AMINO ACIDS AND PEPTIDES. CXIV.*

PROTON MAGNETIC RESONANCE STUDIES OF CYCLODIPEPTIDES CONTAINING PIPECOLIC ACID, PROLINE AND/OR 2-AZETIDINE-CARBOXYLIC ACID

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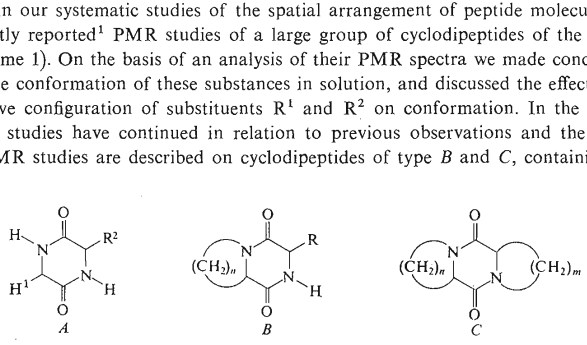
Twenty-five 2,5-piperazinediones with annelation of one or two 6-membered, 5-membered or 4-membered rings (derivatives of pipecolic acid, proline and/or 2-azetidinecarboxylic acid) are analysed in terms of molecular geometry on the basis of PMR spectra. Compounds with annelation of one or two 6-membered rings and with two *trans*-anneled 5-membered rings have a nearly planar piperazinedione moiety, the other compounds have this moiety in a boat conformation. The boat form is deepened with the decreasing size of the anneled ring. A benzyl side-chain in bicyclic 2,5-piperazinediones containing a phenylalanine residue is oriented outside of the 2,5-piperazinedione ring in *cis*-cyclo(2-azetidinecarbonyl-phenylalanyl) and in *cis*-cyclo-(prolyl-phenylalanyl), whereas in the other cyclodipeptides studied in the same category the benzyl side-chain is oriented predominantly over the 2,5-piperazinedione ring.

Within our systematic studies of the spatial arrangement of peptide molecules, we recently reported[1] PMR studies of a large group of cyclodipeptides of the *A* type (Scheme 1). On the basis of an analysis of their PMR spectra we made conclusions on the conformation of these substances in solution, and discussed the effect of the relative configuration of substituents R^1 and R^2 on conformation. In the present work studies have continued in relation to previous observations and the results of PMR studies are described on cyclodipeptides of type *B* and *C*, containing one



SCHEME 1

* Part CXIII: This Journal *38*, 1073 (1973).

or two residues of pipecolic acid, proline or 2-azetidinecarboxylic acid.* The presence of one, and particularly two, 4- to 6-membered rings anneled to the 2,5-piperazinedione ring results in a considerable conformational rigidity of the cyclodipeptide molecule of types *B* and *C* as opposed to *A*. Compounds of type *B* and *C* are therefore attractive model substances for the study of relations between spectroscopic properties and geometric parameters of systems with *cis*-amide bonds. Previous PMR studies of cyclodipeptides of type *A* have shown[1,3-7] interesting conformational behaviour of amino-acid residues with an aromatic ring in the β-position (the side-chain takes on a folded conformation). Since we wished to determine in which manner this behaviour is influenced by the presence and relative configuration of anneled cyclic residues Pip, Pro, Aze, we synthesized diastereoisomeric pairs of cyclodipeptides *VIIa*, *VIIb*, *VIIIa*, *VIIIb* and *IXa*, *IXb*. The synthesis of the other cyclodipeptides studied here has been described elsewhere[8,9]. Some of the results of studies of some of the cyclodipeptides of types *B* and *C* by means of PMR, IR and CD spectra have been described in a preliminary communication[10]. Siemion has described the conformation of the piperazinedione ring in cyclo(L-prolyl-glycyl) (*I*), *cis*- and *trans*-cyclo(prolyl-prolyl)[11] (*XIIIa,b*) and differences between signals of pseudoaxial and pseudoequatorial substituents of the piperazinedione ring in *cis*- and *trans*-c(Pro-Ala), c(Pro-Val), c(Pro-Leu) (*Va,b*) and c(Pro-Phe) (*VIIIa,b*) (ref.[12]).

The cyclodipeptides were prepared by cyclisation of the corresponding dipeptide methyl esters in methanol with the addition of methanolic ammonia. The cyclodipeptide was separated from the remnants of non-cyclised dipeptide methyl ester by ion-exchange chromatography. The dipeptide methyl esters were prepared using 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline as coupling agent and benzyloxycarbonyl group for N-protecting. Condensation of amino acids of the same absolute configuration was carried out in ethyl acetate. However, by mixing the ethyl acetate solutions of benzyloxycarbonyl-amino acids and methyl esters of phenylalanine of the opposite absolute configuration, the salts of these compounds precipitate out from solution. In such cases, a mixture of ethyl acetate and chloroform was used.

EXPERIMENTAL

Melting points were determined on a Kofler block. Sublimation was carried out at a pressure of 1 Torr and at a temperature 10°C less than the temperature of the melting point. Samples for analysis and for physical measurement were dried for 24 h at a pressure of 0·5 Torr over phosphorus pentoxide. Optical rotation was measured on a photoelectric polarimeter. Mass spectra (AEI MS-902 instrument) of all cyclodipeptides corresponded to the presumed structure.

Linear Peptide Intermediates

As an example the synthesis of the methyl ester of N-benzyloxycarbonyl-D-2-azetidinecarbonyl--D-phenylalanine is presented: To a solution of N-benzyloxycarbonyl-D-2-azetidinecarboxylic

* Standard abbreviations[2] are used for amino-acid residues and protecting groups, Pip for pipecolic acid, Aze for 2-azetidinecarboxylic acid.

acid (235 mg) in ethyl acetate (10 ml) at room temperature we added with stirring the D-phenylalanine methyl ester (produced from the hydrochloride of the D-phenylalanine methyl ester (220 mg)) and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (270 mg). After stirring over night, the solution was washed with 1M-HCl, 0·5M-$NaHCO_3$, water, dried with Na_2SO_4 and azeotropically with benzene. The methyl ester of N-benzyloxycarbonyl-D-2-azetidinecarbonyl--D-phenylalanine was crystallised by grinding with light petroleum and was recrystallised from ethyl acetate–light petroleum. In the same manner the other substances presented in Table I were prepared.

