AMINO ACIDS AND PEPTIDES. CXV.* **

2,5-PIPERAZINEDIONES WITH AN ANNELED AZETIDINE RING; SYNTHESIS AND INFRARED SPECTRA

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A series of bicyclic and tricyclic 2,5-piperazinediones (cyclodipeptides) derived from the 2-azeti-dinecarboxylic acid in combination with further amino acids (proline, pipecolic acid, glycine or leucine) were synthesized in both diastereoisomeric forms. The *trans*-isomers cyclo(L-2-azeti-dinecarbonyl-D-2-azetidinecarbonyl) and cyclo(D-2-azetidinecarbonyl-L-prolyl) could not be prepared. The infrared spectra of these substances were measured, along with diastereoisomers of cyclodipeptides derived from phenylalanine and amino acids with 4,5 and 6-membered rings. The spectroscopic data were interpreted from the viewpoint of the conformation of the 2,5-pipe-razinedione ring with changes in bond and torsion angles of the amide group. In compounds containing a phenylalanine residue there was evidence for a hydrogen bond between the N—H group and the aromatic ring.

Previous work[2] has shown that annelation of one or two 5-membered rings to the 2,5-piperazinedione ring results in an increase of the $\nu(C=O)$ wavenumber and a decrease in the $\nu(C_{(0)}—N)$ wavenumber in comparison with 2,5-piperazinedione with an anneled 6-membered ring. These changes in wavenumber have been attributed to a) changes in the bond angle ϱ, b) changes in the conformation of the 2,5-piperazi-nedione ring and c) deviations from planarity of the amide bond due to the additional 5-membered ring which is rigid. The present work constitutes a study of analogous compounds with an added 4-membered ring — cyclodipeptides containing a residue of 2-azetidinecarboxylic acid*** *I–V, VIII*. Changes in the geometric parameters should be even more marked in these polycyclic systems because of the geometry of the 4-membered azetidine ring. Variations in the geometric parameters were obtained by preparation of diastereoisomeric compounds, differing in the relative configuration at the bridgehead atoms (*cis* or *trans*-disubstituted 2,5-piperazinedione rings). Primary attention in discussing the infrared spectra of these compounds was

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paid to stretching vibrations of groups C=O and $C_{(0)}$—N, to complete the information presented in the previous paper[2].

The compounds studied were prepared by cyclisation of linear dipeptide methyl esters. 2-Azetidinecarboxylic acid was prepared according to Fowden[4]. An optically pure preparation was obtained using the hydrazide of L-tyrosine[5]. Due to the lability of the azetidine ring in an acid medium esterification was carried out with diazomethane, the methyl ester of D-2-azetidinecarboxylic acid was isolated as a salt with bis(p-toluenesulphonyl)amine. The dipeptide methyl ester was prepared by condensation using 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline[6]. The cyclodipeptides were prepared by cyclisation of the appropriate dipeptide methyl esters in methanol with addition of methanolic ammonia.

Cyclisation to a 2,5-piperazinedione system with annelation of a 4-membered ring was more difficult than cyclisation to 2,5-piperazinediones so far described. Whereas 2,5-piperazinedione systems with acyclic substituents or with anneled 5- or 6-membered rings can be synthesized in high yield from linear dipeptide methyl esters in a single day (in the extreme case *trans*-c(L-Pip-D-Pip) can be produced in less than 15 minutes[2]) cyclisation to compounds with an anneled 4-membered ring is not complete even after 4 days. Particularly difficult is the cyclisation to 2,5-piperazinedione with two four-membered rings (*IIIa*) the yield being about half that of the other 2,5-piperazinediones. We did not succeed at all in preparing the compound cyclo-(D-2-azetidinecarbonyl-L-prolyl) (*IIb*) or cyclo(L-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*IIIb*). A system with two *trans* anneled small rings is highly strained and these same steric strains put the transition state of the cyclisation reaction at a disadvantage. After processing the reaction mixtures in the latter synthetic attempts we isolated small amounts of cyclo(D-2-azetidinecarbonyl-D-prolyl) (*IIa*) and cyclo-(L-2-azetidinecarbonyl-L-2-azetidinecarbonyl) (*IIIa*) in yields of 8 and 2%, resp. The reason for this phenomenon may be that with molecules which cannot come into a transition state for cyclisation to a *trans* derivative there is an inversion of con-

I, n = 2
II, n = 1
III, n = 0

IV, R = H
V, R = i-C_4H_9

VI, n = 2
VII, n = 1
VIII, n = 0

In compounds *I— III* and *V— VIII a cis*-3,6-disubstitution, *b trans*-3,6-disubstitution.

figuration at the α-carbon of the cyclic amino-acid residue (with a preference for proline) so that cyclisation to a *cis* derivative can occur to a slight degree. An analogous isomerisation of *trans*-c(Pro-Pro) to the *cis* isomer was studied elsewhere[7].

