Tetrahedron Letters No.51, pp. 4463-4466, 1970. Pergamon Press. Printed in Great Britain.

INDIRECT MASS SPECTROMETRIC DIFFERENTIATION OF MESO AND RACEMIC DIAMINOPIMELIC ACID

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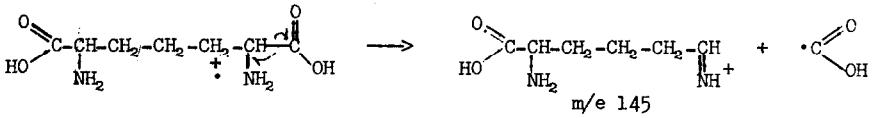
(Received in USA 29 September 1970; received in UK for publication 5 October 1970)

The mass spectra of almost all commonly occurring amino acids have been reported[1,2,3] and the decay mechanisms which lead to the observed spectra are well understood. In the process of acquiring reference spectra of all available amino acids, we also obtained the mass spectrum of an epimeric mixture of α,ε-diamino pimelic acid (DAP), an amino acid which is found in the cell wall of many bacteria.

The mass spectrum could not be interpreted by the usual "amine type" fragmentation mechanisms. It appeared that DAP would vaporize only after a cyclization process, which leads to the loss of one or two molecules of water. It seemed likely from a consideration of this spectrum that it would be possible to distinguish the meso isomer from the mixture of D,D and L,L isomers (racemate) on the basis of the thermal process, which leads to the loss of one or two molecules of H_2O. The epimeric mixture of DAP was fractionated to the pure meso compound and the racemate via their carbobenzoxy derivatives[4] and converted back to the DAP·HBr salts by treatment with glacial acetic/HBr.

Figure 1 provides a comparison of the mass spectra of the racemate hydrobromide (A) and meso DAP·HBr (B). The spectra were obtained by introducing the samples directly into the ion source of a Hatachi RMU-7 mass spectrometer and heating the samples slowly until satisfactory spectra were obtained.

If unmodified DAP were to get into the vapor phase to an appreciable extent, it could be expected in analogy to other amino acids[2,3] that the spectrum would contain an intense peak at m/e 145 arising as shown below. Ionization of either of the two nitrogens would lead to similar fragments. A peak with very low intensity can be observed at m/e 145, when DAP is heated rapidly and scanned quickly. The peak disappears on subsequent scanning.



m/e 145

If the decay of the meso isomer and of the racemate of DAP were initiated from a structure as shown above, no difference would be expected between the mass spectra of the isomers because the ion at m/e 145 has now only one center of asymmetry. However, the mass spectrum of the meso isomer of DAP shows peaks at m/e 172 and m/e 154 corresponding to the loss of one and two molecules of water, while the racemate shows predominantly an ion at m/e 154 confirming the loss of two molecules of water (Table 1).

Table 1 - High Resolution Mass Measurement of Selected Peaks of a Mixture of All Isomers of α,ε-DAP (Measured on an MS-9 Mass Spectrometer).

Calculated Mass	Error	C	H	N	O	Measured Mass
172.0847	-.88	7	12	2	3	172.0838
154.0742	.00	7	10	2	2	154.0742
127.0871	-1.29	6	11	2	1	127.0858
126.0793	+.40	6	10	2	1	126.0797
111.0683	-.53	6	9	1	1	111.0678

Both spectra change with temperature and were obtained by heating the samples slowly to a temperature at which the ratio of the peaks at 172 to 154 is a maximum for the meso isomer. Rapid heating and heating at temperatures above those required for a good spectrum produce spectra in which the racemate shows a small peak at m/e 172, while the ratio of the 172 to 154 peak of the meso compound declines somewhat. But even under these conditions the spectra can be clearly distinguished. Thermal cyclization of the ethyl esters of aspartic and glutamic acid with the loss of a molecule of ethanol[1] and the equivalent loss of H_2O from the free acids[3] has been reported previously. The loss of H_2O from dipeptides, which leads to cyclodipeptides, has been investigated in detail by Svec and Junk[5]. In analogy to these earlier reports, we are suggesting the thermal cyclization of DAP in the ion source of a mass spectrometer to account for the observed differences in the mass spectra of the meso isomer and of the racemate of DAP as shown in Figure 2.

The pyro-DAP species formed from the meso compound (1a) cannot form a second internal amide link, unless epimerization occurs at one of the α carbons, while the corresponding species 1b of the racemate can readily form a second amide link giving 2. (Figure 2). The conversion of 1a to 2 is uncertain, hence a dashed line arrow was utilized to indicate this fact.