Cyclodipeptides

As an example we present the synthesis of cyclo(D-2-azetidinecarbonyl-D-phenylalanyl) (*XIa*): The methyl ester of N-benzyloxycarbonyl-D-2-azetidinecarbonyl-D-phenylalanine (300 mg) was hydrogenolysed in methanol (40 ml) over palladium on activated charcoal. After the end of hydrogenolysis the catalyst was filtered off, to the filtrate a saturated solution of ammonia in methanol (0·5 ml) was added and the mixture was left for 4 days at room temperature. The methanol was evaporated off, the residue was dissolved in 50% methanol (50 ml) and this solution was filtered through a column of Dowex 50 (25 ml, H^+ cycle) and Amberlite IR-4 B (10 ml, OH^- cycle). The ion-exchangers were washed with 50% methanol (80 ml) and the pooled eluates were evaporated. The product *IXa* was crystallised from ether, recrystallised from the solvent given in Table II and sublimated. In the same manner the other substances presented in Table II were prepared.

Spectroscopic Measurements

The PMR spectra of substances *I*—*XVa* were measured on the Varian HA-100 instrument in deuteriochloroform with tetramethylsilane as an internal standard. The concentrations of the measured solutions were about 0·07M. Signals of protons of the non-cyclic residues (Gly, Leu, Phe) were assigned by double resonance experiments on the sequences of interacting protons NH—C_αH with glycine residues and NH—C_αH—C_βH (with phenylalanine and leucine residues). Signals of protons of cyclic residues (Pip, Pro, Aze) were assigned by the same technique on the sequences of interacting protons C_αH to C_ωH (as far as the character of the spectra allowed) and by comparison of the values of the chemical shifts measured with values expected for protons in the given structural fragments. In cyclodipeptides of type *C* with two cyclic residues, the signals of individual residues were assigned on the basis of comparisons with spectra of cyclodipeptides of type *B*. The complex nature of the spectrum cyclo(pipecolyl-prolyl) diastereoisomers *XIa*, *XIb* did not allow detailed analysis. The characteristic parameters of the PMR spectra of 2,5-piperazinediones of type *B* are summarised in Table III, of type *C* are summarised in Table IV.

RESULTS AND DISCUSSION

The aim of analysis of the parameters of the PMR spectra of cyclodipeptides *I*—*XVa* is to acquire detailed information on a spatial arrangement of these substances in solution. An important question, but one difficult to answer only from PMR spectra, is planarity or possible torsion of the amide bond. The only substance of these series so far studied by X-ray diffraction is cyclo(L-prolyl-L-leucyl) (*Va*) where a boat conformation of the piperazinedione ring with a 6° torsion at both amide groups was found[15]. Cyclodipeptides *I*—*XVa* were studied[8,9] by infrared

spectroscopy. The increases in wavenumber of the ν(C=O) vibration and the decreases in wavenumber of the $\nu(C_{(O)}—N)$ vibrations in compounds with anneled 4-membered rings and in *cis*-c(Pro-Pro) are interpreted in the sense of non-planarity of the amide group. However, a pyramidal local conformation on bridgehead nitrogen atom in proline residue and particularly in 2-azetidinecarboxylic acid residue is a general phenomenon operating in all compounds under study. The presence of an anneled 4- or 5-membered ring can also produce some degree of deformation (decrease) of bond angles ϱ (angle $C_\alpha—C_{(O)}—N$) as was demonstrated by X-ray structural analysis in compound c(L-Pro-L-Leu) (values found[15] $\varrho = 114°$ as opposed to $\varrho = 119°$ in compound c(Gly-Gly)[16]. From the point of view of conformation of the 2,5-piperazinedione ring changes in the bond angles ϱ result in changes of the puckering of the 2,5-piperazinedione ring. The decrease in size of the anneled ring induces the deepening of the boat form. Simultaneously the pyramidal arrangement on bridgehead nitrogen atom is augmented.

From analysis of models it would appear that the 2,5-piperazinedione ring with both planar amide bonds can take on either a planar or a more or less deep boat conformation. For *cis* and for *trans*-3,6-disubstituted 2,5-piperazinediones three

TABLE I

Properties of Linear Peptide Intermediates

Dipeptide (solvent[d])	M.p.[a], °C yield, %	M.p.[b], °C solvent[e]	Formula (m.w.)	Calculated/Found % C	% H	% N	$[\alpha]_D^{25}$][c] (c), %
Z-D-Pip--D-Phe-OMe (A)	54—61 71	57—61 C	$C_{24}H_{28}N_2O_5$ (424·5)	67·91 67·55	6·65 6·59	6·60 6·54	+46·4° (0·5)
Z-D-Pip--L-Phe-OMe (B)	104—106 87	106—107 D	$C_{24}H_{28}N_2O_5$ (424·5)	67·91 67·70	6·65 6·65	6·60 6·71	+13·7° (0·5)
Z-L-Pro--D-Phe-OMe (B)	65—71 87	72—74 D	$C_{23}H_{26}N_2O_5$ (410·2)	67·33 67·59	6·39 6·59	6·83 6·72	−59·2° (0·5)
Z-D-Aze--D-Phe-OMe (A)	57·5—61 91	61—63 D	$C_{22}H_{24}N_2O_5$ (396·4)	66·65 66·92	6·10 6·06	7·07 7·11	+72·7° (0·5)
Z-D-Aze--L-Phe-OMe[f] (B)	103·5—106 90	104·5—106·5 D	$C_{22}H_{24}N_2O_5$ (396·4)	66·65 66·58	6·10 6·13	7·07 7·12	+71·8° (0·5)

[a] Melting points of compounds obtained in the given yield; [b] melting points of samples for analysis; [c] in methanol; [d] solvent for condensation A ethyl acetate, B ethyl acetate–chloroform 1 : 1; [e] solvent for crystallisation C ether–light petroleum, D ethyl acetate–light petroleum; [f] also prepared by means of N,N'-dicyclohexylcarbodiimide in an 85% yield.

