AMINO ACIDS AND PEPTIDES. CXVI.*

CONFORMATIONAL FACTORS IN THE ISOMERISATION OF CYCLODIPEPTIDES

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Configurational inversion in alkaline media has been studied with diastereoisomers of 3,6-di-substituted bi- and tricyclic 2,5-piperazinediones (cyclodipeptides containing residues of 2-azetidinecarboxylic acid, proline, and pipecolic acid). The course of the configurational change was shown to be controlled by conformational factors due to the size of the anneled ring.

The molecules of some naturally occurring substances, *e.g.*, the ergotamine and ergotoxine groups of ergot alkaloids, comprise the 2,5-piperazinedione moiety containing a proline residue. After the thermal decomposition of these alkaloids, 2,5-piperazinediones (cyclodipeptides) containing D-proline residue were isolated[1−3]. In the original material, however, both the amino-acid residues which constitute the 2,5-piperazinedione ring, are of the L-configuration, as shown by synthesis of the corresponding alkaloids. The inversion of the absolute configuration at the α-carbon atom of the proline residue in the course of the degradation has been explained by asymmetric induction due to the vicinal cyclol system[4]. In the literature, the conversion of cyclo(L-prolyl-L-phenylalanyl) into cyclo(D-prolyl-L-phenylalanyl) by the action of 0·5M-NaOH has been reported[5]. In the equilibrium mixture, the *trans* isomer cyclo(D-Pro-L-Phe)** predominates by 90 to 95%. The steric interactions in the molecule of this *trans* isomer (as indicated by inspection of Dreiding models) are lower than in the molecule of the *cis* isomer cyclo(L-Pro-L-Phe).

Since these isomerisations appeared of general importance, it was interesting to examine the alkali treatment of some other analogous compounds. The observations are described in this paper. Substrates prepared earlier in connection with the conformational investigations on di- and tricyclic 2,5-piperazinediones[7,8] were used. In alkaline media, two reactions of 2,5-piperazinediones may be observed, namely, the change of the spatial arrangement at the chiral carbon atom[9] and hydrolysis to the linear dipeptide[10]. The rate of the latter reaction is significantly dependent on the nature of substituents at the 2,5-piperazinedione ring. The rate of the former reaction, *i.e.*, of the configurational change at α-carbon atoms (positions 3 and 6)

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**   The cyclodipeptides are designated as usual in the chemistry of peptides. For abbreviations see ref.[6]; Aze, 2-azetidinecarboxylic acid; Pip, pipecolic acid.

has been so far examined with the use of compounds carrying acyclic substituents and has proved considerably slow. In view of similar rates of the configurational change at both chirality centers, the overall result consisted in racemisation of the starting compound, accompanied by decrease of the optical rotation to the zero value. We wish, however, to demonstrate that the structural factors can not only increase the rate of the configurational change at the α-carbon atoms, but also exert a marked influence on the stereochemical course. When there is a considerable difference in the rates of the configurational change at the (two) chiral atoms, the structural factors in the molecule might cause in the first stage inversion of the absolute configuration at one of the chirality centers. This inversion manifests itself as isomerisation of one optically active diastereoisomer into the other one, *i.e.*, of the *cis* isomer into the *trans* isomer and *vice versa*. This process was examined with the use of 3,6-disubstituted bi- and tricyclic 2,5-piperazinediones *I−IV* containing the residue of a cyclic imino acid such as 2-azetidinecarboxylic acid, proline or pipecolic acid.

The epimerisation in 0·1M-NaOH was examined by means of the change in optical rotation (Fig. 1). The steep part of the curve corresponds to inversion of the absolute configuration at α-carbon atoms of the proline and 2-azetidinecarboxylic acid residues respectively in the *cis* isomers cyclo(L-Pro-L-Leu) (*IIIa*) and cyclo(D-Aze-D-Leu) (*IIa*) which are converted to the *trans* isomers cyclo(D-Pro-L-Leu) (*IIIb*) and cyclo(L--Aze-D-Leu) (*IIb*). After 15 min of the treatment with 0·1M-NaOH, the following compounds were isolated from the reaction mixture and the content of the *cis* and *trans* isomers was determined by PMR spectra: 93% of *IIIb* and 7% of *IIIa*;
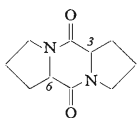
I, cyclo(Pro-Pro)
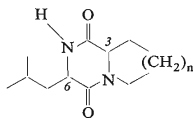
II; n = 0, cyclo(Aze-Leu)
III; n = 1, cyclo(Pro-Leu)
IV; n = 2, cyclo(Pip-Leu)

In formulae *I−IV*: *a cis* isomer, *b trans* isomer.