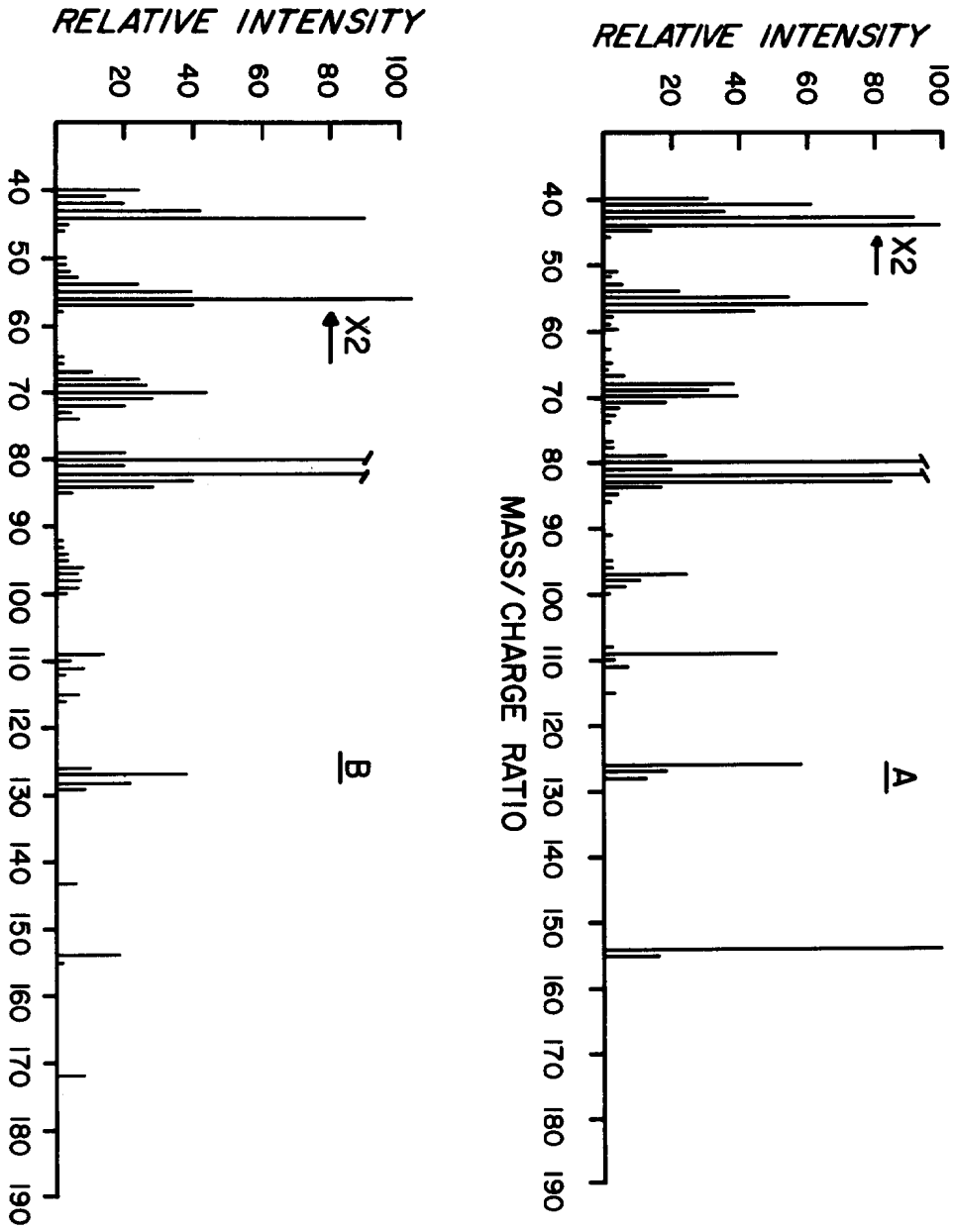


Figure 1. Mass spectra of (A) racemic DAP and (B) meso-DAP.

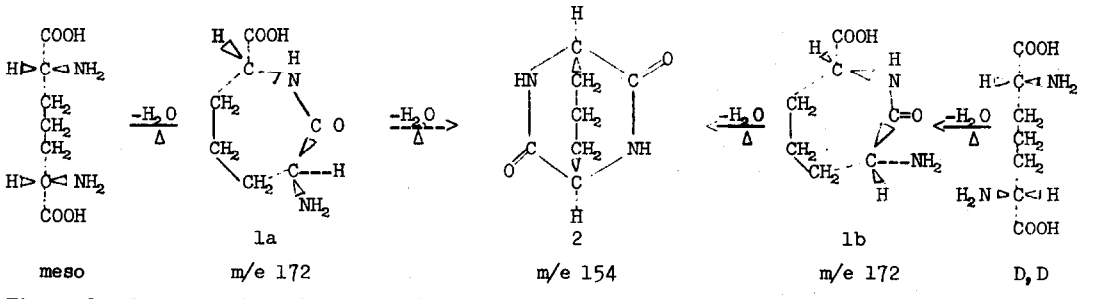


Figure 2. Proposed thermal process for DAP. L,L is not shown.

Some of the intense peaks in the spectrum of the racemate (Fig. 1A) can be interpreted quite readily by analogy to the mass spectra of cyclodipeptides and decay mechanism, which Svec and Junk formulated for these[5]. Among these are the peaks at m/e 126 and 111, which correspond to the loss of CO and HNCO from the parent ion at m/e 154. The intense peak at m/e 127 of the meso isomer corresponds to the loss of COOH from the parent ion at m/e 172.

We are currently investigating to which extent mass spectrometry can be used for the similar, but somewhat more subtle problem of distinguishing dipeptide diastereomers. The principle demonstrated in this report was first utilized to deduce the stereochemistry of a 4-hydroxymethylproline where only one isomer was available[6]. In general, however, it would be advisable to have all of the critical isomers since we unexpectedly did obtain an m/e 154 from the meso-DAP. It could be anticipated that differences will be relative rather than absolute when mass spectra of diastereomers of most compounds are compared.

Acknowledgment. This work was supported by grants from the American Cancer Society (P-343), National Science Foundation (GP 8490), and USPHS (ES-00159). HF was a trainee of a Biomedical Grant (PHS FR7075) and RAD is a Career Development Award Grantee (1-K4-GM-7654). We thank Mr. Lee Harkness for providing us with access to the MS-9 mass spectrometer at Argonne National Laboratory.

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