TABLE II
Properties of Cyclodipeptides

Cyclodipeptide	M.p.[a], °C yield, %	M.p.[b], °C solvent[d]	Formula (m.w.)	Calculated/found % C	% H	% N	$[\alpha]_D^{25}$[c] (c)
c(D-Pip-D-Phe)	148—150	150—151	$C_{15}H_{18}N_2O_2$	69·74	7·02	10·84	−13·3°
VIIa	35	A	(258·3)	69·85	7·05	10·86	(0·7)
c(D-Pip-L-Phe)	122—130	134—135	$C_{15}H_{18}N_2O_2$	69·74	7·02	10·84	−17·5°
VIIb	42	A[e]	(258·3)	69·66	7·06	10·90	(0·7)
c(L-Pro-L-Phe)[f]	130—133	134—136	$C_{14}H_{16}N_2O_2$	68·83	6·60	11·47	−82·3°[g]
VIIIa	58	B	(244·3)	68·83	6·68	11·62	(0·2)
c(L-Pro-D-Phe)[h]	140—149	150—153	$C_{14}H_{16}N_2O_2$	68·83	6·60	11·47	−94·2°[g]
VIIIb	74	B	(244·3)	69·02	6·65	11·55	(0·2)
c(D-Aze-D-Phe)	123·5—125·5	125·5—127	$C_{13}H_{14}N_2O_2$	67·81	6·13	12·17	+64·2°
IXa	49	C	(230·3)	67·70	6·14	12·30	(0·5)
c(D-Aze-L-Phe)[i]	oil	—	$C_{13}H_{14}N_2O_2$	67·81	6·13	12·17	+11·4°
IXb	60		(230·3)	67·19	6·51	11·82	(0·5)

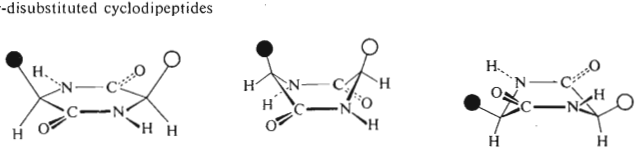
[a] Melting points of compounds obtained in the given yield; [b] melting point of samples for analysis and physical measurement; [c] in methanol, unless stated otherwise; [d] A ethyl acetate–ether–light petroleum, B ethyl acetate–ether, C ethanol–ether–light petroleum; [d] first sublimated, then recrystallised; [f] prepared from HBr.H-L-Pro-L-Phe-OMe (ref.[13]), the literature presents m.p. 133°C (ref.[14]), 125—127°C (ref.[12]), $[\alpha]_D^{20}$ −83° (*c* = 0·2, water)[14]; [g] in water; [h] the literature gives[12] for compound prepared from Z-D-Phe-L-Pro-OMe m.p. 145—147°C, for c(D-Pro-L-Phe) m.p. 148—150°C, $[\alpha]_D^{20}$ +92° (*c* = 0·2, water)[14]; [i] hygroscopic, dried for 3 days at 0·5 Torr over phosphorus pentoxide gives a solid material.

various conformational types could be derived (Fig. 1) of which each represents one member of an enantiomeric pair. Our concept of the conformation of the piperazinedione ring and the position of the C_β atom (or of C_ω in cyclic residues) determined by this was obtained from values of interaction constants between NH and $C_\alpha H$ protons ($J_{N\alpha}$), chemical shifts of $C_\alpha H$ protons ($\delta_{\alpha H}$) and long-range interaction between $C_\alpha H$ protons of both residues (J_{α_1,α_2}). Concepts of the conformation of the side chains of acyclic residues (primarily phenylalanine) and of the conformations of anneled rings were derived from the values of interaction constants of $C_\alpha H$ and $C_\beta H$ protons ($J_{\alpha\beta}$), $C_\omega H$ and $C_{\omega-1}H$ protons ($J_{\omega,\omega-1}$) and from chemical shifts of $C_\beta H$ protons ($\delta_{\beta H}$) and $C_\omega H$ protons ($\delta_{\omega H}$). With derivatives of phenylalanine we also used the degree of shielding by the aromatic ring.

Conformation of the 2,5-*Piperazinedione Ring*

The relationship between the size of $J_{N\alpha}$ and the torsion angle Θ of interacting protons NH and $C_\alpha H$ has recently been studied by a number of investigators[7,17-19]. Semi-empirical relationships $J_{N\alpha} = f(\Theta)$, described by a number of investigators, are not very different and were applied for conformational studies of a number of cyclic oligopeptides in solution (for a review see[20]). In the present study we have used a four-coefficient equation, derived in this institute in relation with studies of other peptides[19]. Cyclic peptides *I–III* contain a residue of glycine, the two $C_\alpha H$ protons of which (or, more precisely, their $J_{N\alpha}$) give information (independent

cis-disubstituted cyclodipeptides



trans-disubstituted cyclodipeptides

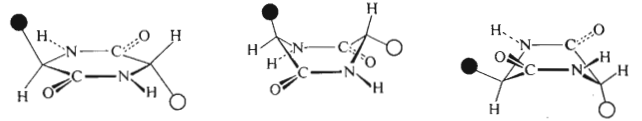


FIG. 1

Possible Conformations of the 2,5-Piperazinedione Ring in Cyclodipeptides with Planar Amide Bonds

TABLE III

Parameters of PMR Spectra of Cyclodipeptides in Deuteriochloroform[a]