EXPERIMENTAL

Melting points were determined on a Kofler block. Sublimation was carried out at pressure of 1 Torr and temperature 10°C below melting point. Samples for analysis and physical measurement were dried 24 h at 0·5 Torr over P_2O_5. Optical rotations were measured on a photoelectric polarimeter. Mass spectra (AEI MS-902 instrument) of all cyclopeptides corresponded to the assumed structure. Preparation of the phenylalanyl cyclodipeptides *VIa—VIIIb* is described elsewhere[2], as is the preparation of compounds containing no residue of 2-azetidinecarboxylic acid[8].

Benzyloxycarbonyl-D-2-Azetidinecarboxylic Acid

D-2-Azetidinecarboxylic acid was prepared according to Fowden[4]. Dowex 50 was used to free the acid from the Ba^{2+} salt. The resulting acid showed an $[\alpha]_D^{25}$ between +97·3 and +116·9₀ (c 0·5, water). (Fowden[4] presents for L-2-azetidinecarboxylic acid isolated from natural material $[\alpha]_D^{20}$ of −108° (c 3·6, water) and for the synthetic acid $[\alpha]_D^{20}$ +102° (c 3·6, water)). From not completely optically pure D-2-azetidinecarboxylic acid we prepared the N-benzyloxycarbonyl derivative, and from this the salt of N-benzyloxycarbonyl-D-2-azetidinecarboxylic acid with L-tyrosinehydrazide m.p. 208·5—211°C. (Rodebaugh and Cromwell[5] give for this compound m.p. 205—206·5°C, for the free D-2-azetidinecarboxylic acid $[\alpha]_D^{20}$ +107·5° (c 3·5, water)). From this salt the N-protecting group was removed[5] to yield D-2-azetidinecarboxylic acid with $[\alpha]_D^{25}$ +123°; a preparation supplied by Calbiochem showed $[\alpha]_D^{25}$ +122° (both measured at c 0·5 in water). To a solution of the salt of N-benzyloxycarbonyl-D-2-azetidinecarboxylic acid with L-tyrosinehydrazide (2·65 g) in water (12 ml) concentrated hydrochloric acid was added (3 ml) and the mixture was extracted with ethyl acetate. The ethyl acetate extract was evaporated and the remainder dried azeotropically with benzene. The dried material was ground up with light petroleum and crystallised to yield 1·40 g (96%) of benzyloxycarbonyl-D-2-azetidinecarboxylic acid, m.p. 60—63°C, up to the present reported as an oil.[5]. Recrystallisation from ethyl acetate–light petroleum gave a yield of 1·24 g (85%) of a product with m.p. 61—63°C, $[\alpha]_D^{25}$ + + 101·4° (c 0·5, methanol). For $C_{12}H_{13}NO_4$ (235·2) calculated: 61·27% C, 5·57% H, 5·95% N; found: 61·44% C, 5·61% H, 5·88% N.

The Salt of the Methyl D-2-Azetidinecarboxylate with Bis(p-toluenesulphonyl)amine

Benzyloxycarbonyl-D-2-azetidinecarboxylic acid (565 mg) in ether (15 ml) was esterified with a solution of diazomethane in ether. The ether was evaporated off, and to the remainder bis(p-toluenesulphonyl)amine (780 mg) and methanol (30 ml) were added and the mixture was hydrogenolysed over palladium on activated charcoal. Following this, the catalyst was filtered off, the methanol evaporated off, the residue was triturated with ether, filtered and rewashed with ether. The yield was 1 010 mg (94%) m.p. 118—122°C. Recrystallisation from methanol–ether gave a yield of 900 mg (85%) of a product with m.p. 124—126·5°C. The sample for analysis was again recrystallised, m.p. 125·5—127·5°C, $[\alpha]_D^{25}$ +22·4° (c 0·5, methanol). For $C_{19}H_{24}N_2O_6S_2$ (440·5) calculated: 51·80% C, 5·49% H, 6·36% N; found: 51·74% C, 5·54% H, 6·15% N.

