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MASS-SPECTROMETER INVESTIGATION OF 3, 6-DIALKYL-2, 5-DIKETOPIPERAZINES

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Analysis of mass-spectra of various 2, 5-diketopiperazines gives the basic fragmentation rules for their molecular ions, and the effects of nature and positions of substituents on the process.

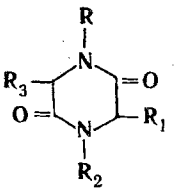
As was previously shown [1, 2], one of the modes of fragmentation of cyclodepsipeptide ions under mass-spectrometric conditions is formation of ions of 3, 6-dialkyl-2, 5-diketomorpholines (morpholine mode of fragmentation). In this connection, investigation of model 3, 6-dialkyl-2, 5-diketomorpholines [3] made it possible to identify uniquely, fragments of morpholine origin in mass-spectra of cyclodepsipeptides of various ring size [1, 2, 4]. In mass-spectrometer study of some cyclohexadepsipeptides of non-regular structure [2], not only were there fragments corresponding to splitting of ions of the appropriate 2, 5-diketomorpholines, but also fragments which might be ascribed to formation and subsequent splitting of ions of 3, 6-dialkyl-2, 5-diketopiperazines.

Table 1

Compound	R	R_1	R_2	R_3
Ia	H	H	CH_3	H
Ib	H	CH_3	H	Me
Ic	H	H	H	i-C_3H_7
Id	H	i-C_3H_7	H	i-C_3H_7
Id'	H	$(CD_3)_2CH$	H	$(CD_3)_2CH$
Ie	H	i-C_3H_7	H	i-C_4H_9
Ie'	D	i-C_3H_7	D	i-C_4H_9
If	H	i-C_4H_9	H	i-C_4H_9
Ig	H	i-C_3H_7	CH_3	i-C_4H_9
Ig'	D	i-C_3H_7	CH_3	i-C_4H_9
Ih	CH_3	i-C_3H_7	CH_3	i-C_4H_9
Ii	H	i-C_3H_7	H	$CH_2C_6H_5$
Ij	H	CH_2OH	H	CH_2OH
Ij'	D	CH_2OD	D	CH_2OD
Ik	H	H	H	$CH_2C_6H_5$
Il	H	CH_3	H	$CH_2C_6H_5$
Im	H	$CH_2C_6H_5$	H	$CH_2C_6H_5$
IIa	H	$-(CH_2)_3-$		H
IIa'	D	$-(CH_2)_3-$		H
IIb	H	$-(CH_2)_3-$		i-C_4H_9
IIc	H	$-(CH_2)_3-$		$CH_2C_6H_5$
IIc'	D	$-(CH_2)_3-$		$CH_2C_6H_5$

To establish the nature of these fragments, systematic study of the mass-spectra of a number of 3, 6-dialkyl-2, 5-diketopiperazines was undertaken. The literature contains only brief references to the fragmentation of the simplest 2, 5-diketopiperazines, in connection with mass-spectrometer researches on linear dipeptides [5] and their acetyl derivatives [6].

The present work considers the mode of fragmentation of 2, 5-diketopiperazines (I and II), built up from amino acid residues of the L series (Table 1).



Our results show that fragmentation of diketopiperazines taking place under electron impact, depends substantially on the natures of substituents R_1 and R_3, but is practically independent of the presence of substituents at the nitrogen atoms. Basically, two ways of splitting can be observed: The first is accompanied by splitting of the heterocyclic ring (mode A), and the second begins with splitting off of a substituent from a ring carbon atom in the form of an olefin (mode B), or as a radical (mode B').

Mode A predominates with 2, 5-diketopiperazines completely lacking in substituents at carbon atoms (Ia), or containing C-methyl groups (Ib). It is accompanied by elimination of HN=C=O or CH_3N=C=O as ions, (Φ_1, Φ'_1) or neutral particles.*** In the latter case, linear ion-radicals (Φ_3, Φ'_3) are formed, further scission of which gives rise to ketene (Φ_4, Φ'_4) or amine (Φ_5, Φ'_5) fragments (see Chart 1 and Table 2).