	Compound	1st residue						2nd residue				
		$\delta_{\alpha H}$	$J_{\alpha\beta}$[b]	$\delta_{\beta H}$	$\delta_{\omega H}$	δ_{NH}[c]	$\delta_{N\alpha}$	$\delta_{\alpha H}$	$J_{\alpha\beta}$[b]	$\delta_{\beta H}$	$\delta_{\omega H}$	$J_{\alpha_1\alpha_2}$
I	c(D-Pip-Gly)	3·84 bd	10 4	1·25—2·20 m	2·52 bt 4·69 bd	7·35 bs	2·2	4·02 bs	—	—	—	0·8 $\pm 0 \leqq 0{\cdot}4$
II	c(L-Pro-Gly)	4·11 bt	[d] [d]	1·75—2·55 m	3·58 m	7·15 bs	4·0 $\pm 0 < 1$	3·87 dd 4·10 bd	—	—	—	[e]
III	c(D-Aze-Gly)	4·95 bt	8·5 7	2·54—2·89 m	4·13 bt	7·17 bs	5·0 $\approx 0 \ll 1$	3·73 dd 4·05 bd	—	—	—	$\approx 0 \ll 1$ 1·3
IVa	c(D-Pip-D-Leu)	3·84 bd	11·5 3	1·25—2·20 m	2·50 bt 4·69 bd	7·06 bs	2·2	4·02 bd	[d] [d]	1·25—2·20 m	0·96 d	[e]
IVb	c(D-Pip-L-Leu)	3·83 dd	11·5 3	1·20—2·15 m	2·50 bt 4·69 bd	6·99 bs	2	4·03 bd	[d] [d]	1·20—2·15 m	0·94 d	[e]
Va	c(L-Pro-L-Leu)	4·11 bt	8·5 7	1·36—2·52 m	3·54 m	6·48 bs	$\pm 0 < 1$	4·02 bd	9·5 4	1·36—2·52 m	0·94 d 0·98 d	[e] [e]
Vb	c(L-Pro-D-Leu)	4·08 bt	[d] [d]	1·48—2·50 m	3·57 m	7·18 bs	4·0	3·93 m	[d] [d]	1·48—2·50 m	0·94 d 0·97 d	[e]
VIa	c(D-Aze-D-Leu)	4·91 bt	8·5 7	2·58 q	4·08 bt	6·36 bs	$\approx 0 \ll 1$	3·92 m	9·5 4·5	1·30—2·05 m	0·94 d 0·99 d	$\pm 0 \leqq 1$
VIb	c(D-Aze-L-Leu)	4·92 bt	8 7·5	2·69 m	4·10 m	7·19 bd	4·8	3·83 m	8 7·5	1·45—1·90 m	0·92 d 0·97 d	$\approx 0 \ll 1$

VIIa	c(D-Pip-D-Phe)	3·60 bd	12·0 2·6	2·35 bt 1·05—2·00 m	2·35 bt 4·65 bd	7·25 bs	2·2	4·34 bt	5·0 4·3	2·95 dd 3·32 dd	7·26 m	0·7
VIIb	c(D-Pip-L-Phe)	2·96 bd	11·5 3	2·23 bt 1·15—2·00 m	2·23 bt 4·63 bd	6·60 bs	1·8	4·27 bt	5·3 4·5	3·07 dd 3·20 dd	7·26 m	0·5
VIIIa	c(L-Pro-L-Phe)	4·07 bt	8·5 7	1·75—2·40 m	3·61 m	5·70 bs	$\pm 0<1$	4·27 dd	10·5 4·0	2·79 dd 3·61 dd	7·28 m	$\pm 0<1$
VIIIb	c(L-Pro-D-Phe)	2·90 m	9·5 6	1·50—2·25 m	3·52 m	6·67 bs	3·9	4·22 bd	4·5 5·5	3·02 dd 3·19 dd	7·26 m	0·6
IXa	c(D-Aze-D-Phe)	4·90 bt	8·5 7	2·69 bq	4·13 bt	5·57 bs	$\pm 0<1$	4·19 bq	11 4	2·75 dd 3·52 dd	7·27 m	$\pm 0<1$
IXb	c(D-Aze-L-Phe)	4·01 m	*d* *d*	2·49 m	4·01 bt	7·45 d	4·0	4·01 m	6 5·5	2·87 dd 3·01 dd	7·24 m	*e*

[a] Chemical shifts are given in the δ-scale (p.p.m.) and interaction constants in Hz. [b] Values of $J_{\alpha\beta}$, were obtained from the multiplets of C_α-H protons (with the exception of the phenylalanine residue) and can show a marked inaccuracy. [c] Chemical shifts of the NH protons are strongly dependent upon concentration in deuteriochloroform. With reference to the varied solubility of the studied compounds all samples were not measured at the same concentrations, and values of shifts of the NH protons are therefore not always comparable. [d] Values of $J_{\alpha,\beta}$ could not be determined from the spectra. [e] Values of $J_{\alpha_1\alpha_2}$ could not be determined.

TABLE IV

Parameters of PMR Spectra of Cyclodipeptides in Deuteriochloroform[a]

Compound	1st residue				2nd residue				
	$\delta_{\alpha H}$	$J_{\alpha\beta}$[b]	$\delta_{\beta H}$	$\delta_{\omega H}$	$\delta_{\alpha H}$	$J_{\alpha\beta}$[b]	$\delta_{\beta H}$	$\delta_{\omega H}$	$J_{\alpha_1\alpha_2}$
Xa c(D-Pip-D-Pip)[c]	3·81 bd	11 3	2·42 bd 1·15—2·30 m	2·50 bt 4·68 bd	—	—	—	—	—
Xb c(D-Pip-L-Pip)	3·82 bd	11 3	2·45 bd 0·95—2·38 m	2·50 bt 4·69 bd	—	—	—	—	—
XIa c(L-Pip-L-Pro)	3·35—4·15 m	*d* *d*	1·15—2·95	4·61 bd	3·35—4·15 m	*d* *d*	1·15—2·95 m	3·35—4·15 m	*e*
XIb c(D-Pip-L-Pro)	3·33—4·18 m	*d* *d*	1·18—2·75 m	4·63 bd	3·33—4·18 m	*d* *d*	1·18—2·75 m	3·33—4·18 m	*e*
XIIa c(D-Pip-D-Aze)	3·83 dd	10 4	1·20—2·40 m	2·55—2·90 m 4·40 bd	4·87 bt	8·5 7	2·55—2·90 m	4·09 bt	$\pm 0 < 1$
XIIb c(D-Pip-L-Aze)	3·86 bd	12 2·5	1·25—2·25 m	2·40—2·97 m 4·59 bd	4·94 bt	8 7·5	2·40—2·97 m	4·11 q	$\approx 0 \ll 1$
XIIIa c(L-Pro-L-Pro)	4·20 t	7·5 7·5	1·70—2·55 m	3·53 q	—	—	—	—	—
XIIIb c(L-Pro-D-Pro)	4·08 bt	5·5 6·5	1·53—2·60 m	3·30 m 3·96 m	—	—	—	—	—
XIVa c(D-Pro-D-Aze)	4·05 bt	8·5 7	1·75—2·40 m	3·55 m	4·92 bt	8·5 7	2·64 q	4·02 t	$\pm 0 < 1$
XVa c(D-Aze-D-Aze)	4·98 bt	7·5 7·5	2·69 m	4·10 m	—	—	—	—	—

