N-Terminal Groups in Mass Spectrometry of Peptides. A Study Including Some New and Useful Derivatives

RICHARD A. DAY, HERMANN FALTER,* JAMES P. LEHMAN, AND ROBERT E. HAMILTON

Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221

Received October 12, 1972

In an effort to find volatile peptide derivatives with mass spectrometric fragmentation characteristics suitable for peptide sequencing studies, 20 new N-terminal blocking groups were used to derivatize the test peptide Val-Ile-Ala. Electron impact mass spectra were obtained for the derivative esters and compared to the previously reported spectra of the test peptide in terms of relative intensity of molecular and N-terminal sequence ions. Thirty-four derivatives were compared in all. The most successful of these in terms of ease of interpretation were the 5-(*N*,*N*-dimethylamino)naphthalenesulfonyl, *p*-dimethylaminobenzylidene, and 4-(*N*,*N*-dimethylamino)naphthylidene derivatives. The intensities of the molecular ions were 10–100 times greater relative to the base peak than in previously reported spectra of derivatives of Val-Ile-Ala. The M − 56 ions, ascribed as arising from a McLafferty rearrangement and loss of C_4H_8 from the isoleucyl residue, did not appear from most of the derivatives displaying relatively intense molecular ions. The apparent inverse relationships between the relative intensities of molecular ions and the corresponding M − 56 ions was attributed to ionization potential effects. Selection of the appropriate derivatives of the more complex peptides, Pro-Val-Ile-Ala, Met-Val-Ile-Ala, Glu-Try-Glu, Gly-Pro-Gly-Gly, Gly-Gly-Gly-Gly-Gly-Gly, and the gastrin C-terminal fragment, Try-Met-Asp-Phe-NH_2 led to mass spectra containing sufficient information to allow sequence assignment in every instance; however, the amino acid composition was required in some cases.

The application of mass spectrometry to structure elucidation of complex molecules is limited by a number of factors, one of which is the tendency of the molecule to fragment along an increasing number of routes with increasing size and complexity of the molecule. There appears to be an inverse relationship between molecular weight within a homologous series and the probability of observing the parent ion or larger fragments in the electron-impact (EI) induced mass spectra of any given series. This problem is acute in mass spectrometry of peptides and is further accentuated by the occurrence of side-chain related fragmentation patterns.

It would appear that any factor tending to yield stable ionic species in which the charge and/or energy is localized to a significant degree on some center in the ion that does not participate in elimination or cleavage processes should result in a relative increase in the intensity of parent and some large fragment ions. It would be expected that any such center possessing a low ionization potential could be, in fact, the site of charge localization. We report here three peptide derivatives which give parent and sequence ions of 10–100 times the relative intensity of previously reported derivatives as well as diminished side-chain cleavage. These are the dansyl [5-(*N*,*N*-dimethylamino)naphthalenesulfonyl] (DNS), *p*-dimethylaminobenzylidene (DMB), and the 4-(*N*,*N*-dimethylamino)naphthylidene (DMN) derivatives of the peptide amino groups.[1]

Experimental Section

Materials.—The peptides Val-Ile-Ala and Met-Phe-Gly were obtained from Cyclo Chemical Corp. The peptides Glu-Try-Glu and Try-Met-Asp-Phe-NH_2 and DNS-Gly were obtained from Mann Research Laboratories. The peptide Leu-Ala was obtained from Nutritional Biochemicals Corp. DNS-Pro and DNS-Met were obtained from Sigma Chemical Co. The aldehydes and ketones were obtained from Aldrich Chemical Co., except *p*-dimethylaminobenzaldehyde and acetylacetone (Matheson Coleman and Bell) and 4-dimethylamino-1-naphthaldehyde which was prepared as described.[2]

Syntheses.—The phthalyl and naphthalene-1,8-dicarboxyl peptide derivatives were prepared essentially by the method of King and Kidd.[3] Dansyl amino acids and peptides and 1-naphthalenesulfonyl-Val-Ile-Ala-OCH_3 were prepared essentially by the method of Gray.[4] The Schiff base peptide ester derivatives were prepared by heating equivalent amounts of the aldehyde and peptide ester in glacial acetic acid at 118° for 5–15 min. The 1- and 2-naphthoyl peptide esters were prepared from the *N*-hydroxysuccinyl esters of the acids and Val-Ile-Ala-OC_2H_5. Permethylation of the peptide DNS-Val-Ile-Ala-OCH_3 was accomplished essentially by the method of Thomas.[5] Esterification of peptides was accomplished by refluxing the peptide in the appropriate alcohol for 10 min after addition of thionyl chloride.

The peptide derivatives DNS-Gly-Val-Ile-Ala-OC_2H_5, DNS-Pro-Val-Ile-Ala-OC_2H_5 and DNS-Met-Val-Ile-Ala-OC_2H_5 were synthesized from the corresponding *N*-hydroxysuccinyl-DNS-amino acid and Val-Ile-Ala-OC_2H_5. DNS-Leu-Ala-Val-Ile-Ala-OC_2H_5 was prepared by coupling equimolar amounts of DNS-Leu-Ala and Val-Ile-Ala-OC_2H_5 with an equivalent amount of dicyclohexylcarbodiimide; the hexapeptide derivatives DMB-Val-Ile-Ala-Val-Ile-Ala-OC_2H_5 and DMB-Val-Ile-Ala-Met-Phe-Gly-OC_2H_5 were prepared in an analogous fashion.

Mass Spectra.—The spectra reported were obtained with a Hitachi Perkin-Elmer RMU-7 mass spectrometer. The samples were introduced through the solid inlet system. Inlet temperatures at which the spectra were recorded are indicated in the description of the spectra. In all cases the inlet temperature was raised gradually until the relative intensities of the peaks were nearly constant; in some cases higher temperatures were required, presumably because of some impurities of greater volatility. An ionizing voltage of 70 eV was used in obtaining all spectra reported.

