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AMINOACYL INCORPORATION INTO LINEAR AND CYCLIC PEPTIDES

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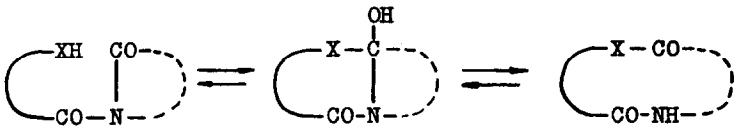
IT was shown earlier in our Institute (1-5) that linear and cyclic N-hydroxyacylamides (I, X=O), depending upon their structure, are capable of reversible or irreversible isomerization to cyclols (II, X=O) and further to cyclodepsipeptides (III, X=O).



(I) (II) (III)

X=O or NH

This new reaction, called hydroxyacyl incorporation into the amide group, served as the basis for a method we have developed for synthesizing linear and cyclic depsipeptides containing amino and hydroxy acid residues (4,6,7).

The next stage of our studies in this field was devoted to an investigation of the intramolecular isomerization

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of N-aminoacylamides, in order to see whether analogous incorporation of amino acid residues into peptides (I→II→III, X=NH) would occur. The first examples we took were N-acylphthalyldipeptides, which we prepared by acylation of phthalyldipeptide esters by azidoacyl chlorides. The latter are highly convenient forms of activated N-protected amino acids, being relatively stable under acylating conditions (refluxing in toluene, 12 hrs.) and sufficiently active as acylating agents. For instance, acylation of ethyl phthalyldiglycinate (IV) by azidoacetyl chloride yielded the corresponding N-azidoacetyl derivative (V) (Table 1), which by the action of HBr in glacial acetic acid was converted into the hydrobromide of the N-aminoacyldipeptide ester (VI). The latter was then treated with DEAE-cellulose or Ag_2O in aqueous alcohol solution to give phthalyltriglycine ethyl ester (VII). The structure of this compound was confirmed by comparison with a specimen obtained by counter synthesis.

$$\underset{\text{(IV)}}{\text{PhthNCH}_2\text{CONHCH}_2\text{COOEt}} \longrightarrow \underset{\text{(V)}}{\text{PhthNCH}_2\text{CON(COCH}_2\text{N}_3\text{)CH}_2\text{COOEt}} \longrightarrow$$

$$\longrightarrow \underset{\text{(VI)}}{\text{PhthNCH}_2\text{CON(COCH}_2\text{NH}_2\cdot\text{HBr)CH}_2\text{COOEt}} \longrightarrow \underset{\text{(VII)}}{\text{PhthNCH}_2\text{CONHCH}_2\text{CONHCH}_2\text{COOEt}}$$

We investigated the incorporation of amino acid residues into cyclopeptides on the example of N-β-aminoacyllactams. It is known (8,9) that N-α-aminoacyllactams very easily pass over to the corresponding dicyclic amidines (XI, n=1),

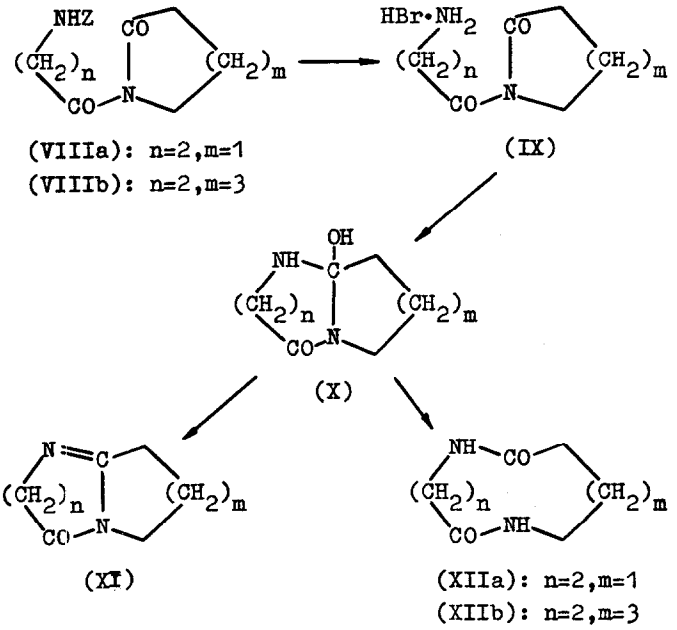
TABLE 1

Constants and Characteristic I.R. Frequencies[a]

