MASS SPECTROMETRY OF DNP-AMINO ACIDS COMBINATION WITH PAPER CHROMATOGRAPHY*

Martin H. Studier and Leon P. Moore
Chemistry Division, Argonne National Laboratory
Argonne, Illinois 60439

Ryoichi Hayatsu and Sumiko Matsuoka
Enrico Fermi Institute, University of Chicago
Chicago, Illinois 60637

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SUMMARY - The DNP(2,4-dinitrophenyl) derivatives of twenty amino acids were prepared and their mass spectra determined. The analytical application of the combination of mass spectrometry and paper chromatography was demonstrated.

The combination of mass spectrometry with paper chromatography has resulted in a powerful technique for the analyses of purine and pyrimidine bases (1,2). However, the application of this technique to the analyses of free amino acids is limited as follows:

1. Free amino acids cannot be detected on paper without the addition of reagents which jeopardize subsequent mass analyses.
2. In general the mass spectra of free amino acids or their esters or acyl derivatives lack specificity. Parent ions are usually small and "base peaks" (most intense) are in the low mass regions where backgrounds from paper, reagents or the residual gases in the spectrometer are high (3).

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Although DNP(2,4-dinitrophenyl) derivatives of amino acids are not as readily separated by paper chromatography as are the free acids (4), these derivatives have been used extensively for separation and identification of amino acids for the following reasons:

1. During the reaction of proteins and peptides with 2,4-dinitrofluorobenzene to form the DNP derivatives only the terminal amino acid is attacked and thus tagged (5).
2. The DNP derivatives are readily detected by their intense color and by absorption of ultraviolet light.
3. The majority of DNP-amino acids can be purified by extraction with ether.

In this paper we shall show that the combination of paper chromatography of the DNP-amino acids with mass spectrometry is a promising analytical technique.

Mass Spectrometry

Because mass spectral data were not available (6), the DNP derivatives of twenty amino acids were prepared and mass spectra were determined with a modified time-of-flight mass spectrometer (7). The mass spectra of several by-products of the dinitrophenylation reaction were determined also. The mass data are summarized in Table 1. The principal peaks for each derivative are listed in order of decreasing intensity. The data were taken with an ionizing electron beam of 70 volts at the lowest temperature which produced a definitive spectrum. All showed characteristic fragmentation patterns with prominent peaks in higher mass regions where backgrounds in general are low.

DNP Monoamino Monocarboxy Acids - For the class of amino acids represented as $R\text{-}CHNH_2CO_2H$ the mass spectra of the DNP derivatives are simple. In general the molecular ion peak, M, represented as $(R\text{-}CHNHDNPCO_2H)^+$ is prominent, but exceeded in intensity by the base peak which may be represented as $(R\text{-}CHNH\text{-}DNP)^+$ derived from the parent by loss of CO_2H. Lesser peaks are produced by fragmentation of "R" or the DNP group. It is of

TABLE I

Mass Spectra of DNP-Amino Acids and By-Products. (m/e) Values of the More Prominent Peaks are Given with Relative Abundances Beneath Each Value

Mol. wt.	DNP-Amino Acid								
241	Glycine	196	241	77	104	150	92	166	
		100	21	18	18	14	12	8	
255	Alanine	210	164	118	255	134	91		
		100	26	22	19	18	17		
269	α-Amino Butyric	224	269						
		100	16						
271	Serine	226	134	241	166	271	179		
		100	58	48	42	41	39		
281	Proline	236	190	189	144	281	143		
		100	17	15	15	13	11		
283	Valine	238	43	134	55	283	166	194	146
		100	45	23	17	16	13	8	7
297	Leucine	252	43	196	134	297	210		
		100	96	28	13	12	10		
297	Isoleucine	252	57	69	134	297	196		
		100	41	23	17	13	11		
297	Norleucine	252	43	41	134	164	297	196	
		100	40	35	19	14	9	8	
331	Phenylalanine	91	240	178	194	166	331		
		100	24	13	13	13	10		
255	β-Alanine	196	191	176	255	104	150	219	
		100	85	79	48	37	26	12	
313	Glutamic	250	222	55	268	85	313		
		100	83	73	53	42	7		
370	Tryptophan	130	43	103	77	58	370		
		100	24	9	9	6	1		
513	Tyrosine	167	273	107	79	91	183	240	
		100	56	50	36	23	17	6	
487	Histidine	247	276						
		100	25						
298	Ornithine	280	167	236	264				
		100	92	56	33				

340	Arginine	86 100						
341	Citrulline	70 100	234 44	176 36	193 26	280 19	222 15	
478	Lysine	183 100						
572	Cystine	208	240					
184	Dinitrophenol	184 100	63 32	91 28	53 25	107 24	154 21	79 18
183	Dinitroaniline	183 100	91 38	52 30	64 27	63 16	153 15	107 14
212	DNP Ethylether	184 100	168 45	107 44	212 39	91 33	154 32	

interest to note that the three isomeric leucine derivatives, not separable by paper chromatography, are distinguishable from each other as a result of variations in the fragmentation of the aliphatic part, R, of the molecule.

Phenylalanine-DNP is characterized by a base peak at m/e = 91 as the benzyl group is split from the molecule. In this case the ion formed by loss of CO_2H from the parent is much smaller than for the case of the free acid, its ester, or for DNP-alanine.

For DNP-β-alanine at 70 eV the base peak is at m/e = 196 corresponding to a loss of CH_2CO_2H from the parent molecule. At 30 eV the parent ion (m/e = 255) itself is the most intense peak.