Results

Figure 1 shows the mass spectra of the 1-naphthalenesulfonyl, DNS, and permethylated DNS esters of Val-Ile-Ala (**1**). The relative intensities of the sequence and molecular ions show a significant variation among the derivatives. The spectrum of 1-naphthalenesulfonyl-Val-Ile-Ala methyl ester contains a molecular ion (*m/e* 505) of relatively low intensity and a prominent ion of *m/e* 449 corresponding to the product ion of the McLafferty rearrangement of the Ile side chain

* Department of Chemistry, Laurentian University, Sudbury, Ontario.

(1) For preliminary accounts of this work, see J. P. Lehman, H. Falter, and R. A. Day, Abstracts, Third Central Regional Meeting of the American Chemical Society, Cincinnati, Ohio, June 6–8, 1971, No. 178; H. Falter, J. P. Lehman, and R. A. Day, Abstracts Volume, XXIII International Congress of Pure and Applied Chemistry, Boston, Mass., July 25–30, 1971, p 90.

(2) J. C. Banerji and S. N. Sanyal, *Indian J. Chem.*, **6**, 346 (1968).

(3) F. King and D. Kidd, *J. Chem. Soc.*, 3315 (1949).

(4) W. R. Gray, "Methods in Enzymology," Vol. 11, Academic Press, New York, N. Y., 1967, pp 142, 143.

(5) D. W. Thomas, *Biochem. Biophys. Res. Commun.*, **33**, 483 (1968).

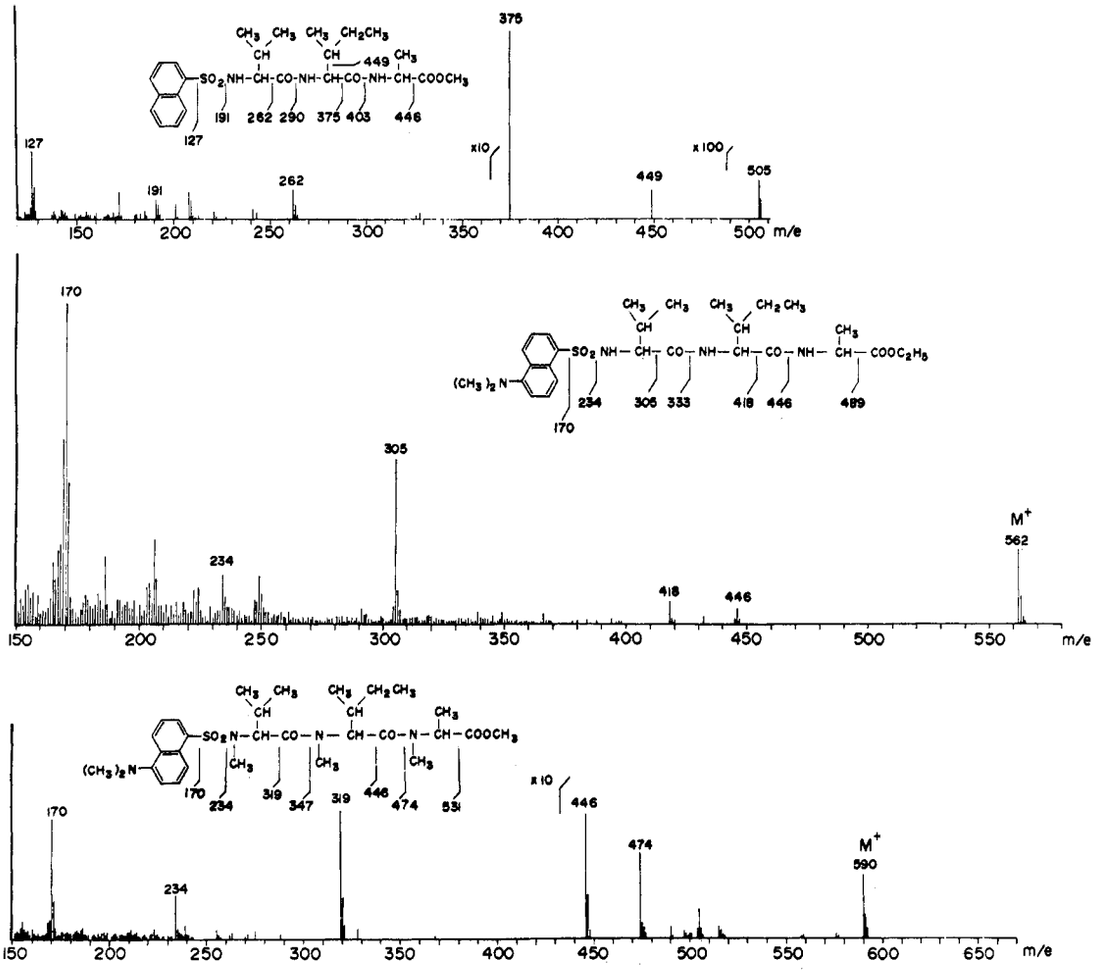


Figure 1.—Mass spectra of naphthalenesulfonylvalylisoleucylalanine derivatives: top, spectrum of 1-naphthalenesulfonyl-Val-Ile-Ala-OCH_3 obtained with an inlet temperature of 320°; middle, spectrum of DNS-Val-Ile-Ala-OC_2H_5, inlet temperature of 440°; bottom, spectrum of permethylated DNS-Val-Ile-Ala-OCH_3, inlet temperature of 250°.

with loss of isobutylene. The DNS derivative has a relatively more intense molecular ion (*m/e* 562) and no detectable ion at *m/e* 506, the expected value of the McLafferty product ion. All derivatives show many, but not all, of the sequence ions. The permethylated derivative was significantly more volatile[6] and the distribution of ion intensities was similar to that of DNS-Val-Ile-Ala-OC_2H_5 except that the relative intensity of the molecular ion was less.