The second mode of decomposition of a molecular ion Ia, b is elimination of a CO group. In the case of compound Ia, the resultant 5-membered ring cyclic ion radical (Φ_2) can vary in structure, depending on whether the CO group is eliminated from position 2 or from position 5. However, the contribution of this mode of fragmentation to the mass-spectra of compounds Ia and Ib is small. This mode of fragmentation does not obtain in the mass spectra of the rest of

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***This type of fragmentation of diketopiperazines is analogous to the COX type fragmentation of cyclodepsipeptides which we previously described [2].

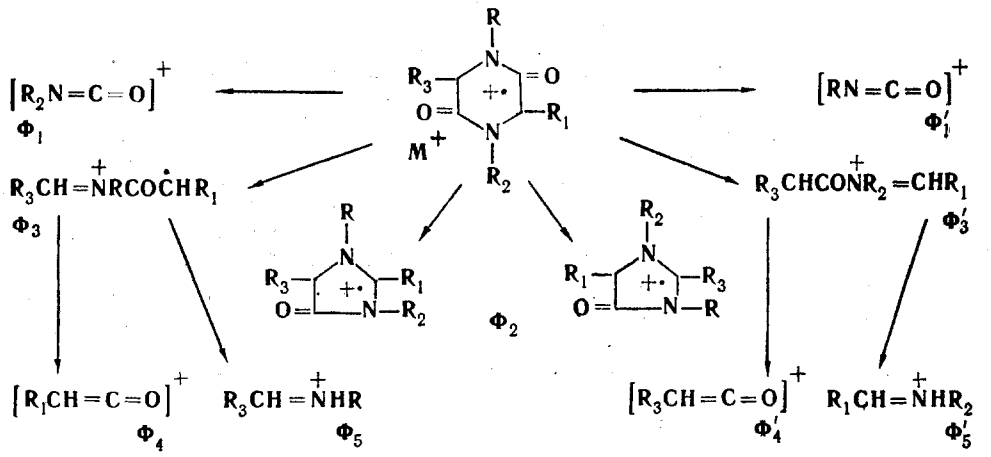
Table 2

m/e Values and Relative Intensities of Fragments

Compound	M^+	Φ_1 Φ'_1	Φ_2	Φ_3 Φ'_3	Φ_4 Φ'_4	Φ_5 Φ'_5
Ia	128 (100)	57 43 (16) (40)	100 (8)	78 85 (50) (8)	42 (30)	30 44 (20) (30)
Ib	142 (13)	43 (10)	114 (4)	99 (66)	56 (13)	44 (100)
Ic	156 (0.4)	43 (8)		113 (13)	84 42 (1) (8)	30 72 (2) (4)
Id	198 (1.2)	43 (4)	170 (4)	155 (9)	84 (4)	72 (44)
Id'	210 (1.5)	43 (3)		167 (6)	90 (2)	78 (36)
Ie	212 (0.4)	43 (9)	184 (0.2)	169 (10)	84 98 (3) (11)	86 72 (32) (43)
Ie'	214 (0.7)	44 (7)		170 (12)	84 98 (7.5) (6)	87 73 (28) (22)
If	226 (5.7)	43 (6)		183 (9)	98 (22)	86 (35)
Ig	226 (4)	57 43 (9) (4)		183 169 (6) (22)	84 98 (4) (5)	100 72 (17) (20)
Ig'	227 (0.5)	57 44 (11) (27)		183 170 (4) (100)	84 98 (10) (14)	100 73 (9) (5)
Ih	240 (0.3)	57 (6)		183 (4)	84 98 (6) (3)	100 86 (36) (11)
Ii	246 (88)	43 (5)		203 (6)	84 132 (2) (4)	120 72 (10) (14)
Ij		43 (32)		131 —	72 (4)	60 (36)
Ij'		44 (22)		134 (3)	73 (2)	62 (8)
Ik	204 (30)	43 (1)		161 (2)	132 (1)	30 120 (6) (1)
Il	218 (47)	43 —		175 (2.5)	56 132 (1) (2)	120 44 (5) (62)
Im	294 (100)	43 (1)		251 (1)	132 (3)	120 (12)
IIa	154 (75)	43 (2)	126 (9)	111 (100)	42 (7)	70 (33)
IIa'	155 (47)	44 (39)	127 (8)	111 (100)	42 (12)	71 (4)
IIb	210 (0.3)	43 (10)		167 (5)	98 (4)	70 (65)
IIc	244 (4)	43 (1)		201 (3)	132 (3)	70 (43)
IIc'	245 (5.5)	44 (1)		201 (3.5)	132 (0.8)	71 (89)