86% of *IIb* and 14% of *IIa*. With compound *IVa*, containing the residue of pipecolic acid, the course of the change in optical rotation is quite different: no inversion occurs at the α-carbon atom of the cyclic residue and the small decrease of the optical activity corresponds in this special case to a very slow racemisation at both centers of chirality. When attached to 2,5-piperazinedione, the cyclic residue of pipecolic acid (six-membered ring) behaves as an acyclic amino-acid residue. A similar observation has been made when investigating the chiroptical properties of 2,5-piperazinediones[11]: the behaviour of pipecolic acid derivatives was more similar to that of acyclic amino-

acid derivatives (*e.g.*, leucine) than of other imino-acid derivatives such as proline and 2-azetidinecarboxylic acid. As indicated by the course of change in optical rotation with the *cis* isomer cyclo(L-Pro-L-Pro) (*Ia*), no inversion of the absolute configuration takes place at the α-carbon atom and the chiral system remains untouched. On the other hand, the inversion occurs with the *trans* isomer cyclo-(D-Pro-L-Pro) (*Ib*); as shown by IR spectroscopy, the optically inactive *trans* isomer (*meso* form) is converted into the racemic *cis* isomer. The proof of the intermolecular mechanism of the inversion at the α-carbon atom of the proline residue or 2-azetidinecarboxylic acid residue was effected with the use of the isotope exchange. Thus, treatment of compounds *I−IV* with 0·1M-NaOD for 15 min resulted in replacement of all hydrogen atoms at α-carbon atoms in the proline or 2-azetidinecarboxylic acid residues with deuterium whereas the hydrogen atoms at α-carbon atoms of pipecolic acid or acyclic amino-acid residues were not exchanged.

The 2,5-piperazinedione ring can exist in the planar or boat conformation, the conformational features of which are closely similar to those of 1,4-cyclohexadiene[12]. When the 2,5-piperazinedione ring is monosubstituted (R = H), the conformation *B* with the substituent in the pseudoaxial position is more stable (Fig. 2), probably due to the interaction of the carbonyl group oxygen atom and the amide group
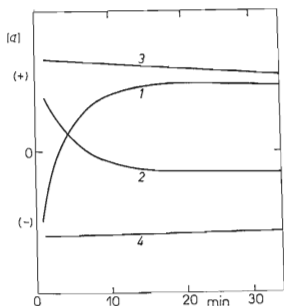
FIG. 1

Change in Optical Rotation of 2,5-Piperazinedione Solutions in 0·1M-NaOH on Standing at 26°C

1 Cyclo(L-Pro-L-Leu); *2* cyclo(D-Aze-D--Leu); *3* cyclo(D-Pip-D-Leu); *4* cyclo(L-Pro--L-Pro).
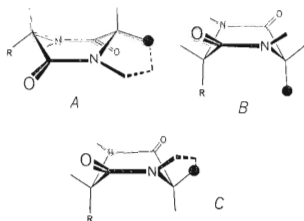
FIG. 2