The DMB ethyl (Figure 2, top) and the DMN methyl (Figure 2, bottom) esters of **1** display intense molecular ions. The distribution of intensity among sequence and molecular ions for the latter is perhaps the most uniform of any derivative of **1** shown or tabulated in Table I. It was amenable to gas–liquid partition chromatography. Table I contains a comparison of the relative intensities of the sequence and molecular ions for a number of drivatives of **1**. The spectrum of the phthalyl methyl ester of **1** was prepared in this laboratory for comparison with that reported by Prox and Sun[7] in order to determine differences in observed spectra due to differing instrumental and operational parameters.

Mass spectra of the DNS ethyl esters of Pro-Val-Ile-Ala (Figure 3), Met-Val-Ile-Ala (Figure 4), Gly-Val-Ile-Ala, and Leu-Ala-Val-Ile-Ala were obtained at inlet temperatures higher than that required for DNS-Val-Ile-Ala-OC_2H_5. The mass spectra of these derivatives contain relatively intense molecular ions with the exception of the last one, and none contain the ion fragments expected from a McLafferty rearrangement. A sequence peak at one or both sides of each carbonyl group was found in all cases.

The mass spectra of the DMB triethyl ester (Figure 5) and the DMN trimethyl ester (Figure 6) derivatives of Glu-Try-Glu both contain the molecular and sequence ions. Side-chain cleavage of the tryptophan residue from the former as the indolylmethyl is indicated by the presence of an *m/e* 547 (M − 130). The *m/e* 577 corresponds to loss of $CH_2CHCO_2C_2H_5$ from glutamyl side chains by a McLafferty rearrangement. The *m/e* 447 corresponds to a sequence peak and loss of both fragments from the molecular ion. High-resolution analysis would be required to determine the processes contributing to *m/e* 447. The spectrum contains several prominent ions which are not N-terminal fragments. Most of these may be rationalized as corresponding to C-terminal fragments and to fragments having arisen by two single bond cleavages.[8] The second derivative gave a spectrum (Figure 6) with a smaller number of ions; the most

(6) This derivative was readily purified by gas–liquid partition chromatography. Experiment performed by Dr. J. MacGee, V. A. Hospital, Cincinnati, Ohio.

(7) A. Prox and K. K. Sun, *Z. Naturforsch. B*, **21**, 1028 (1966).

(8) Patil, *et al.*, *Org. Mass Spectrom.*, in press.

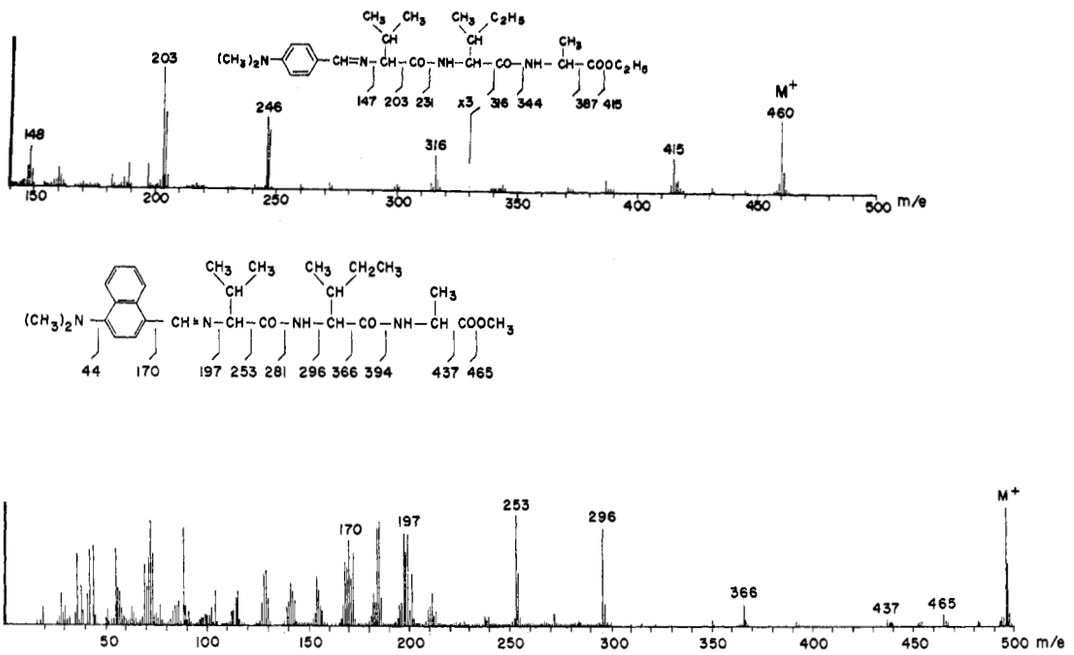


Figure 2.—Spectra of DMB-Val-Ile-Ala-OC_2H_5 and DMN-Val-Ile-Ala-OCH_3; inlet temperatures 220 and 120°, respectively.

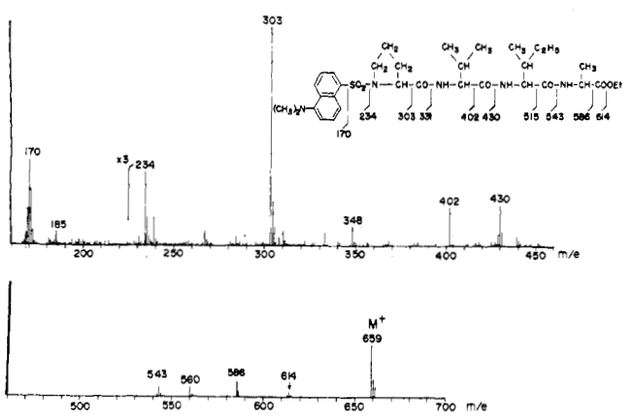


Figure 3.—Spectrum of DNS-Pro-Val-Ile-Ala-OC_2H_5; inlet temperature 380°.

