COMMUNICATIONS

Mass Spectra of Some Cyclic Dipeptides (2,5-Diketopiperazines)

A mechanism is proposed for the electron-impact induced fragmentations of cyclic dipeptides (2,5-diketopiperazines) with side chains larger than methyl. The mechanism is illustrated with the mass spectrum of *cyclo*-(Ile-Val).

It is known that the predominant peak in the mass spectra of most amino acids and their ethyl esters is the so-called amine peak: $(RCH{=}NH_2)^+$ (Biemann et al., 1961; Junk and Svec, 1963a). The presence of another group in the molecule (e.g., heteroatom, carboxylic group, aromatic ring) increases the tendency for cleavage of other bonds either in the original molecule or in the amino fragment, both leading to a lower abundance of the latter (Biemann et al., 1961).

Junk and Svec (1963b) have reported on the use of mass spectrometry in the sequential analysis of underivatized dipeptides. They utilized the amine peak which is characteristic of the N-terminal amino acid and the molecular ion in their analysis. Later, they published the mass spectra of the symmetrical cyclic dipeptides (Gly-Gly) and (Ala-Ala) (Svec and Junk, 1964). Here also, the dominant peak was the amine peak, but other important peaks occurred from the elimination of CO and HNCO. The same was found for *cyclo*-(Ala-Gly) by Heyns and Grutzmacher (1963b). Svec and Junk (1964) predict these characteristic peaks for several cyclic dipeptides and in the case of the cyclic dipeptide (Leu-Val) predicted that m/e 169 and 184 would be the fragments formed by the loss of HNCO and CO, respectively.

Heintz and Grutzmacher (1963b) have shown that the introduction of linear dipeptides into the mass spectrometer yields 2,5-diketopiperazines via thermal cyclization. Therefore, the fragmentation patterns of 2,5-diketopiperazines have particular importance in the complete interpretation of mass spectra of dipeptides. In this communication, we report a proposed mechanism for the fragmentation pattern of 2,5-diketopiperazines with side chains larger than methyl. The mechanism is illustrated with the mass spectrum of *cyclo*-(Ile-Val).

MATERIALS AND METHODS

Mass Spectrometry. Mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6L in the single focusing mode. Samples were introduced by means of the direct introduction probe with the source temperature at 210 °C. Spectra were obtained at 80 eV.

Sample Purification. The *cyclo*-(Ile-Val) was isolated from acid-hydrolyzed soy protein by liquid–liquid extraction with 1-butanol as described by Dakin (1918). The extracted material was recrystallized twice from 95% ethanol and once from 50% acetic acid. The identity of the compound was confirmed by comparison of its infrared spectrum with the spectrum published by Obata and Mizutani (1959), comparison of its NMR spectrum with Sadtler NMR spectra 13611M, 15096M, and 16538M (Nuclear Magnetic Resonance Spectra, 1972) and by gas chromatography (Adams, 1974) of the constituent amino acids after hydrolysis for 70 h with 6 N HCl at 110 °C.

RESULTS AND DISCUSSION

We have found three major peaks in the mass spectrum of the cyclic dipeptide (Ile-Val), m/e 170, 156, and 113 (Figure 1). This can only be explained by a cyclic elimination of the side chains preceding the elimination of HNCO. This is substantiated by the fact that the mass spectrum obtained at 10 eV contains only two peaks, m/e 156 and 170.

In the proposed mechanism (Figure 2), the 4-hydrogen atom is transferred through a sterically favorable six-membered ring transition state (McLafferty rearrangement). In contrast to the usual sequential cleavage of the 2,3 carbon–carbon bond, it is suggested that this cleavage is occurring simultaneously with the McLafferty rearrangement leading to the loss of an olefin. The resonance stabilization of the production is part of the driving force in the cyclic elimination of the side chain.

This cyclic rearrangement is known to occur with amino acids if there is a hydrogen atom in the 4 position to the carbonyl group (Heyns and Grutzmacher, 1963a). Since this reaction is favored by the number of hydrogen atoms in the 4 position and the stability of the neutral, unsaturated fragment, it follows that valine, isoleucine, and threonine are specially reactive. Junk and Svec (1963a) found this rearrangement to occur for the amino acids isoleucine and valine and to an insignificant degree for leucine, norleucine, norvaline, and α-aminobutyric acid. The degree of reactivity of cyclic dipeptides is determined by the constituent amino acids. That is, *cyclo*-(Ile-Val) is highly reactive whereas *cyclo*-(Leu-Leu) is relatively unreactive in forming the cyclic transition state. The above rearrangement does not take place with amino acid ethyl esters (Heyns and Grutzmacher, 1963a). Because the cyclic elimination of the side chains in *cyclo*-(Ile-Val) can occur with isoleucine and the valine residues alone or together before the ring opens, the fragments corresponding to the mass spectra of *cyclo*-(Gly-Val), *cyclo*-(Ile-Gly), and *cyclo*-(Gly-Gly) are also present in the mass spectrum of *cyclo*-(Ile-Val). Several metastable ions appear in the mass spectrum of *cyclo*-(Ile-Val), m/e 81.9, 76.5, 75.1, and 64. For the transition, $m_1 \rightarrow m_2$, $m^* = m_2^2/m_1$. The ion m/e 76.5 which arises from the 56 loss from the m/e 170 ion ($114^2/170 = 76.45$) confirms the cyclic elimination scheme of Figure 2. The ion m/e 75.1 indicates that simple cleavage of the side chain occurs in addition to the cyclic elimination, $170 \rightarrow 113$ ($113^2/170 = 75.11$). The m/e 113 in this case is a cyclic ion and different from the m/e 113 ion arising as a result of HNCO loss from m/e 156. This