Conformation of the 2,5-Piperazinedione Ring

hydrogen atom with a substituent which would be situated in the pseudoequatorial position (A). In the case of an additional substitution with the residue R, inter-actions of the 1,4-type are involved resulting in distorsion of the bond or flattening of the ring. The bridging of the nitrogen atom with the α-carbon atom by means of a trimethylene or ethylene bridge is possible only in the pseudoequatorial posi-tion; in the case of the *cis* isomer, also the second substituent R must be situated in the unfavourable pseudoequatorial position. The system of 2,5-piperazinedione with anneled five- or four-membered ring is capable of a quick inversion of the absolute configuration at the α-carbon atom because of the advantageous sp^2 ar-rangement of the activated reaction complex in the case of a five- or four-membered ring and the facile removal of a proton from the α-carbon atom of the proline or 2-aze-tidinecarboxylic acid residue under the formation of a carbanion[11]. The resulting planar system of the remaining three bonds can be aproached by hydrogen or deute-rium from both sides. Consequently, the thermodynamically more stable *trans* isomer with the substituent R in the favoured pseudoaxial position (C) is formed under the equilibrium control. An easy removal of protons from the α-carbon atoms may be assumed also in the case of a 2,5-piperazinedione with two anneled five-membered rings. On the other hand after removal of the hydrogen atom from one of the α-car-bon atoms of the *cis* isomer cyclo(L-Pro-L-Pro), the original conformation remains untouched since the second anneled ring prevents planarisation of the three bonds of the monocarbanion formed. Hydrogen or deuterium enter then the molecule from the side of the original hydrogen atom and a product is obtained the optical activity of which remains unchanged. With the *trans* isomer cyclo(D-Pro-L-Pro), the (step-wise) removal of hydrogen from the α-carbon atoms is accompanied by the change of conformation and a racemic mixture of the *cis* isomers cyclo(D-Pro-D-Pro) and cyclo(L-Pro-L-Pro) is obtained.

This observation thus constitutes the required[13] experimental proof that the system of 2,5-piperazinedione with two anneled five-membered rings is less strained and thermodynamically more stable in the *cis* configuration than in the *trans* form. It is necessary to expect the thermodynamical drawback of the activated complex in the cyclisation leading to 2,5-piperazinediones with *trans* dianneled rings. Both the *cis* isomers have been obtained[8] in the synthesis of 2,5-piperazinediones anneled with one five-membered ring and one four-membered ring, *i.e.*, in the synthesis of cyclo(Pro-Aze), and with two four-membered rings, *i.e.* in the synthesis of cyclo-(Aze-Aze). On the other hand, in the attempted synthesis of *trans* isomers only a small amount of *cis* isomers was obtained, but no *trans* isomer. The failure of the synthesis of *trans* isomers is ascribed to the considerable strain which must be expected with compounds of this type and which must manifest itself even at the stage of the activated complex of the cyclisation reaction.

EXPERIMENTAL

The test substances have been described elsewhere[7,8]. The change in optical rotation has been measured on a Jasco UV/ORD-5 spectropolarimeter at such a wavelength (in the 226—250 nm region) to get at least 80% rotation of the apparatus range. The concentration of solutions was approximately $3 . 10^{-3}$M of the particular 2,5-piperazinedione in (exactly) 0·12M-NaOH. Cell compartment temperature, $26 \pm 2°C$; cell thickness, 1 cm. In the isotope exchange measurements, the concentration was $2 . 10^{-2}$M of the particular 2,5-piperazinedione in 0·12M-NaOD (in 99·5% D_2O). The isotope exchange was performed at room temperature. After 15 min of reaction, the 2,5-piperazinediones were separated from sodium hydroxide or sodium deuteroxide with the use of the Dowex 50 ion exchange resin. The ratio of *cis* and *trans* isomers in the equilibrium mixture was determined[14] from the intensity ratio of N—H protons in PMR spectrum on a Varian HA 100 apparatus; error, $\pm 0·5\%$. The substitution of hydrogen with deuterium was checked by mass spectrometry on an AEI MS 902 apparatus.

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Note added in proof: In a recently published paper (Steyn P. S.: Tetrahedron *29*, 107 (1973)) the epimerization of cyclo(L-tryptophyl-L-prolyl) was studied in connection with stereochemical studies on the metabolites of *Aspergillus ustus*. Treatment of this substituted 2,5-piperazinedione in boiling ethanol and triethylamine yielded an equilibrium mixture of *cis* and *trans* isomers. Epimerization on the tryptophan α-carbon atom resulting in formation of cyclo(D-tryptophyl- -L-prolyl) was assumed. However, from our results it is highly presumptive that in this case the epimerization occurs at proline α-carbon atom.