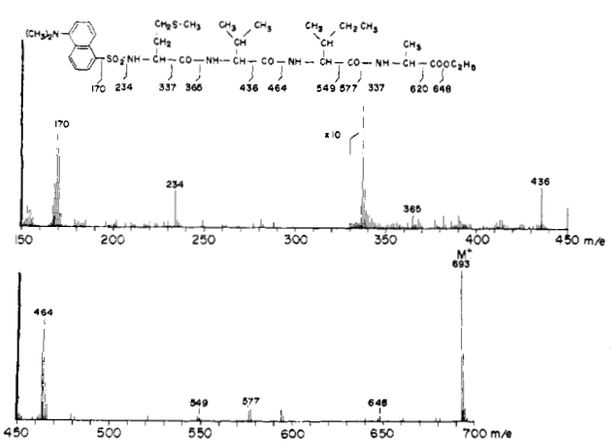


Figure 4.—Mass spectrum of DNS-Met-Val-Ile-Ala-OC_2H_5; inlet temperature 420°.

prominent ions include N-terminal fragment and molecular ions.

The tetrapeptide derivative, DMN-Gly-Pro-Gly-Gly-OEt (not shown), displays the molecular ion and some sequence ions in its spectrum. In addition there

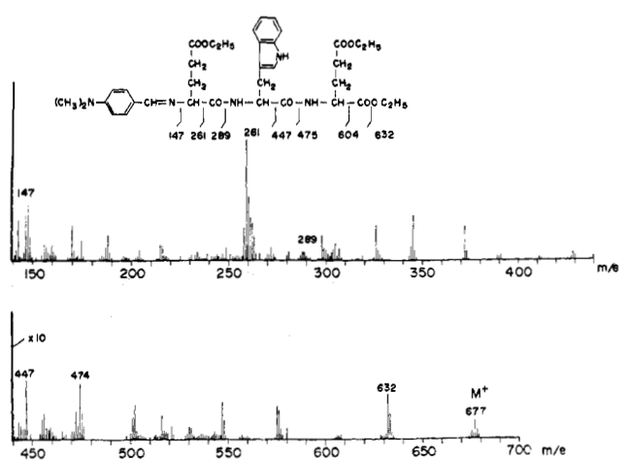


Figure 5.—Mass spectrum of DMB-Glu-Try-Glu-$(OCH_3)_3$; inlet temperature 400°.

are prominent ions at m/e 114 and 154 having the m/e of glycylglycyl and glycylprolyl or prolylglycyl, or the corresponding diketopiperazines. Also, m/e 211 is observed, corresponding to Gly_2-Pro. Additional fragments corresponding to these but plus or minus CO or NH are seen.

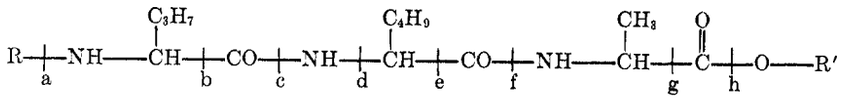
Figure 7 shows the spectrum of the DMB derivative of the C-terminal gastrin fragment, DMB-Try-Met-Asp-(OC_2H_5)-Phe-NH_2. The observed spectrum contains few N-terminal sequence ions. Many of the prominent peaks can be rationalized as arising from two single bond cleavages.[8]

In Figure 8 is shown the mass spectrum of DMB-Val-Ile-Ala-Val-Ile-Ala-OC_2H_5 which may be rationalized in terms of the peptide sequence. The spectrum contains a prominent molecular ion and many of the N-terminal sequence ions are prominent. A McLafferty rearrangement of the Ile side chain may contribute to the observed, low intensity m/e 687.

Figure 9 shows the mass spectrum of DMB-Val-Ile-Ala-Met-Phe-Gly-OC_2H_5, which contains a prom-