the diketopiperazines which we have investigated, except for Id, Ie, and IIa, where th Φ_2 ion peak is of low intensity (see Table 2).

Chart 1



The character of the fragmentation of the molecular ion is sharply altered by introducing more complex substituents into the 2,5-diketopiperazine molecule. Although all the compounds which we have studied show mass spectrum peaks due to fragments due to mode A, their intensities are small, and, in many cases, they are resolvable (see Tables 2 and 3).

Chart 2

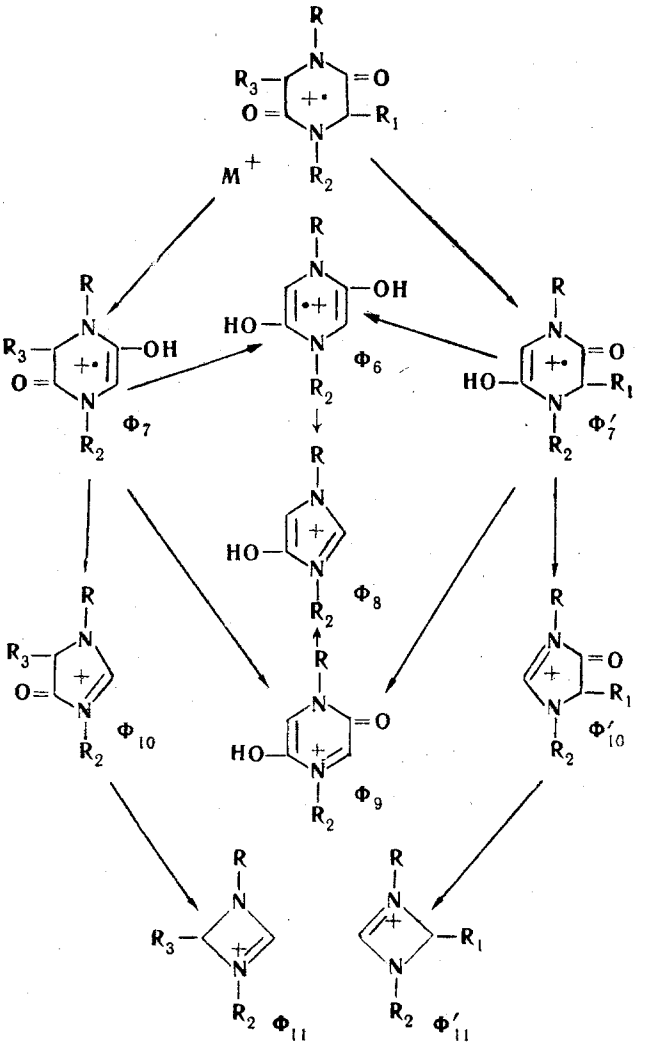
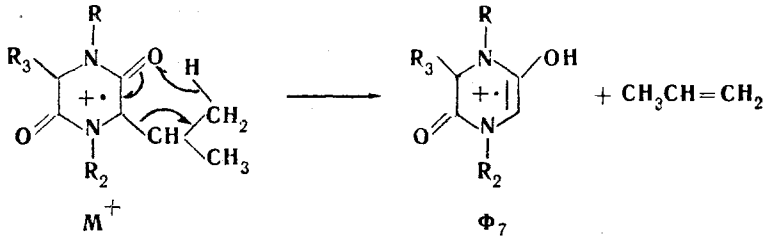