Cyclodipeptides

As an example the preparation of cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*IIIa*) is presented. To a solution of benzyloxycarbonyl-D-2-azetidinecarboxylic acid (215 mg) in ethyl acetate (12 ml) at room temperature we added with continuous stirring the methyl ester of D-2-

azetidinecarboxylic acid (obtained from the salt of the methyl ester of D-2-azetidinecarboxylic acid with bis(p-toluenesulphonyl)amine (400 mg) after treatment with a saturated solution of ammonia in chloroform; in the other cases we used the hydrochloride of the amino acid methyl esters) and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (235 mg). This mixture was stirred overnight, and then washed with 1M-HCl, 0·5M-NaHCO$_3$, water, and finally dried over Na$_2$SO$_4$. The ethyl acetate was evaporated and the residue was hydrogenolysed in methanol (40 ml) over palladium on activated charcoal. After completing the hydrogenolysis the catalyst was filtered off and saturated ammonia in methanol (0·5 ml) was added. The mixture was left for 4 days at room temperature. Methanol was then evaporated off, the residue was dissolved in 50% methanol (50 ml) and the 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 to give cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl), which was sublimated after recrystallisation. In the same manner all of the other substances (Table I) were prepared.

TABLE I

Properties of Synthesized Substances

Cyclodipeptide	M.p.[a], °C yield[d], %	M.p.[b] °C	Formula (m. w.)	Calculated/Found			$[\alpha]_D^{25c}$ (c), %
				% C	% H	% N	
c(D-Aze-D-Pip)[e] *Ia*	135·5—138 40	135—138	C$_{10}$H$_{14}$N$_2$O$_2$ (194·2)	61·84 61·56	7·27 7·32	14·42 14·46	— 20·1 (0·3)
c(L-Aze-D-Pip) *Ib*	118·5—121 56	120·5—122·5	C$_{10}$H$_{14}$N$_2$O$_2$ (194·2)	61·84 61·92	7·27 7·37	14·42 14·60	+ 43·9 (0·4)
c(D-Aze-D-Pro) *IIa*	121·5—125·5 42	131—133	C$_9$H$_{12}$N$_2$O$_2$ (180·2)	59·99 60·04	6·71 6·81	15·55 15·85	+136·8 (0·3)
c(D-Aze-D-Aze) *IIIa*	200—210 23	208—209	C$_8$H$_{10}$N$_2$O$_2$ (166·2)	57·82 58·06	6·07 6·17	16·86 16·99	+ 53·8 (0·3)
c(D-Aze-Gly) *IV*	182—185 50	184—186	C$_6$H$_8$N$_2$O$_2$ (140·1)	51·42 51·25	5·75 5·68	19·99 20·02	+121·4 (0·3)
c(D-Aze-D-Leu)[f] *Va*	147—153 51	154—154·5	C$_{10}$H$_{16}$N$_2$O$_2$ (196·3)	61·20 61·15	8·22 8·23	14·27 14·24	+ 73·1 (0·4)
c(D-Aze-L-Leu) *Vb*	144·5—150·5 60	151—152	C$_{10}$H$_{16}$N$_2$O$_2$ (196·3)	61·20 61·19	8·22 8·20	14·27 14·15	+ 51·5 (0·5)

[a] Melting point in given yield; [b] melting point of a sample for analysis and physical measurement after recrystallisation from ethanol–ether–light petroleum; [c] in methanol; [d] related to benzyloxycarbonyl-2-azetidinecarboxylic acid; [e] first sublimated, then recrystallised; [f] prepared also with N,N'-dicyclohexylcarbodiimide in a yield of 65% for a product of m.p. 140—150°C. During synthesis of this compound the crystalline intermediate Z-D-Aze-D-Pip-OMe was obtained, m.p. 108—110°C $[\alpha]_D^{25}$ 163·4° (c 0·5 in methanol); for C$_{19}$H$_{24}$N$_2$O$_5$ (360·4) calculated: 63·32% C, 6·71% H, 7·77% N; found: 63·69% C, 6·61% H, 7·76% N;

TABLE II

Wavenumbers of $\nu(CO)$ Bands (cm^{-1}) of Substances $I-VIII$ in Tetrachloromethane

Spectra measured with Perkin-Elmer model 621 instrument calibrated with water and ammonia; precision ± 0.5 cm^{-1}; spectral slit width 2 cm^{-1}; values directly read off. Tetrachloromethane solutions used for measurement had for substances $I-II$ a concentration of 0.07M (cell 0.05 mm), for substance $IIIa$ 0.0033M (cell 1 mm), for substance IV saturated solution (cell 1 cm), for substance V 0,0005M (cell 1 cm), for substances $VI-VIIa$ concentration 0.0003M (cell 1 cm), substance $VIIIb$ was measured in a saturated solution (cell 1 cm).