TABLE I

RELATIVE INTENSITIES OF SEQUENCE IONS[a] FOR DERIVATIVES OF VALYLISOLEUCYLALANINE ESTERS



Registry no.	Amino blocking group	Ester[b]	a	b	c	d	e	f	g	h	M − 56	M	Source of data[c]
37580-54-4	None	M		100			3.9	1.6	0.5	0.3	0.13	0.3	a
37580-55-5	Phthalyl	M		100	15		48	3		0.4	2.3	0.15	a
	Phthalyl	M		100	33		100	7.2		1.9	10	1.3	b
37580-56-6	Phthalyl	E		100	70		100	19	1	5.3	22	2.1	b
37580-57-7	Cyclohexanecarboxyl	M	11	42	100		10	4	0.07	0.5	2.8	0.5	a
37580-58-8	Benzoyl	M	100	39	27	na[e]	5	2	0.04	0.2	na[e]	0.1	a
37580-59-9	Pentafluorobenzoyl	M	100	54	5	na	10	6	0.3	0.5	na	0.04	a
37580-60-2	*p*-Chlorobenzoyl	M	100	39	13		2.5	2.5	0.04	0.1	1.6	0.1	a
37580-61-3	Acetyl	M	21	100	36		26	12	0.3	0.5	9.8	0.3	a
37580-62-4	Trifluoroacetyl	M		31			100	7	1.5	0.8	33	0.1	a
37580-63-5	Formyl	M	20	100	10	na	89	19	1.4	1.3	na	0.4	a
37580-64-6	*n*-Decanoyl	M	8.4	54	100		9	7	0.09	0.9	4.5	0.5	a
37580-65-7	*n*-Stearoyl	M	8.1	41	100	na	1.4	6	0.2	0.7	na	0.2	a
37580-66-8	Chlorodifluoroacetyl	M		55	5	na	100	11	1.1	0.4	na	0.1	a
6686-82-4	Carbobenzoxy	M		28	100	na	67	10	0.2	1.4	na	1.3	a
37580-68-0	2,4-Dinitrophenyl	M		100		na	3	0.9		0.4	na	0.8	a
37580-69-1	1-Naphthoyl	M	100	65	93		20	9		2	10	8	b
37580-70-4	1-Naphthoyl	E	99	90	100		11	14	<1	2	10	5	b
37580-71-5	2-Naphthoyl	M	100	33	61		4.5	3		1	2	3.1	b
28415-47-6	Adamantoyl	M	19	35	100		3	2		0.4	10	0.6	c
37580-73-7	Naphthalene-1,8-dicarboxyl	M		46	100		8	4					b
37580-74-8	Naphthalene-2,3-dimethylene	M		100									b
37580-75-9	Benzenesulfonyl	M	45	100			85	5		0.4	4.1	0.2	a
37580-76-0	Dansyl	M	100	30	<1	1	5	2				24	b
37580-77-1	1-Naphthalenesulfonyl	M	68	100			54				16	3.2	b
37580-78-2	Benzylidene	E		100		37	37	5		3.7	1.0	0.5	b
37495-93-5	Salicylidene	E		100	21.3	73	35	13		7.8		23.0	b
37580-24-8	β-Indolylmethylidene	E	9.5	100		37	64	2.8	0.74	3.2	0.7	3.2	b
37580-25-9	*p*-Nitrobenzylidene	E	32	37		25	29	15		4	4.4	0.4	b
	p-Nitrobenzylidene[d]	E	27	100		27	9	4	1.7	2.9		0.9	b
37580-26-0	*p*-Cyanobenzylidene	E		94		44	100	47		7.0	1.34	1.07	b
37580-27-1	α-Phenyl-*p*-dimethylaminobenzylidene	E		100								7.3	b
37580-28-2	*p*-Dimethylaminocinnamylidene	E	77	100		37	3			9.2		24	b
37580-29-3	*p*-Diethylaminocinnamylidene	E		100		68	18		3.2	6.8		40	b
37580-30-6	*p*-Methoxybenzylidene	E	12	100		83	67	9	1.2	11		1.2	b
37580-31-7	2-Pyridylmethylidene	E	15	79	31	100	11	12	6.7	11		8.0	b
37580-32-8	3-Pyridylmethylidene	E		79		100	12	5		2.3	1.1	1.2	b
37580-33-9	4-Pyridylmethylidene	E		100		59	98	11	2.2	5.4	1.7	1.1	b
37580-34-0	Acetylacetonyl	E		100	12			4.6	1.0	2.9		6.0	b
37580-35-1	*p*-Dimethylaminobenzylidene	E	29	100	8	44	6	6	22	6		29	b
37580-36-2	4-Dimethylamino-1-naphthylidene	M	77	92		72	9.4		6.0	8.5		100	b
37580-37-3	2-Hydroxy-1-naphthylidene	E		100	3.8	5.7	3.6	2.1	1.2	3.4		29	b

[a] The values were obtained from spectra obtained on an RMU-7 mass spectrometer or from literature cited. Cleavage "a" is between N^{α} and C^{α} of valyl residue for many of the Schiff bases and cyclic imides; R is amino blocking group and may be bonded to the valyl nitrogen by one single bond, one double bond, or two single bonds; R′ is methyl or ethyl. [b] M = methyl, E = ethyl. [c] (a) From an AEI MS-9 mass spectrometer, Prox and Sun;[7] (b) from a Hitachi Perkin-Elmer RMU-7 mass spectrometer in this laboratory; (c) from a Hitachi Perkin-Elmer RMU-6D mass spectrometer, Lengyel, *et al.*[9] [d] Peaks corresponding to *m/e* for cleavage indicated in column heading accompanied by a loss of NO. [e] The term "na" means not available from reference cited.

inent molecular ion and ions whose *m/e* correspond to sequence ions, some of relatively high intensity. The spectrum contains many prominent ions which were not rationalized as N-terminal fragments. The prominent *m/e* 643 can be rationalized as side-chain cleavage of both C_7H_7 and C_2H_5S from the molecular ion. DMN-Gly_6-OCH_3 (spectrum not shown) gave an M − 31 and a small M^+ ion; in addition, it displayed peaks having the nominal masses of Gly_2 and Gly_3 and some sequence ions.

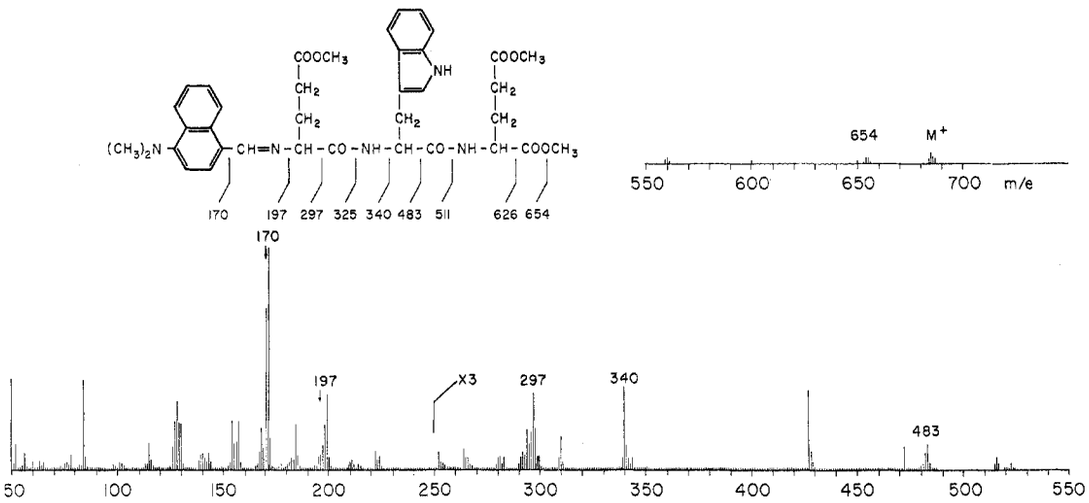


Figure 6.—Mass spectrum of DMN-Glu-(γ-OCH_3)-Try-Glu-(OCH_3)$_2$; inlet temperature 140°.