Table 3

m/e and Relative Intensity for Fragments

Compound	M^+	Φ_6	Φ_7	Φ'_7	Φ_8	Φ_9	Φ_{10}	Φ'_{10}	Φ_{11}	Φ'_{11}	Φ_{12}	Φ'_{12}	Φ_{13}	Φ'_{13}
Ic	156 (0.4)		114 (100)					85 (21)		57 (16)	43 (cf Φ_1)			113 (cf Φ_3)
Id	198 (1.2)	114 (8)	156 (100)		85 (11)	113 (51)	127 (22)			99 (7)	43 (cf Φ_1)		155 (cf Φ_3)	
Id'	210 (1.5)	116 (11)	163 (100)		86 (13)	114 (64)	133 (17)			105 (6)	49 (2)		161 (9)	
Ie	212 (0.4)	114 (22)	170 (34)	156 (100)	85 (11)	113 (42)	141 (17)	127 (10)	113 (cf Φ_9)	99 (4)	43 (cf Φ_1)	57 (7)	169 (cf Φ_3)	155 (1)
Ie'	214 (0.7)	116 (16)	172 (38)	158 (100)	87 (cf Φ_5)	115 (36)	143 (16)	129 (7)	115 (cf Φ_9)	101 (3)	43 (21)	57 (20)	171 (4)	157 (61)
If	226 (5.7)	114 (9)	170 (100)		85 (1)	113 (17)	141 (12)		113 (cm. Φ_9)	57 (3)			169 (2)	
Ig	226 (4)	128 (2)	184 (32)	170 (100)	99 (18)	127 (33)	155 (7)	141 (42)	127 (cf. Φ_9)	113 (5)	43 (cf Φ'_1)	57 (cf Φ_1)	183 (cf Φ_3)	169 (cf Φ'_3)
Ig'	227 (0.5)	129 (9)	185 (16)	171 (55)	100 (cf Φ_5)	128 (22)	156 (3)	142 (15)	128 (cf Φ_9)	114 (2)	43 (81)	57 (cf Φ_1)	184 (28)	170 (cf. Φ'_3)
Ih	240 (0.3)	142 (15)	198 (12)	184 (70)	113 (57)	141 (cf Φ_{11})	169 (36)	155 (11)	141 (100)	127 (4)	43 (8)	57 (cf Φ_1)	197 (18)	183 (cf Φ_3)
Ii	246 (88)		204 (21)		85 (20)	113 (21)	175 (4)	127 (94)	147 (2)	99 (20)	43 (cf Φ_1)	91 (100)	203 (cf Φ_3)	155 (50)
Ij		114 (46)	144 (100)		85 (54)	113 (54)	115 (8)				31 (8)			
Ij'		118 (14)	148 (100)		88 (13)	116 (57)	118 (14)				32 (2)			
Ik	204 (30)							85 (9)		57 (1)		91 (100)		113 (6)
Il	218 (47)						175 (2)	99 (22)	147 (1)	71 (4)		91 (100)		127 (55)
Im	294 (100)						175 (60)			147 (6)	91 (55)		203 (88)	
IIb	210 (0.3)			154 (100)				125 (8)		97 (2)		57 (1)		97 (cf Φ'_{11})
IIc	244 (4)			154 (4)				125 (100)		97 (2)		91 (50)		153 (60)
IIc'	245 (5.5)			155 (11)				126 (100)		98 (1)		91 (98)		154 (48)

With the aliphatic substituents (i–C_3H_7, i–C_4H_9), mode B splitting (Chart 2) predominates. Olefin splits off, and an ion-radical of enol-ketone structure (Φ_7, Φ'_7) is formed through elimination of R_1 or R_3 with simultaneous migration of a hydrogen atom from the carbon atom of a side chain to oxygen of a carbonyl group.