Substance	$\nu(CO)$ tert-amide		$\nu(CO)$ sec-amide	Substance	$\nu(CO)$ tert-amide		$\nu(CO)$ sec-amide
Ia	1 671·2	1 692·7	—	Ib	1 668·5	1 689·1	—
IIa	1 671·6	1 695·6	—	IIb	—		—
$IIIa$	1 668·7	1 690·0	—	$IIIb$	—		—
IVa		1 699		IVb			—
Va	1 682·4 sh	1 699·4		Vb		1 681·8 sh	1 695·3
VIa	1 659·4		1 693·6	VIb	1 661·2		1 694·2
$VIIa$	1 676·6		1 704·0	$VIIb$	1 670·4		1 699·0
$VIIIa$		1 697·6		$VIIIb$		1 679·5 sh,	1 697·4

TABLE III

Wavenumbers of $\nu(CO)$ Bands (cm^{-1}) in Chloroform

Substance[a]	$\nu(CO)$ cm^{-1}			Substance[a]	$\nu(CO)$ cm^{-1}		
c(Pip-Pip)cis		1 648·6	1 656·6 A	c(Pip-Leu)cis	1 648·2	1 655·9	1 689·9 B
c(Pip-Pip)trans		1 647·6	1 649·6 A	c(Pip-Leu)trans	1 647·3	1 655·6	1 697·7 B
c(Pip-Pro)cis		1 654·6	1 663·6 A	c(Pro-Leu)cis	1 667·2		1 687·2 B
c(Pip-Pro)trans		1 656·8	A	c(Pro-Leu)trans	1 660·4	1 668·7	1 682·8 B
c(Pro-Pro)cis		1 659·4	1 668·4 A	Va	1 685·3		B
c(Pro-Pro)trans		1 658·1	1 671·1 A	Vb	1 680·2		1 693·2 B
Ia		1 656·8	1 671·8 A	VIa	1 647·3	1 654·2	1 680·6 B
Ib	1 652·3	1 667·3	1 682·3 A	VIb	1 654·3	1 683·3	B
IIa	1 657·6	1 667·6	1 691·6 A	$VIIa$	1 671·3	1 686·4	B
$IIIa$		1 668·4	1 686·4 A	$VIIb$	1 657·9	1 666·2	1 684·4 B
c(Pip-Gly)	1 654·0	1 661·4 1 683·4	1 691·4 B	$VIIIa$	1 685·1		B
c(Pro-Gly)		1 666·3	1 689·3 B	$VIIIb$	1 681·1	1 693·0	B
IV		1 685·5	1 703·5 B				

[a] For measurements see Table II. Directly measured values. Concentrations of chloroform solutions: A 0.05M (cell 0.07 mm), B 0.002M (cell 2 mm).

In attempts to synthesize cyclo(D-2-azetidinecarbonyl-L-prolyl) (*IIb*) after 4 days of cyclisation of the methyl ester of D-2-azetidinecarbonyl-L-proline, filtration through the ion exchangers, sublimation and recrystallisation, we achieved a 8% yield of cyclo(D-2-azetidinecarbonyl-D-prolyl) (*IIa*), m.p. 121—129°C. In an attempt to prepare cyclo(L-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*IIIb*) after 4 days of cyclisation of the methyl ester of L-2-azetidinecarbonyl-D-2-azetidinecarboxylic acid, ion exchanger filtration, sublimation and recrystallisation we obtained a 2% yield of cyclo(L-2-azetidinecarbonyl-L-2-azetidinecarbonyl) (*IIIa*), m.p. 204—206°C.