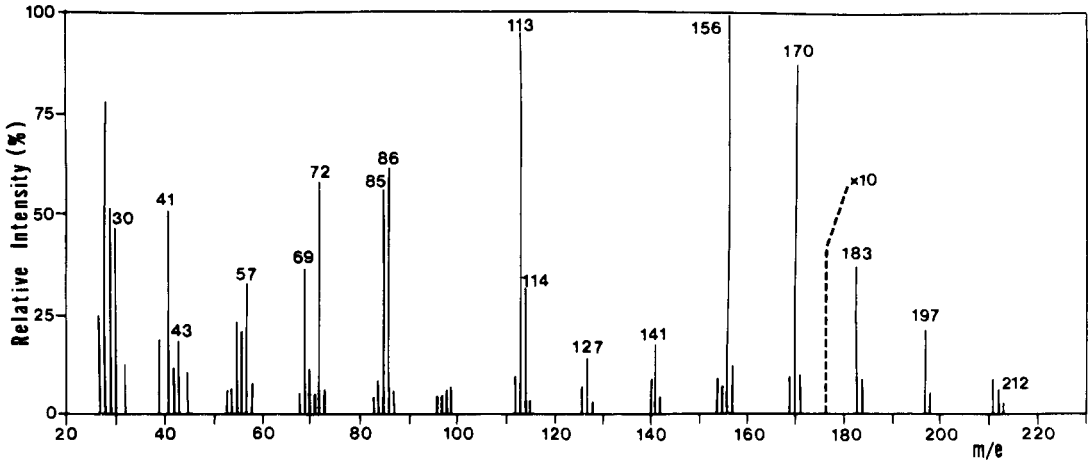

Figure 1. Mass spectrum of *cyclo*-(Ile-Val); mol wt 212.

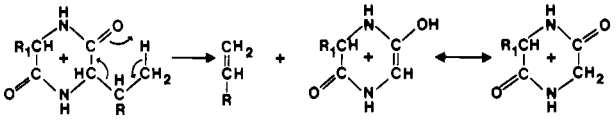

Figure 2. Suggested mechanism of cyclic elimination; R = CH_3-Val, R = C_2H_5-Ile, R_1 = amino acid residue.

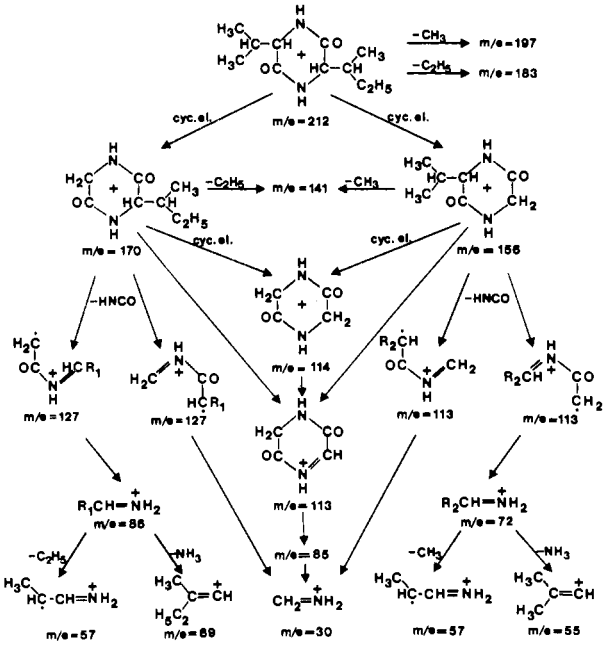

Figure 3. Proposed reaction scheme for the formation of the major ions from *cyclo*-(Ile-Val); R_1 = 2-butyl, R_2 = 2-propyl.

transition is verified by the ion m/e 81.9 ($113^2/156$ = 81.85). The 43 loss from m/e 156 in this case could also be a loss of 2-propyl leaving the cyclic ion m/e 113 as the result. Finally, the metastable ion m/e 64 verifies a loss of 28 (CO) from m/e 113 ($85^2/113$ = 63.94), which in this case must be the cyclic ion. The proposed reactions are summarized in Figure 3. This fragmentation mechanism of *cyclo*-(Ile-Val) can be employed in the identification of other cyclic dipeptides containing residues capable of splitting off side chains by cyclic elimination.

In fact, the cyclic elimination (Figure 2) as a major reaction occurring in the mass spectral fragmentation of these cyclic dipeptides has also been found to occur for *cyclo*-(Val-Val), *cyclo*-(Ile-Ile), and *cyclo*-(Val-Phe).

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