[a] Notes see Table III.

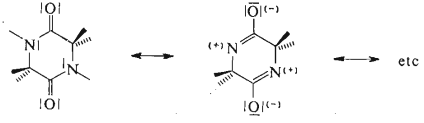
of the exact relation $J_{N\alpha} = f(\Theta)$) on the dihedral angle Φ_1, and therefore on the degree of planarity of the 2,5-piperazinedione ring. One value of $J_{N\alpha} = 2{\cdot}2$ Hz and the same values $\delta_{\alpha H}$ of both glycine $C_\alpha H$ protons suggests planarity of the 2,5-piperazinedione ring in *I*. The same value of $J_{N\alpha}$ was found[1,4] in c(Gly-Gly) for which X-ray structure analysis demonstrated a planar arrangement in crystal[16]. On the other hand, in substances *II* and *III* we can observe non-equivalence of both glycine $C_\alpha H$ protons and markedly different values of their $J_{N\alpha}$, with even greater differences than have been thus far observed[1,4,7] in cyclodipeptides of type *A*. In substance *III* the reported value $J_{N\alpha} = 5{\cdot}0$ Hz (so far the highest observed value in cyclodipeptides) approximates the value $J_{N\alpha} = 5{\cdot}6$ Hz for 2-azabicyclo[2,2,2]octane-3-one with a dihedral angle $\Theta = 0°$. This quite clearly demonstrates a boat conformation of the 2,5-piperazinedione ring in *II* and *III* with glycine $C_\alpha H$ in pseudoaxial and pseudoequatorial positions as was found earlier for *II* by Siemion[11]. From comparison of found values of $J_{N\alpha}$ with the known course of $J_{N\alpha} = f(\Theta)$ it would appear that boat conformation in substance *III* is deeper than in *II*.

This experimental result is in agreement with analysis of models which shows that a decrease in size of the anneled rings from 6-membered to 4-membered results in gradual planarisation of fragments C_ω—N—C_α—C_β, and thus to a deepening of the boat form of the 2,5-piperazinedione moiety. In substances *II* and *III* this effect can be accompanied by a decrease in valence angles ϱ and to a certain degree of non-planarity of the amide grouping arising from the deviation of the bond N—C_ω from the plane of C_α—C(O)—N. Markedly different values of $J_{N\alpha}$ in *II* and *III* allow a clear assignment of both glycine $C_\alpha H$ protons: $J_{N\alpha}$ near to zero must correspond with pseudoaxial $C_\alpha H$ protons with an angle Θ about 90° and $J_{N\alpha}$ about 4·0 Hz or 5·0 Hz (in *II* and *III* respectively) must correspond with pseudoequatorial $C_\alpha H$ protons with an angle Θ near to zero. From the point of view of chemical shifts this means that the signal of pseudoaxial $C_\alpha H$ is manifest in substances *II* and *III* at a lower magnetic field than the signal of pseudoequatorial $C_\alpha H$. Similar conclusions have been arrived at by previously reported[1,4] $J_{N\alpha}$ constants of glycine residues in cyclodipeptides of the type c(Gly-X), where *X* is an acyclic residue not containing an aromatic ring on the β carbon atom. The equivalence of both $C_\alpha H$ protons in substance *I* shows that the effect of the anneled ring (non-symmetrically arranged in relation to the plane of the 2,5-piperazinedione ring) on shielding of $C_\alpha H$ protons of the opposite residue is negligible.

The degree of shielding of both glycine $C_\alpha H$ protons is therefore determined by their position to magnetically anisotropic amide groups. Neither theoretical calculations nor empirical measurements with model compounds with known geometry lead to a satisfactory description of the shape of the shielding field in the surroundings of the amide group[21]. In general it is assumed[21] that protons lying above and below the plane of the amide bond are shielded more than protons lying in the same plane (in analogy with the shielding by carbonyl groups in ketones). Chemical shifts

of glycine protons in the above cyclodipeptides show exactly the opposite trend, C_α—H protons lying above the 2,5-piperazinedione ring (pseudoaxial) are less shielded than C_α—H protons lying near to the plane of amide bonds (pseudoequatorial). In agreement with this conception the degree of shielding of C_α—H in cyclic residues decreases in the series *I*, *II*, *III* (Table III) with increasing axial nature of C_α—H. Some contribution to this trend is also due to a change in the size of the ring of the cyclic residue causing a change in shift in the same direction. It is a question to what degree the course of the shielding field is influenced by the presence of two antiparallel *cis*-amide bonds in the ring. Without regard to this, differences in shielding have been described which are generally applicable to the behaviour of C_α—H protons in cyclodipeptides.