The difference between the mass spectrum of Id, starting with the ion peak $(M-42)^+$, and that of the mass spectrum of Ic, with a diketone structure (Fig. 1), is indication of enol structure of fragments Φ_7, Φ'_7 (in the case of the diketo structure of fragment Φ_7, these spectra should have been similar). A demonstration of the correctness of the splitting mechanism put forward is displacement, by 2 and 7 mass units, respectively, of the peaks of the Φ_6 and Φ_7 ions in the spectrum of the dodecadeutero analog (Id'). Another confirmation was obtained in the case of compound Ij, where Φ_7 is formed by splitting off of CH_2O, with migration of the hydrogen atom of the hydroxyl group (also at the β position of the side chain) to the oxygen of a carbonyl group. In this case, determination of the mass spectrum of the deutero analog Ij' showed that the mass number of the fragment Φ_7 is displaced by 4 mass units, like the molecular ion too.

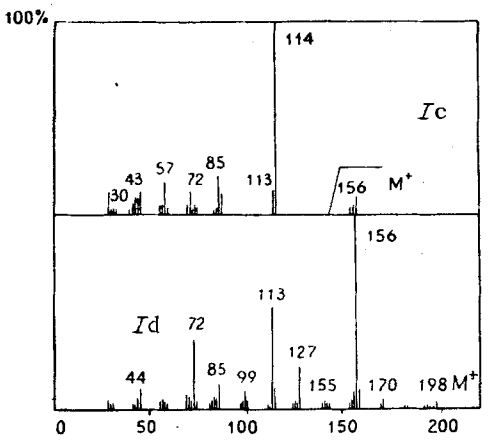


Fig. 1. Mass-spectra of 3-isopropyl-2,5-diketopiperazine (Ic), and 3, 6-diisopropyl-2,5-diketopiperazine (Id).

In the case of the mono-substituted diketopiperazine Ic, further splitting of fragment Φ_7 is accompanied by ejection of the HCO group Φ_{10}, and then of the group CO Φ_{11}. With substituted diketopiperazines containing various alkyl groups (Ie, Ig, Ih) isobutene is primarily eliminated, which is indicated by the considerably greater intensity of fragment Φ'_7 as compared with fragment Φ_7 (see Table 3). Obviously, this is due to the greater reactivity of the tertiary β hydrogen atom in the isobutene residue as compared with the primary β hydrogen atom in the isopropyl residue.

Apart from the mode described above, $\Phi_7 \rightarrow \Phi_{10} \rightarrow \Phi_{11}$ (and correspondingly $\Phi'_7 \rightarrow \Phi'_{10} \rightarrow \Phi'_{11}$), further splitting of the disubstituted diketopiperazines Id–e, is accompanied by splitting off of the second substituent either as olefin, by the mechanism described above (formation of fragment Φ_6) with subsequent loss of the group Φ_8, or as the radical Φ_9 followed by loss of CO Φ_8 (see Chart 2). Ejection of the HCO group by Φ_6 and Φ_7 fragments was confirmed by the spectrum of the deutero analog Id' (ejection of 30 mass units instead of 29).

Further fragmentation of the ion radical Φ_7 proceeds in a basically similar manner. Φ_7 arises through splitting off of the CH_2O group from the molecular ion Ij. However, here there is an additional mode, comprising loss of a molecule of water by the ion-radical Φ_7, to give a fragment with m/e 126 (relative intensity 27%), also followed by loss of a CHO group (fragment with m/e 97).

