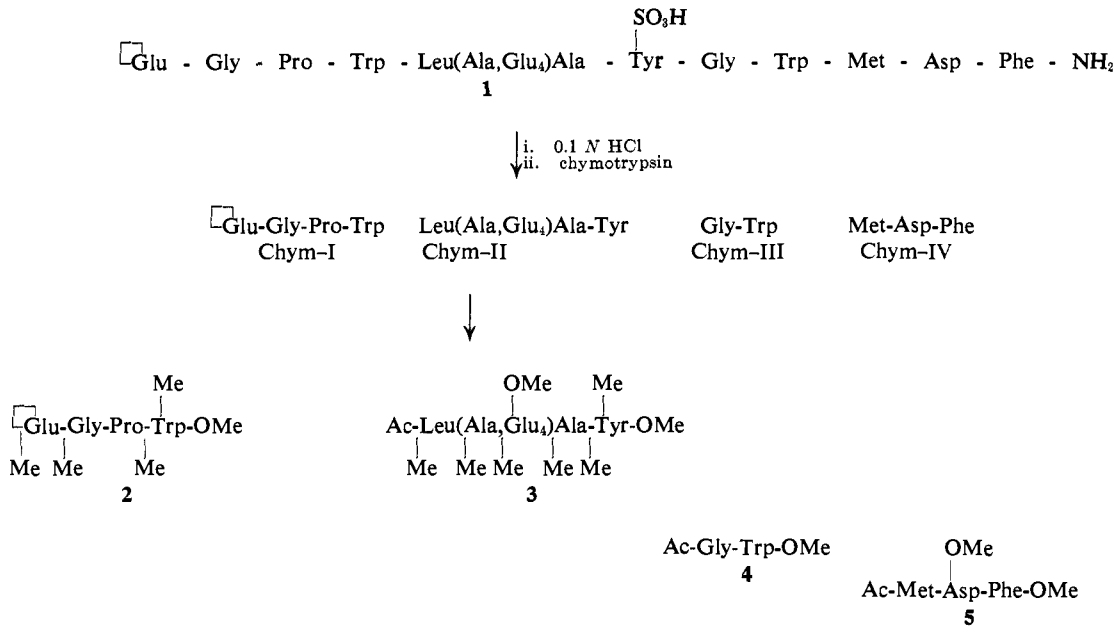
(3) R. A. Gregory, *Gastroenterology*, **51**, 953 (1966); *Proc. Roy. Soc.* (London), B **170**, 81 (1968).

(4) Review: G. W. Kenner and R. C. Sheppard, *ibid.*, B**170**, 89 (1968). Individual gastrins: J. C. Anderson, G. W. Kenner, J. K. MacLeod, and R. C. Sheppard, *Tetrahedron Suppl.*, **8**, 39 (1966); J. Beacham, P. H. Bentley, G. W. Kenner, J. K. MacLeod, J. J. Mendive, and R. C. Sheppard, *J. Chem. Soc., C*, 2520 (1967); K. L. Agarwal, J. Beacham, P. H. Bentley, R. A. Gregory, G. W. Kenner, R. C. Sheppard, and H. J. Tracy, *Nature*, **219**, 614 (1968); K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *Experientia*, **25**, 346 (1969).

(5) Both sulfated and unsulfated forms of feline gastrin were isolated from antral mucosa. The former predominated and was used in the present investigation.

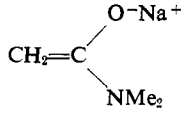
(6) *Cf.* B. C. Das, S. D. Géro, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **29**, 211 (1967); D. W. Thomas, B. C. Das, S. D. Géro, and E. Lederer, *ibid.*, **32**, 199 (1968); K. L. Agarwal, R. A. W. Johnstone, G. W. Kenner, D. S. Millington, and R. C. Sheppard, *Nature*, **219**, 498 (1968).

(7) C-Methylation can in general be avoided by reducing the amount of base present in the methylation reaction.



established the expected amino acid sequence, and it was identical with that of a similarly methylated authentic sample of the tetrapeptide Chym-I. The sequences of Chym-I, Chym-III, and Chym-IV thus deduced are common to all the gastrins so far examined. The key octapeptide Chym-II was esterified, acetylated, and permethylated. The resultant octa-N-methylpentamethyl ester monomethyl ether (mol wt 1190) did not yield a molecular ion in the mass spectrum because of dominant fragmentation at the terminal aromatic tyrosine residue. However the spectrum (Figure 1) contained a complete set of sequence ions, and it unambiguously established the part sequence Leu-Glu-Glu-Glu-Glu-Ala-Ala-, to which the C-terminal tyrosine residue can be added by virtue of the origin of Chym-II from a chymotryptic hydrolysate. From the specialized viewpoint of the gastrin researcher, cat is thus a hybrid of man and ox or sheep.[4]

In this work a new technique of N-methylation was employed, and the method may have wider applications. Sodium hydride was dissolved in N,N-dimethylacetamide at 120–125° with gas evolution, possibly yielding the salt



The cold solution (20°) was added to a solution or suspension of the acetylated peptide in dimethylacetamide, followed immediately by methyl iodide. Methylation was complete in 1 hr at 20°, and in other cases it was cleaner than by the Hakamori reagent (sodium hydride, DMSO, methyl iodide).[8] Obviously the catalyst derived from dimethylacetamide and sodium hydride may be a more generally useful reagent in place of methylsulfoxonium methylide and other strong bases.

The problem of sequence analysis of peptides on a submicro scale is of such importance that some remarks on the scope of the mass spectrometric method are in order. The usual conception that mass spectrometry is an exceptionally sensitive method is unjustified in the field of peptide and protein chemistry, where highly sensitive "wet" methods are available. The foregoing work was accomplished with 180 nmol, a substantial quantity by the standards of protein chemistry, and there was none to spare. The advantages of the mass spectrometric method lie in the strength of the sequence assignment for several residues obtained in the one operation.

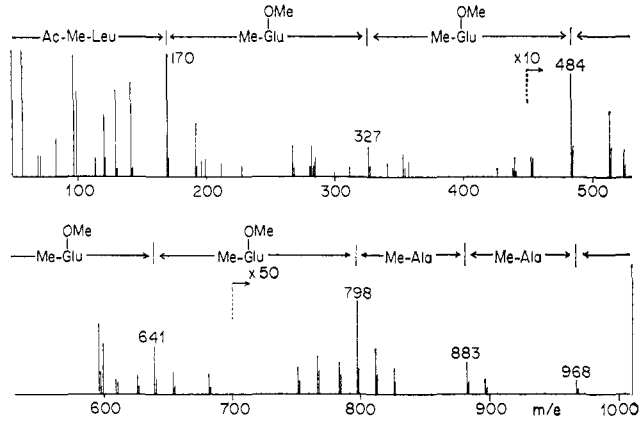


Figure 1. Mass spectrum of **3** derived from Chym-II (A.E.I. MS902 spectrometer, temperature 280°).

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(8) E. Vilkas and E. Lederer, *Tetrahedron Lett.*, 3089 (1968).