Conformational rigidity of the studied cyclodipeptides is confirmed by observations of long-range interactions ($J_{\alpha_1\alpha_2}$) between C_α—H protons of structurally different residues. This is manifest either by splitting or only broadening of the signals of C_α—H protons and was demonstrated by double resonance. The magnitude of observed values of $J_{\alpha_1\alpha_2}$ is 0—1·3 Hz and in the entire series is somewhat greater in *cis* than in *trans* cyclodipeptides. The highest value (1·3 Hz) was found in substance *III* between C_α—H proton in the residue of 2-azetidinecarboxylic acid and the C_α—H proton of the glycine residue in *cis* orientation to it. In the sense of a mesomeric structure *E* of the 2,5-piperazinedione ring it is possible to consider this interaction as a particular type of *cis*-homoallylic interaction with a double interaction pathway.



D *E*

The magnitude is decided by the degree of the σ and π contributions which are dependent both on the influence of separate mesomeric structures *D*, *E etc.* in the ground state and on the orientation of both C_α—H bonds in relation to the amide group. These differences in $J_{\alpha_1\alpha_2}$ are in agreement with results found in 1,4-cyclohexadiene derivatives in boat conformation (for 1,4-cyclohexadiene 9·63 Hz has been reported[22] between the *cis* and 8·04 Hz between the *trans* protons, and in *cis*- resp. *trans*-1,4-dihydro-4-tritylbiphenyl 11 Hz resp. 7·5 Hz have been reported[13]). Markedly lower absolute values of $J_{\alpha_1\alpha_2}$ were observed in cyclodipeptides and this can be explained by a contribution of mesomeric structure *D* and electron dilution in the interaction pathway produced by the presence of heteroatoms. In cyclodipeptides *IVa* to *IVb* the

leucine residue is the contrast to the cyclic amino acid residues. From Table III it would appear that there is a striking similarity of all PMR parameters of cyclic amino acid residues with parameters found in the corresponding glycine cyclodipeptides *I*–*III*. Since even values $\delta_{\alpha H}$ and $J_{N\alpha}$ of leucine residues are very similar to values found for *cis* or *trans*-$C_\alpha H$ protons of glycine of the corresponding cyclodipeptides *I*–*III*, it can be concluded that the 2,5-piperazinedione ring has the same (planar) conformation in substances *I*, *IVa*, *IVb*, and approximately the same (boat) conformation in substances *II*, *Va*, *Vb* and approximately the same (a deeper boat) conformation in substances *III*, *VIa* and *VIb*. The isobutyl substituent takes on a pseudoequatorial position in *cis*-cyclodipeptides *Va* and *VIa* (for *Va* demonstrated by X-ray diffraction[15]) and a pseudoaxial position in *trans*-cyclodipeptides *Vb* and *VIb*. Whereas in both diastereoisomers c(Pip-Leu) the $C_\alpha H$ protons of leucine have the same $\delta_{\alpha H}$ as the $C_\alpha H$ of glycine in c(Pip-Gly), in the pairs of substances c(Pro-Leu) and c(Aze-Leu) there is a decrease in the difference between $\delta_{\alpha H}$ of pseudoaxial and pseudoequatorial protons of leucine in comparison with the difference of glycine $\delta_{\alpha H}$ in c(Pro-Gly) and c(Aze-Gly). In agreement with the described course of shielding these changes are interpretable as a slight flattening of the boat conformation of the 2,5-piperazinedione ring in the pairs c(Pro-Leu) and c(Aze-Leu) in comparison with c(Pro-Gly) and c(Aze-Gly).

The three diastereoisomeric pairs *VIIa*, *VIIb*, *VIIIa*, *VIIIb* and *IXa*, *IXb* contain, in addition to a cyclic amino-acid residue, a phenylalanine residue. The presence of an aromatic ring of phenylalanine influences not only the chemical shift of $C_\alpha H$ proton of the phenylalanine residue itself, but also of C_α—H proton in the opposite anneled ring. The interaction constants $J_{N\alpha}$ of phenylalanyl $C_\alpha H$ protons again in the entire series *VIIa*–*IXa* show a marked similarity with the previous series of leucine (*IVa*–*VIb*) and glycine (*I*–*III*) cyclodipeptides. The PMR parameters of the cyclic amino-acid residues (with the exception of $\delta_{\alpha H}$) also show a marked similarity with previous series, from which we can suggest a similarity of conformation. The observed changes in chemical shifts of $C_\alpha H$ protons of the cyclic residues are produced by a shielding effect of the aromatic ring, which under certain conditions can occur in close proximity. Conformation of the phenylalanine side chain will be discussed later.

The presence of two cyclic amino-acid residues in compounds of type *C* obviously results in even more marked conformational rigidity (as compared with the above discussed compounds of type *B*) but at the same time there is a limitation of sources of conformational information on the 2,5-piperazinedione ring. Chemical shifts of $C_\alpha H$ protons only are available for direct interpretation. There can be some indirect information from chemical shifts and interaction constants of other protons of the cyclic residues, and some information derived from symmetry of spectra. From analysis of models it would appear that cyclodipeptides formed from two structurally identical residues (*Xa*, *Xb*, *XIIIa*, *XIIIb*, *XVa*) can have C_1 or C_2 symmetry in the

case of *cis*-diastereoisomers (*Xa*, *XIIa*, *XVa*) or C_1 or C_i symmetry in the case of *trans*-diastereoisomers (*Xb*, *XIIIb*). Half the number of signals with a doubled relative intensity in the PMR spectra of cyclodipeptides *Xa*, *Xb*, *XIIIa*, *XIIIb*, *XVa* (Table IV) lead to the conclusion that these cyclodipeptides in solution take on a conformation with the highest permissible symmetry (*i.e.* C_2 in *cis*- and C_i in *trans*-cyclodipeptides). Practically the same values of all PMR parameters of both c(Pip-Pip) diastereoisomers *Xa* and *Xb* with corresponding parameters of residues of pipecolic acid in c(Pip-Gly) suggest a similar (planar) conformation of the 2,5-piperazinedione ring in these derivatives. The complex character of the 100 MHz spectrum of c(Pip-Pro) diastereoisomers does not allow a detailed interpretation of the PMR parameters. Analysis of models shows that the presence of a proline residue should result in a boat conformation of the piperazinedione ring, with the $C_\alpha H$ proton of pipecolic acid residue in a pseudoaxial position in the *cis*-derivative *XIa* and a pseudoequatorial position in the *trans*-derivative *XIb*. The PMR spectra of both diastereoisomers show, despite this, a marked similarity. A possible explanation of this can be that the conformation of the 2,5-piperazinedione ring is the result of a compromise of antagonistic conformational requirements of both residues and is similar in both compounds *XIa* and *XIb*.