The mass spectrum of DNP-glutamic acid is characterized by a base peak at m/e = 250. A reasonable explanation is that the carboxy group adjacent to the DNP group is lost in the usual manner followed by ring closure with loss of water. The free acid behaves in an analogous manner (3).

The DNP-tryptophan spectrum shows a base peak at m/e = 130 probably from the $(\text{Indole-}CH_2)^+$ fragment.

The di-DNP-tyrosine spectrum has its base peak at m/e = 167 from the DNP group splitting off at the ether linkage. Two other characteristic peaks occur at m/e = 273 and 240 as a result of the molecule splitting between the α and β carbon atoms.

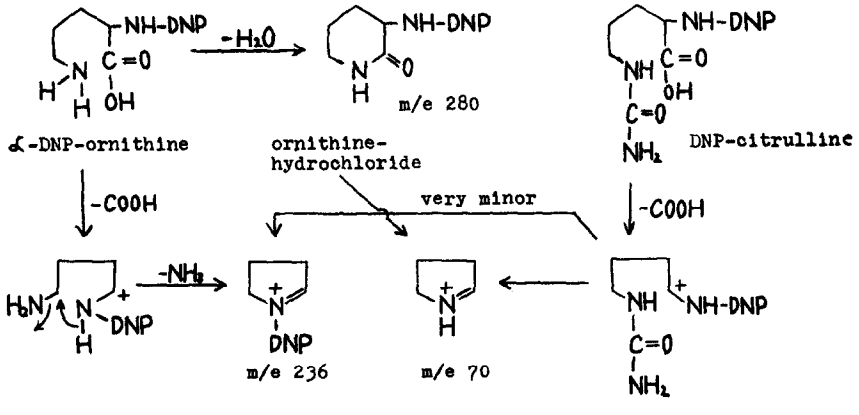
Of the basic amino acids the di-DNP-histidine is the simplest with two very characteristic peaks. One at m/e = 247 is probably due to the DNP-imidazolyl methyl ion. The other at 276 may be derived from the parent by loss of CO_2 and a DNP group.

The DNP-arginine required a relatively high temperature before appreciable volatilization occurred with resulting decomposition. The mass spectrum was very complex but was characterized by a very large peak at m/e = 86. The prominence of this peak suggests that it is due to a thermally stable cyclic structure.

The di-DNP-lysine also suffered decomposition at the high temperature required for volatilization but was characterized by a prominent peak at m/e = 183, probably due to dinitroaniline.

DNP-cystine also decomposes readily and yeilds a sulfur spectrum from S_8, S_7 -- to S_1. Two peaks more closely related to the parent molecule occur at m/e = 208 and 240 probably from the ions $(DNP\text{-}NH\text{-}CH{=}CH)^+$ and $(DNP\text{-}NH\text{-}CH{=}CHS^+)$ respectively.

A probable mechanism to explain part of the mass spectra of α-DNP-ornithine and DNP-citrulline is illustrated in Figure 1. For α-DNP-ornithine the most characteristic peaks are at m/e = 280 and 236. Although the most prominent peaks for DNP-citrulline are in the low mass region (m/e = 43 and 44) mass 70 is used for normalization as a prominent peak more characteristic of the parent molecule. Arginine hydrochloride and ornithine hydrochloride both show a prominent peak at mass 70 also.



Many of the peaks listed in Table 1 may be derived by loss of O, OH, NO or NO_2 groups from the DNP part of the molecule in a manner analogous to mononitrobenzene derivatives for which data are available (8).

Preparation of DNP-Amino Acids - Dinitrophenylation of amino acids was carried out using the methods of Peraino and Harper (4b), and Rao and Sober (9). In addition to the known by-products, dinitrophenol and dinitroanaline, we detected another, dinitrophenyl ethyl ether* by mass spectrometry.

Chromatographic Separations - Separations of mixtures of DNP-amino acids on a microgram scale were made using two dimensional chromatography on Whatman No. 1 paper and the solvent system described by Peraino and Harper (4b). Spots on the paper were revealed by their yellow color and by absorption of ultraviolet light. Spots of ether soluble DNP derivatives were cut out and extracted with dilute HCl and ether. The ether was separated and evaporated to dryness, transferred to platinum filaments with water, and placed in the source of the mass spectrometer. The "water soluble" compounds were transferred directly with an aqueous extract of the paper spot.

A mixture of 14 DNP-amino acids (two micrograms each) with trace impurities were spotted on Whatman No. 1 paper and developed exactly according to the procedure described by Peraino and Harper (4b). Fourteen compounds were discernible. Not all were clearly separated but all were readily resolved by mass spectrometry. Half the spots were cut out and the DNP-amino acid verified by mass spectrometry. One spot contained DNP-ethyl ether.

In another experiment 4 micrograms each of free acids glycine, valine, tryptophan and glutamic acid were put into solution and the DNP derivatives prepared in the usual manner. The final ether extract was evaporated over water and one fourth plated on a platinum filament and placed in the mass spectrometer. Marked fractionation with temperature was observed and each

*No doubt formed from ethyl alcohol used as a solvent in the syntheses.

derivative was clearly identifiable. The order of volatility of the DNP derivatives was valine > glycine > glutamic acid tryptophan. No impurities were detected.

The analytical procedures described above are being successfully applied in the abiotic synthesis of deuterated amino acids from gas mixtures of D_2, ND_3, and CO.

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