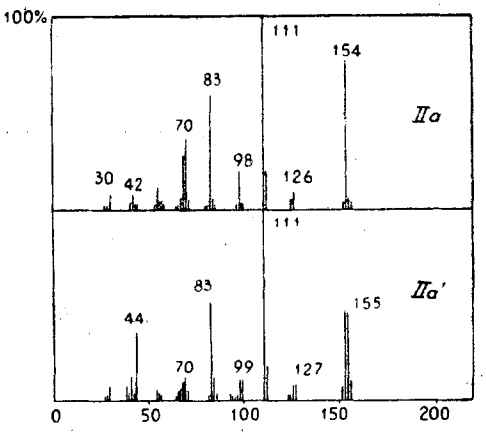
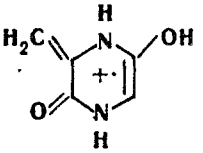


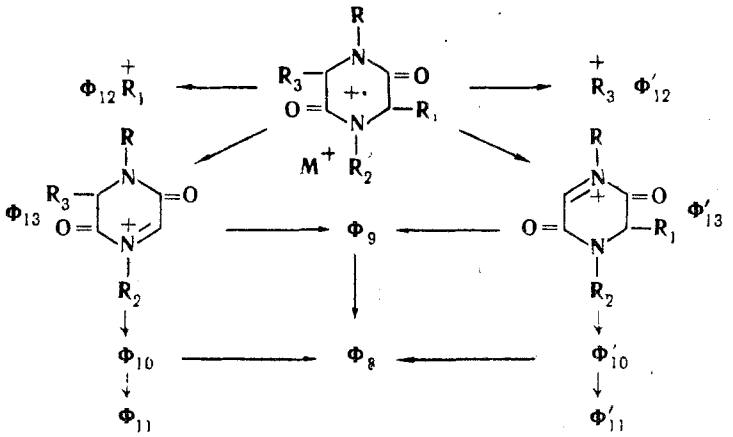
Fig. 2. Mass-spectra of 3,4-trimethylene-2,5-diketopiperazine (IIa) and its 1-deutero analog (IIa').



The correctness of the envisaged mode of decomposition of the molecular ion Ij was confirmed by determining the mass spectrum of its deutero analog Ij'.

Less characteristic is elimination by the molecular ion of 2, 5-diketopiperazines, containing aliphatic substituents, of a side chain as a radical (mode B', Chart 3), to give the ions Φ_{13}, Φ'_{13}. Generally, the contribution of these fragments to the mass-spectra of the compounds enumerated is small, and the peaks due to them are apparently compound.

Chart 3

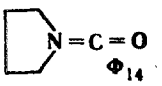


However, this mode of decomposition becomes absolutely predominant when the 2,5-diketopiperazine contains even one benzyl substituent. Then, as a rule, the maximum peak corresponds to the tropylium ion (Φ_{12}, m/e 91), but peaks Φ_{13}, Φ'_{13} usually have considerable intensity. Further decomposition of the ions Φ_{13}, Φ'_{13} is accompanied by subsequent ejection of the CO group, with formation of fragments Φ_{10}, Φ'_{10} and Φ_{11}, Φ'_{11} (Chart 3).

The modes of fragmentation of 2,5-diketopiperazines shown in Charts 1-3 were confirmed by investigating the mass-spectra of a number of deutero analogs (Id', Ie', Ig', Ij').

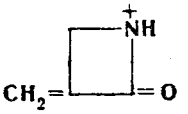
Fragments having m/e 44 (or correspondingly 58) present in the mass spectra of almost all the type I compounds studied, evidently have a structure $H_2\overset{+}{N}=C=O$ (or $CH_3\overset{+}{N}H=C=O$). These ions can be formed by various routes, so the latter are now shown in Charts 1-3.

Basically, fragmentation of diketopiperazines containing proline units (IIa-c) proceeds analogously to those described above (cf. Tables 2 and 3). One-stage ejection of the neutral particle HNCO and formation of fragment Φ'_3 are confirmed by the mass spectrum of IIa having a metastable peak m/e 80.3 (calculated 80.1). However, the presence of a bicyclic system in IIa-c also superimposes some specific peculiarities on their mode of fragmentation. Thus, in addition to the fragment Φ_1, the mass-spectra of IIa,b have a m/e 98 (Φ_{14}) ion peak.