From Table IV it is obvious that there is also a similarity of PMR parameters in compounds *XIIa* and *XIIb*, the values of which are also similar to values found in the corresponding glycine cyclodipeptides *I* and *III*. From conformational analysis of models it would appear that the presence of a residue of 2-azetidinecarboxylic acid should result in a relatively deep boat form of the 2,5-piperazinedione ring with pseudoaxial C_α—H protons of the 2-azetidinecarboxylic acid residues and a pseudoaxial C_α—H proton in the pipecolic acid residue in *cis*-derivative *XIIa* and pseudoequatorial C_α—H proton of the same residue in the *trans*-derivative *XIIb*. Since the PMR spectra do not show the expected marked differences, it is probable that the azetidine ring induces a conformational distortion, but this distortion is partially eliminated by the non-planarity of the amide group (pyramidal arrangement on the nitrogen atom) and both moieties take on energetically advantageous conformations, similarly as in compounds *I* and *III*. This concept is supported by the results of infrared measurements[9], which show a different behaviour of both amide groups, of which only one should be markedly deviated from planarity.

The spectra of c(Pro-Pro) diastereoisomers (*XIIIa* and *XIIIb*) show greater differences in PMR parameters (primarily $C_\alpha H$ and $C_\omega H$ protons) than previously discussed diastereoisomeric pairs. From analysis of models it would appear, that the presence of two proline rings in *cis*-arrangement need not introduce further significant conformational change into the molecule in comparison with c(Pro-Gly). On the other hand, the antagonistic tendencies of two proline rings in the *trans*-isomer (each proline residue tend to impose the enantiomeric boat conformation on the 2,5-piperazinedione ring) should result in taking on a planar character of the central ring

(or a flattened chair-form with non-planar bonds). The observed differences in the PMR spectra of isomers *XIIIa* and *XIIIb* – $C_\alpha H$ proton is less shielded and therefore "more axial" and $\delta_{\omega H}$ is less different so that the $C_\omega H$ protons are more symmetrically situated in relation to the neighbouring amide group in *cis*-derivative *XIIIa* as opposed to *trans*-derivative *XIIIb* – are consistent with conceptions arising from molecular models. Cyclodipeptides of types c(Pro-Aze) and c(Aze-Aze) were synthesized only in the *cis*-series (*XIVa* and *XVa*). Values of the PMR parameters of c(Aze--Aze) (*XVa*) are very near to values of parameters of the 2-azetidinecarboxylic acid residue in c(Aze-Gly) (*III*) and the conformation of the 2,5-piperazinedione ring should correspond to a relatively deep boat conformation. The PMR parameters of cyclodipeptide c(Pro-Aze) (*XIVa*) show a conformational similarity with symmetrical *cis*-cyclodipeptides *XIII* and *XVa* and glycine cyclodipeptides *II* and *III*. The 2,5-piperazinedione ring takes on a boat conformation, the amide group need not be planar due to the different geometrical requirements of two different cyclic amino acid residues.

The Conformation of Side-Chains of the 2,5-*Piperazinedione Ring*

Information on the local conformation of isobutyl and benzyl substituents in cyclodipeptides *IVa–IXb* is contained in the interaction constants $J_{\alpha\beta}$. Values of these constants are obtainable practically only for phenylalanine cyclodipeptides *VIIa–IXb* In leucine cyclodipeptides *IVa–VIb* the complex character of the 100 MHz spectra of strongly interacting systems of protons of the side-chains does not allow in most cases a reliable determination of values of $J_{\alpha\beta}$. The measured values of $J_{\alpha\beta}$ of the phenylalanyl residues show marked differences in dependence on the character and configuration of the cyclic amino-acid residue. Whereas in both isomers c(Pip-Phe) (*VIIa*, *VIIb*) and in *trans*-cyclodipeptides c(Pro-Phe) and c(Aze-Phe) (*VIIIb*, *IXb*) both $J_{\alpha\beta}$ values are small (in the range from 4·0 to 5·0 Hz), *cis*-cyclodipeptides c(Pro-Phe) and c(Aze-Phe) (*VIIIa* and *IXa*) show marked differences in the values of $J_{\alpha\beta}$ (approx. 11 and 4 Hz). Assuming an energetically preferred staggered conformation on the C_α—C_β bond and approximate validity of Pachler's values of $J_{\alpha\beta}$ (ref.[24]) for $J_{180°}$ and $J_{60°}$ (13·6 Hz resp. 2·6 Hz) this indicates that in substances *VIIa*, *VIIb*, *VIIIb* and *IXb* the preferred conformation (to about 60%) is a folded one with the aromatic ring of phenylalanine located above the 2,5-piperazinedione ring with possibilities of interaction with both *cis*-amide groups. The same behaviour of the aromatic side-chain was found by various investigators[1,3–7] in cyclodipeptides of type *A* with residues of phenylalanine, tyrosine, *p*-methoxyphenylalanine, histidine and tryptophane. In *cis*-cyclodipeptides *VIIIa* and *IXa* the situation is different and there is a marked preference (about 75%) for a unfolded conformation with the aromatic ring pointing away from the 2,5-piperazinedione ring.