Compound	M.p. °C	Band position (cm^{-1})							Integral intensity[b] $l \cdot mole^{-1} \cdot cm^{-2}$
		-CONCO-	CO Ester	CO Urethane	AmideI	AmideII	NH	N_3	
V	115-116	1780, 1720[c]	1750					2100	
VII	228-229	1780,1710	1735		1650	1567	3300,3100		
VIIIa	94-95	1740,1715		1680		1535	3380		
VIIIb	60-61	1720		1690		1545	3340		
XIIa[d]	174				1)1660 2)1650 3)1635	1570	3200,3080		$10,8 \cdot 10^4$
XIIb[d]	258-259				1)1650 4)1650	1560	3300,3090		$8,2 \cdot 10^4$

[a] The analytical and molecular weight data of the compounds given in the table correspond to those calculated. [b] In EtOD; For 1-Oxo-5-azacyclodecan-4,10-dione containing a single amide bond, the value is $4,2 \cdot 10^4$. [c] The CONCO bands of the phthalyl and acylamide group are superposed. [d] 1) In nujol, 2) in EtOH, 3) in EtOD, 4) deuterated compound in nujol.

which may be regarded as dehydration products of intermediately formed azacyclols (X, n=1).+ However, by analogy with the behavior of N-hydroxyacyllactams (2,4,5) we expected the unstable azacyclols of type (X, n=2) to decompose also by different route, with the formation of cyclopeptides (XII).



Indeed, we synthesized the cyclopeptides (XII) as follows. Acylation of pyrrolidone or of caprolactam by N-carbobenzoxy-β-alanyl chloride afforded the acyl derivatives (VIIIa) and (VIIIb). Subsequent removal of the benzyloxycarbonyl group by HBr in AcOH and then treatment of the resultant hydrobromides (IX) by Ag_2O in aqueous solution or hydrogenolysis of initial acyl derivatives in EtOH led

+ The only report of the isolation of azacyclols as individual compounds (10) was later refuted (11).

to isolation of the expected cyclopeptides (XIIa) and (XIIb). The compounds do not possess free amino or carboxyl groups. Analytical data and molecular weight determination (cryoscopy in water) are in agreement with their molecular formula. In contrast with I.R. spectra of N-acyllactams, the I.R. spectra of these compound exhibited no CONCO bands (in the region of 1720 cm^{-1}), but did show the amide I and amide II frequencies (for the compound (XIIa) the latter band is observed only in solution, cf. (2)). The amide II band (in the region of 1560 cm^{-1}) disappears on deuteration of compounds (XIIa) and (XIIb). Finally, intensity measurements of the amide carbonyl showed two amide groups to be present. All these data give sufficient reliability to the conclusion that compounds (XIIa) and (XIIb) are cyclodipeptides.

Finally, the readiness of dehydration of type (XII) cyclodipeptides, displayed during their mass spectrometry[+] should be mentioned. The main fragmentation pathway in both cases (XIIa and XIIb) is elimination of water according to the Scheme (XII)→(X)→(XI), subsequent degradation being identical with that of amidines of type (XI). The tendency of the 9-membered cyclopeptide (XIIa) and 11-membered cyclopeptide (XIIb) to cyclolize and then dehydrate is similar to the behavior under mass spectrometric conditions of the 10-membered cyclodepsipeptide we studied earlier (12).

Our data show that the aminoacyl residue is readily incorporated into the activated amide bond. Hence a new me-

[+] The mass spectra were studied in our Institute by N.S. Wulfson and V.A. Puchkov. Details will be published in a separate communication.

thod has been discovered for the synthesis of some linear and cyclic peptides which have so far been difficult to prepare by the usual methods of peptide synthesis. It should be mentioned that aminoacyl incorporation into the peptide chain or ring may be one of the ways for the biogenesis of a number of naturally occurring peptides, and also a way for the transformation of peptides and proteins in the organism.

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