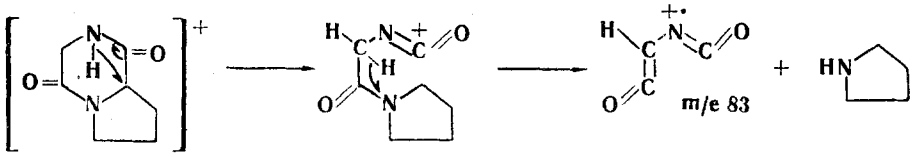


Its formation from a molecular ion in one stage by homolytic breaking of C_2-C_3 and C_5-C_6 bonds, followed by migration of a hydrogen atom from the N_1 atom to C_3 in the piperazine ring, is confirmed by the mass-spectrum of IIa having a metastable peak m/e 62.2 (calculated 62.3). The structure of the Φ_{14} ion is also confirmed by displacement by 1 mass unit of the fragment peak m/e 98 on switching to the deutero analog IIa' (see Fig. 2).

The spectrum of Ia shows an intense peak m/e 83, ascribed [5] to the cyclic structure



The absence of shift of this peak in the mass spectrum of the deutero analog IIa', indicates that this fragment does not contain the NH group (see Fig. 2). For that reason, we assume that it is linear, and that its formation can be explained by elimination of a molecule of pyrrolidine from the molecular ion:



The mass spectrum of IIb has an analogous fragment m/e 139, though its intensity is negligible.

As in the case of type I compounds, the presence of an aliphatic substituent at position 5 in type II molecules conditions decomposition of the molecular ion by mode B, while introduction of a benzyl group brings about mode B' (see Table 3). Furthermore, the mass-spectra of IIa-c have a m/e 69 fragment which is not displaced in the spectra of the deutero analogs, when it can be ascribed a Δ^1-pyrroline structure.

As can be seen from what has been said, analysis of the mass-spectra of the various diketopiperazines made it possible to establish basic rules regarding fragmentation from molecular ions and to elucidate the effects of nature and position of substituents on this process, thus making it possible to obtain sufficiently complete information regarding the structures of amino acid units from which diketopiperazine molecules are built up.

Experimental

Synthesis of the Deutero-Analogs

Ie'. Prepared similarly to Ig [7], starting from the Me ester of hexadeuterovaline, which was in its turn synthesized from hexadeuteroisopropyl bromide and N-acetylaminomalonic ester [8].

Ig'. 0.05 g Ig was dissolved in 0.5 ml AcOD (97% enriched), heated for 25 hr at 50° C, vacuum-evaporated to dryness, and the operation repeated twice more. IR spectrum data showed that the deuterium content of the Ig' was 82%.

Ie', IIa', IIc'. 0.03 g Ie and 0.2 ml EtOD were heated together in a sealed tube for 7 hr at 90° C, the products vacuum-evaporated to dryness and the operation repeated twice more. The enrichment in deuterium reached 80%. IIa' and IIc' were prepared similarly.

Ij'. 0.05 g Ij in 1 ml D_2O was heated for 10 hr at 90° C and pH 7, the products vacuum-evaporated to dryness, and the operation repeated twice more; the Ij' was 78% deuterium-enriched.

Mass spectra were determined with a MKh-1303 series instrument, using a feed system at 200-240° C, ionizing voltage 25-30 electron volts, (Ia-Ii, IIa-c, IIa'), or by the same instrument with direct introduction of the sample into the ion source, at 125° C and 60-70 eV (Ie, IIc), or 190° C and 30 eV (Ij, Ij'). To reduce reverse deuterium exchange, the instrument was first washed out with D_2O, then the deuterium analog taken.

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