Confirmation of the preference of the folded conformation in compounds *VIIb*,

VIIIb and *IXb* is the shift of the signal C_α—H of the cyclic residue to higher field values as a result of shielding by the aromatic nucleus in comparison with the corresponding leucine cyclodipeptides *IVb*, *Vb*, *VIb*. This difference is greatest (1·18 p.p.m.) in c(Pro-Phe) (*VIIIb*) in which molecular models show an optimal spatial arrangement for shielding by the aromatic nucleus. In planar *trans*-c(Pip-Phe) (*VIIb*) the difference is less (0·87 p.p.m.) due to the greater distance and the less advantageous orientation of C_α—H and the aromatic nucleus. A similar difference (0·91 p.p.m.) was also found in *trans*-c(Aze-Phe) for which models show a decreased distance of C_α—H and the aromatic ring in the folded conformation (as opposed to *VIIIb*) but at the same time also a less advantageous orientation. The decrease in population of folded forms also contributes to this difference (as compared with *VIIIb*) as it would appear from values of $J_{\alpha\beta}$. Shift of C_α—H to higher field values manifests itself even though to a lesser degree (0·24 p.p.m.) in *cis*-c(Pip-Phe) (*VIIa*) with a preferred folded conformation. In *cis*-c(Pro-Phe) and *cis*-c(Aze-Phe) (*VIIIa*, *IXa*) for which the measured values of $J_{\alpha\beta}$ exclude preference of folded conformations, this shift is negligible (0·04 and 0·01 p.p.m.). The similarity of the appearance of the spectra of phenylalanyl C_β—H protons (C_β—H at higher field values shows a larger value of $J_{\alpha\beta}$) in a series of linear and cyclic peptides and amino acids suggests to us an analogous assignment of signals (for detailed discussion see reference[19]) and to the conclusion concerning the preference of the unfolded conformation with aromatic ring oriented towards the neighbouring nitrogen atom of the secondary amide bond in substances *VIIIa* and *IXa*. Approximate values of $J_{\alpha\beta}$ measured in three leucine cyclodipeptides *Va*, *VIa*, *VIb* show that in these substances there is a probable preference for the unfolded conformation.

Detailed analysis of the conformation of rings of pipecolic acid, proline, and 2-azetidinecarboxylic acid would require complete analysis of all PMR parameters, and this has not yet been possible because of the complex nature of the spectra. For approximate conformational analysis of anneled rings we therefore used available values of chemical shifts of C_β—H and C_ω—H protons and approximate values of interaction constants $J_{\alpha\beta}$ and $J_{\omega,\omega-1}$, which, however, can be very uncertain (first order analysis). In all cyclodipeptides containing residues of pipecolic acid there is a large difference (about 2·2 p.p.m.) between chemical shifts of both C_ω—H protons. This difference cannot be caused only by the variance between axial and equatorial positions of both protons in the neighbourhood of the nitrogen atom (in quinolizidine derivatives difference of 0·7 to 0·8 p.p.m. have been reported[25] between axial and equatorial protons in the α position to nitrogen atom). One can explain this difference in chemical shifts only by different orientation of both C_ω—H protons in respect to the amide bond. This is in agreement with the conception of the planar 2,5-piperazinedione ring and the chair conformation of the anneled six-membered ring, in which (according to molecular models) one $C_\omega H$ proton lies approximately in the plane and the second perpendicular to both amide bonds.

Very much smaller differences between the shifts of both $C_\beta H$ protons in c(Pip-X) derivatives ($C_\beta H$ lie in the region 1·5–2·5 p.p.m.) are also consistent with this model. The observed splitting of the signals of $C_\omega H$ leads to assignment of the lower-field signals to protons lying in the plane of the amide bond and high-field $C_\omega H$ signals to protons above this plane, therefore in agreement with present concepts of anisotropy of the amide group[21]. Because shielding of the $C_\omega H$ protons is obviously influenced very much more by the proximate amide bond, there is an anomalous magnitude of shielding of $C_\alpha H$ protons (*vide supra*) produced probably by a simultaneous effect of two neighbouring antiparallel amide bonds. Similar values reported for $J_{\alpha\beta}$ and $J_{\omega,\omega-1}$ in the whole series of pipecolic acid cyclodipeptides would allow for only small conformational changes in the six-membered anneled ring. The existence of an alternative boat conformation of the six-membered ring of pipecolic acid is excluded on the basis of the observed values of $J_{\omega,\omega-1}$.

Differences between chemical shifts of C_ω—H protons are very much less or completely disappear in cyclodipeptides containing proline residues. Both C_ω—H protons in all these substances are manifest in the region about 3·60 p.p.m. (*i.e.* in the middle of shifts of C_ω—H in c(Pip-X)). This observation is in agreement with a boat conformation of the 2,5-piperazinedione ring and with C_ω—H protons oriented roughly in the same angle above and below the plane of the amide group arising from the ring. Models show that the anneled proline ring should form an "envelope" with the C_β carbon outside of the plane of C_α—N—C_ω—$C_{\omega-1}$ in agreement with observed values of $J_{\alpha\beta}$ (for *cis*-c(Pro-Leu) the same conformation of the proline ring has been reported[15] in crystals). Similarity of available PMR parameters in the whole series c(Pro-X) derivatives demonstrates only moderate conformational deviations of the proline ring. An exception to this is formed by *trans*-c(Pro-Pro) (*XIIb*) in which there is a more marked difference in shifts of C_ω—H protons and, particularly, in $J_{\alpha\beta}$ values in agreement with previous conclusions on planarisation of the 2,5-piperazinedione ring, which also allows conformational changes of the proline ring. (Similar conclusions were reached for *trans*-c(Pro-Pro) by Siemion[11]).

Cyclodipeptides containing residues of 2-azetidinecarboxylic acid again show a striking similarity to the PMR parameters in the whole series. Differences between shifts of C_ω—H protons are of a lesser degree than in c(Pro-X) derivatives and the position of their signals (at lower field values as opposed to c(Pro-X)) there is an obvious effect of the altered geometry and hybridisation during transition from a 5- to 4-membered ring. Approximately symmetrical positions of the C_ω—H protons to the amide bond originating in the ring (with practically identical shifts of C_ω—H protons and the approximate values of $J_{\alpha\beta}$ and $J_{\omega,\omega-1}$) are consistent with the boat conformation of the 2,5-piperazinedione ring with annelation of approximately planar four membered ring of the azetidinecarboxylic acid residue.

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