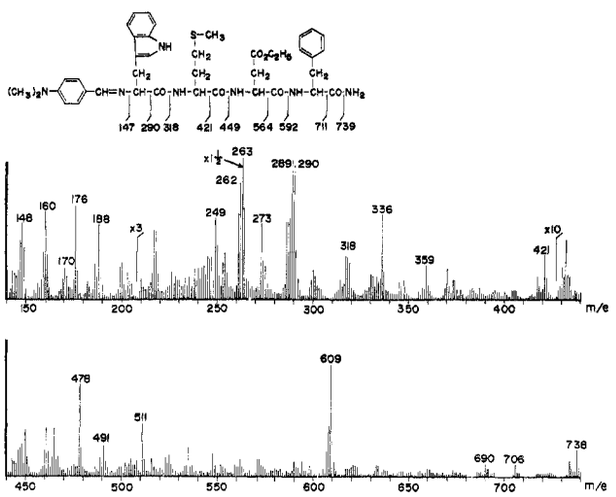


Figure 7.—Mass spectrum of DMB-Try-Met-Asp-(β-OC_2H_5)-Phe-NH_2; inlet temperature 450°.

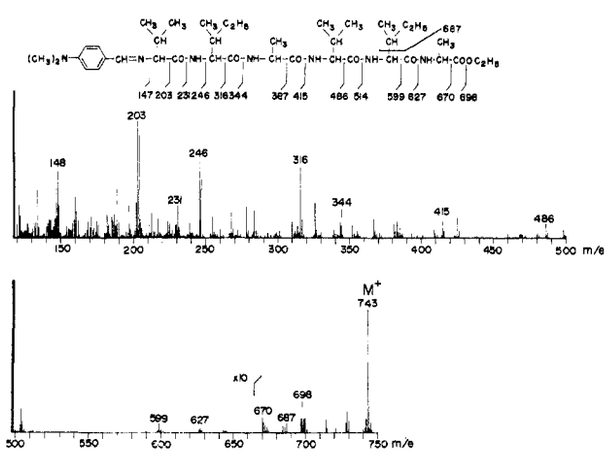


Figure 8.—Mass spectrum of DMB-Val-Ile-Ala-Val-Ile-Ala-OC_2H_5; inlet temperature 400°.

Discussion

It is apparent from the preceding data that some of the peptide derivatives reported here for the first time have important properties relative to the application of mass spectrometry to peptide sequencing. This study, in part an extension of the work of Prox and Sun[7] using **1** as a reference peptide, reveals that certain peptide derivatives give EI mass spectra with relatively more intense molecular and sequence ions than any derivatives reported thus far. DMB-Val-Ile-Ala-OC_2H_5 gave a spectrum containing all of the sequence ions with none less than 6% of the intensity of the base peak of the spectrum. The spectrum of DMN-Val-Ile-Ala-OCH_3 contained the most intense molecular ion of any derivative of **1** reported thus far. Similarly, the DNS derivative of **1**, and its permethylated form, and the naphthoyl derivatives of **1** all give significantly more intense sequence and molecular ions relative to derivatives reported earlier.

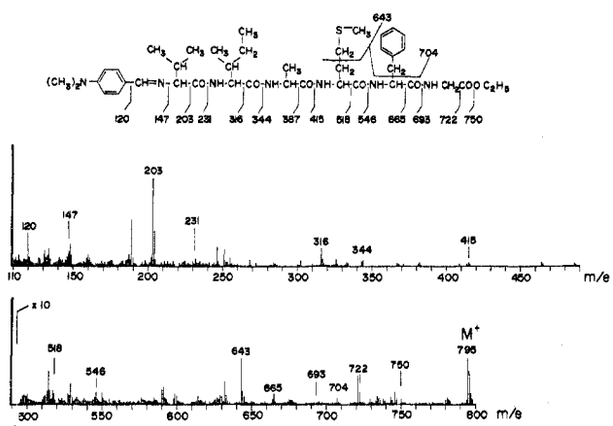


Figure 9.—Mass spectrum of DMB-Val-Ile-Ala-Met-Phe-Gly-OC_2H_5; inlet temperature 175°.

The mass spectra of the DNS, DMB, and DMN peptide esters of **1** are dominated by molecular and sequence ions. More complex peptides gave spectra with varying ease of interpretation. The two DNS tetrapeptide derivatives (Figures 3 and 4) both gave relatively intense molecular ions, 15 and 5.2%, respectively, compared to the base peaks of the spectra. McLafferty product ions arising from rearrangement of the Val or Ile side chains were not found in either instance, nor was cleavage of the Met side chain of the latter observed; the sequence and molecular ions dominate the mass spectra of both. Similar results were obtained for DNS-Gly-Val-Ile-Ala-OC_2H_5 (not shown). The low-resolution mass spectra discussed

above are suitable for the assignment of the sequences of the respective peptides without prior knowledge of the amino acid compositions. The DNS pentapeptide ester of Leu-Ala-Val-Ile-Ala (not shown) gave a mass spectrum containing all of the sequence ions but no molecular ion. This derivative required a very high inlet temperature to obtain the observed spectrum, and it can be suspected that the derivative's vapor pressure was insufficient and that the observed spectrum was that of pyrolysis products. Other DMB and DMN peptide esters gave mass spectra which required knowledge of the amino acid composition in order to assign the amino acid sequences, or they gave spectra which could not be unambiguously interpreted with respect to amino acid sequence on the basis of N-terminal fragments, even with knowledge of the composition of the peptide.[8]

The mass spectrum of DMB-Glu-Try-Glu-$(OC_2H_5)_3$ (Figure 5) would be difficult to interpret on the basis of its N-terminal fragments alone without the knowledge that the amino acid composition was Glu_2-Try. With this knowledge, *m/e* 677 is identified as the molecular ion and *m/e* 632 as the M − OC_2H_5 ion. The expected N-terminal sequence ions for all possible sequences of the composition Glu_2-Try are tabulated as follows: DMB-Glu-Try-Glu-$(OC_2H_5)_3$ 147, 261, 289, 447, 475, 604, 632; DMB-Try-Glu-Glu-$(OC_2H_5)_3$ 147, 290, 318, 447, 475, 604, 632; DMB-Glu-Glu-Try-$(OC_2H_5)_3$ 147, 261, 289, 418, 446, 604, 632. Intense ions corresponding to the expected sequence ions are found only for one of the possible sequences, Glu-Try-Glu. This spectrum illustrates some of the difficulties to be encountered in attempting to assign composition and sequence of peptides on the basis of expected N-terminal cleavages in their low-resolution EI mass spectra. If the amino acid composition of the peptide were unknown, it would be necessary to assign the molecular weight on the basis of the *m/e* 677 and 632 (M − OC_2H_5) ions. The molecular weight in this and parallel cases would be uncertain due to the presence of side-chain carboxyl groups. A detailed analysis of all possible fragment ions which could give rise to *m/e* 677 in conjunction with the observed ions would be necessary if one were to unambiguously assign the composition and sequence of the peptide on the basis of the observed mass spectrum. In addition, the plethora of fragment ions below the highest *m/e* of 677 such as *m/e* 577 (M − $CH_2CHCO_2C_2H_5$) and 547 (M − $C_9H_8N_1$) would render the spectrum more difficult to interpret without prior knowledge of the amino acid composition of the peptide. The sequence assignment is unambiguous if the compositional data and other fragmentation data from the mass spectrum as utilized. In contrast to the spectrum of DMB-Glu-Try-Glu-$(OC_2H_5)_3$ discussed above, the spectrum of DMN-Glu-Try-Glu-$(OCH_3)_3$ (Figure 6) contains fewer ions, and the sequence ions are of greater relative intensity making this spectrum more readily interpreted. We have not observed any significant differences in observed spectra attributable to the choice of either the methyl or ethyl ester derivative in a number of comparative spectra.

The observed mass spectra of the DMN peptide ester derivatives in general contain the sequence and molecular ions in greater relative abundance than do the spectra of the DNS and DMB ester derivatives of the same peptide. The DMN ethyl ester derivative of I (Table I) is a striking example in that the base peak of the observed spectrum (Figure 2) is the molecular ion in contrast to the DMB derivative where the M^+ is 29% of the base peak. Most of the sequence peaks are of relatively high intensity in the spectrum of the DMN derivative of **1** as well. The spectrum of DMN hexaglycine methyl ester (not shown) contained some sequence ions, M^+ and M − 31 ions and fragment ions corresponding to di- and tripeptide fragments of the molecule. This spectrum is notable in that this large high glycine content peptide was not found to be amenable to EI mass spectrometry with other N-terminal blocking groups. The spectra discussed above indicate the potential utility of the DMN derivative in peptide sequencing studies by mass spectrometry.

The spectrum of the DMB derivative of the gastrin fragment (Figure 7) reveals that for this tetrapeptide amide no total sequence assignment can be made on the basis of N-terminal sequence peaks with or without knowledge of the amino acid composition. By applying the "internal fragmentation" concept,[8] the majority of the most prominent peaks can be rationalized and assigned structures. These assignments provide unambiguous data for sequence assignment in this and other peptides from their low-resolution spectra. The spectra shown in Figures 8 and 9 are further representative examples. The spectrum of DMB-Val-Ile-Ala-Val-Ile-Ala-OC_2H_5 (Figure 8) contains a prominent molecular ion and relatively intense sequence ions. Given the amino acid composition, it is possible to assign the amino acid sequence of the peptide unambiguously from the low-resolution spectrum on the basis of the N-terminal fragments. DMB-Val-Ile-Ala-Met-Phe-Gly-OC_2H_5 (Figure 9) gives a strong molecular ion with most of the N-terminal sequence ions. Other prominent ions can be rationalized as C-terminal fragments (*m/e* 550, 591) with an H shift and as arising from two single bond cleavages, *e.g.*, *m/e* 643 from two side chains, *m/e* 514 and 529 from the two ends of the chain.

An important characteristic of the DNS, DMB, and DMN peptide ester derivatives of **1** is the suppression of the McLafferty rearrangement of the Ile and Val side chains. This rearrangement was found operative in all of the other derivatives of **1** examined by Prox and Sun[7] and Lengyel, *et al.*[9] Most of the Schiff base derivatives failed to give the M − 56 peak. Significantly reduced side-chain cleavage of DNS, DMB, and DMN peptide derivatives relative to other derivatives was found for a variety of peptides containing amino acids with readily cleaved side chains such as Met, Phe, Try, and Glu.

Comparison of the spectra of analogous benzene and naphthalene derivatives (Table I) shows that the latter have a more favorable cleavage pattern in general. The 1- and 2-naphthoyl derivatives gave a larger fraction of the total ion current in the sequence and molecular ions than did the benzoyl and several substituted benzoyl derivatives; the McLafferty cleavage manifested as *m/e* M − 56 shows the converse relationship. The naphthalenesulfonyl shows a more intense M^+ than

(9) I. Lengyel, R. A. Salamone, and K. Biemann, *Org. Mass Spectrom.*, **3**, 789 (1970).

the benzenesulfonyl derivative. The DMN derivative displays an M^+ that is the base peak, while the M^+ is 29% of the base peak for the DMB derivative. All of the aromatic Schiff bases examined thus far display a prominent ion corresponding to a cleavage of the N^{α}–C^{α} bond of the second amino acid residue from the N-terminus (Table I, column d). There is no apparent relationship between the electron-withdrawing properties of the aromatic moiety and the relative intensity of the "d" cleavage. The spectra of the *p*-diethyl- and *p*-dimethylaminocinnamylidene ester derivatives of **1** (Table I) were found to be comparable in their ease of interpretation although differences in the distributions of ion intensities were noted. This suggests that dimethylamino and diethylamino substituents should have about the same effect.

Many of the peptide mass spectra discussed above exhibit two properties which are pertinent to the problem of peptide sequencing by EI mass spectrometry. These features are (1) relatively intense molecular and high *m/e* sequence ions, and (2) suppression or elimination of McLafferty fragmentations and other side-chain cleavages in certain derivatives. It has been well documented that substituent groups within a molecule can have a decided effect on the EI mass spectrum of the molecule.[10,11] Numerous studies have shown that the ionization potential (IP) of a molecule with an aryl amino or dimethyl amino group is significantly lower than that of the unsubstituted molecule or one with another substituent.[12,13] It has been suggested that the fragmentation observed is a function of the difference between the IP of the molecule and the appearance potential (AP) of the fragment(s), *i.e.*, the greater the $\Delta(AP - IP)$, the less likely the fragmentation will be observed.[14,15] The Schiff base ester derivatives of **1** (Table I) examined in this study appear to bear out this contention if the IP's of model compounds are taken as estimates of the IP's of the peptide derivatives.[16] Wachs and McLafferty[17] have shown that an aryl substituent greatly affects the relative amount of McLafferty fragmentation through intervening σ bonds and that an aryl amino group almost completely suppresses the fragmentation in the model compound studied.

Audier[18] has demonstrated the generalization that when a fragmentation takes place in EI mass spectroscopy the positive charge remains on the fragment with the lowest IP. Bursey and McLafferty[19] recorded similar observations for a series of para-substituted aceto- and benzophenones. The EI mass spectra reported here of peptide derivatives containing an aryl dimethyl amino group are in contrast with previously reported chemical ionization mass spectra of peptide derivatives which contain both the C- and N-terminal sequence identifying ions.[20]

Acknowledgment.—This work was supported by grants from the National Science Foundation (GP 8490) and the USPHS (ES-00159, GM 18756). H. F. was a trainee of a Biomedical Grant (PHS FR 7075) and R. A. D. is a Career Development Award Grantee (1-K4-GM-7654).

(10) (a) R. G. Cooks, I. Home, and D. H. Williams, *Org. Mass Spectrom.*, **2**, 137 (1969); (b) M. S. Chin and A. G. Harrison, *ibid.*, **2**, 1073 (1969).
(11) F. W. McLafferty, *Chem. Commun.*, 956 (1968).
(12) C. Lageot, *Org. Mass Spectrom.*, **5**, 845 (1971).
(13) M. M. Bursey and F. W. McLafferty, *J. Amer. Chem. Soc.*, **88**, 529 (1966).
(14) M. A. Baldwin and A. G. London, *Org. Mass Spectrom.*, **2**, 549 (1969).
(15) D. A. Lightner and F. S. Steinberg, *ibid.*, **3**, 1095 (1970).
(16) R. W. Kiser, "Introduction to Mass Spectrometry and Its Application," Prentice-Hall, Englewood Cliffs, N. J., 1965, p 301 ff, Appendix IV.
(17) T. Wachs and F. W. McLafferty, *J. Amer. Chem. Soc.*, **89**, 5044 (1967).
(18) H. E. Audier, *Org. Mass Spectrom.*, **2**, 283 (1969).
(19) M. M. Bursey and F. W. McLafferty, *J. Amer. Chem. Soc.*, **88**, 529 (1966).
(20) A. A. Kiryushkin, H. M. Fales, T. Axenrod, E. J. Gilbert, and G. W. A. Milne, *Org. Mass Spectrom.*, **5**, 19 (1971).

An Electrochemical and Spectrophotometric Study of Fluorene and the Fluorene Carbanion in Dimethylformamide, Dimethyl Sulfoxide, and Acetonitrile[1]

JOHN R. JEZOREK

Department of Chemistry, University of North Carolina at Greensboro, Greensboro, North Carolina 27412

AVINASH LAGU, THEODORE M. SEIGEL, AND HARRY B. MARK, JR.*[2]

Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221

Received October 28, 1970

The reduction behavior of the nonalternant aromatic hydrocarbon fluorene is investigated in DMF, DMSO, and acetonitrile, and compared and contrasted to that of alternant hydrocarbons. It is concluded that the normal electrochemical sequence does not occur on formation of the fluorene anion radical at the electrode in protic media, or when self-protonation occurs in aprotic media. Polarographic and coulometric data indicate that, rather than the usual reduction of a double bond, with a two-electron change, three electrons per molecule of fluorene are transferred under protic conditions, and reactive intermediates are formed which yield colored products on addition of oxygen. These products are unstable and decay rapidly to fluorenone under uv light. Spectrophotometric data of the colored intermediates are given, along with that of the fluorene anion radical.

It has been known for several years that chemical and electrochemical reduction of alternant aromatic hydrocarbons in aprotic solvents yields relatively stable anion radicals, which degrade by reaction with solvent and/or impurities. In the presence of electroinert proton donors such as phenol and resorcinol the anion radical abstracts a proton from the donor, and the resultant neutral radical is further reduced and protonated.[3] This sequence is the so-called electrochemical–chemical–

(1) This work was supported in part by the National Science Foundation, Grant No. GP-9307 and GP-27216.
(2) To whom all correspondence should be addressed.
(3) (a) N. H. Velthorst and G. J. Hoijtink, *J. Amer. Chem. Soc.*, **87**, 4529 (1965); (b) *ibid.*, **89**, 209 (1967).