Spectroscopic Measurement

IR-Spectra were measured on a Perkin-Elmer 621 instrument. Calibration was carried out with water vapour and ammonia. The precision of measurement was ± 0.5 cm^{-1}. The spectral slit width used was 2 cm^{-1}. Numerical resolution of the spectra in separate bands was performed by an Elliott 503 or a Tesla 200 computer with the assumption that the shape of the band can be described by a Lorentz distribution (Cauchy method of damped mean squares[9–11]). With substances containing N—H bonds (bicyclic 2,5-piperazinediones) in the region of ν(C=O) we measured with 1 cm cells in a solution of tetrachloromethane at a concentration of $3-5 \cdot 10^{-4}$M, or in a saturated solution if solubility was low. We always excluded the possibility of association. For measurement in the region of ν(N—H) we used a concentration of $3-5 \cdot 10^{-4}$M in tetrachloromethane and 10 cm cells. Tricyclic compounds were measured in tetrachloromethane at a concentration of 0·07M with a 0·5 mm cell, or at lower concentrations if solubility was too low (see footnote to Table II). Chloroform solutions for measurements in the region ν(C=O) of tricyclic compounds were at a concentration of 0·05M in 0·07 mm cells, with substances with a N—H bond at 0·002M with a 2 mm cell (Table III). The same concentration was used for measurement in the ν(N—H) region with 2 cm cells. For measurement between 1 260 and 1 500 cm^{-1} with tricyclic compounds we used a tetrachloromethane solution at 0·07M in 0·1 mm cells, with all other substances a concentration of 0·015M in 0·5 mm cells. In most cases, particularly where the solubility was low, the spectra were also measured under different conditions (*i.e.* lower concentration with a longer optical pathway) in order to get an optimal spectral reading — for details see footnotes to Table IV. Chloroform solutions for measurements in this region were 0·05—0·08M, cell 0·1 mm. Not in all cases was it possible to carry out measurements in the region from 1 260 to 1 500 cm^{-1} under the same degree of association; in tetrachloromethane the substances were partially associated, in chloroform practically not associated at all.

RESULTS AND DISCUSSION

Substances studied in the present work and previously[2] represent a fairly complete series of bi- and tricyclic 2,5-piperazinediones in which structural changes alter geometrical parameters over a wide range. The results of studies on extremely deformed compounds with anneled 4-membered rings are basically in agreement with previous observations on compounds with larger rings. Extreme changes in geometry are manifest by very marked changes in the infrared spectrum which allow us to complete and affirm previous conclusions. In another paper[8] we described the spectra of proton magnetic resonance of all of the substances studied here and on this basis we deduced probable conformations. From these concepts further discussion can be developed.

The ν(C=O) region: In that case where the 2,5-piperazinedione system has anneled

to it two 6- or 5-membered rings, or a combination, the spectrum measured in tetrachloromethane contains only a single band for $v(C=O)$ for both *cis* and *trans* anneled ring[2]. For the determination of intensity by means of a computer it was shown that the shape of the band can for most substances be best approximated by two overlapping bands — a weaker one at lower wavenumbers and a stronger at higher. This manner of curve approximation gave the suitable error of fit. In the spectra of these substances measured in chloroform in the carbonyl region there are two more or less overlapping bands (Table III). The lower wavenumber band is either stronger than or equal to the larger wavenumber band. The appearance of a further band in the spectrum in the $v(C=O)$ region in chloroform solution is considered a manifestation of Fermi resonance. As a result of the shift in wavenumber of the $v(C=O)$ band to lower values due to greater solvent polarity (chloroform) Fermi interactions between this band and the lower wavenumber band is increased and there is an alteration in the ratio of intensities between the two bands. Similar behaviour can be seen clearly in the spectrum of *cis*-c(D-Aze-D-Aze) (*IIIa*) where the half-width of both bands decreases and the bands do not overlap even in the spectrum measured in tetrachloromethane solution. In the latter solution the spectrum contains a weak band at lower wavenumbers and a stronger at higher, in chloroform solution this relation is reversed. With *cis*-c(D-Aze-D-Aze) we calculated the unperturbed value of the $v(C=O)$ vibration and showed that the shift in the wavenumber of this vibration produced by Fermi resonance was $+3$ cm^{-1}. For 2,5-piperazinedione with anneled 4-membered and larger rings (5, 6) in the carbonyl region measured in tetrachloromethane we see two bands, in chloroform solution there are three bands. In these compounds there are two $v(C=O)$ vibrations with different wavenumbers, and in addition there is splitting due to Fermi resonance in the same manner as with the previously discussed molecules.

In a compound in which the 2,5-piperazinedione system has anneled to it one 4-membered and one 6-membered ring (*Ia, Ib*) in the carbonyl region of the IR spectrum in tetrachloromethane solution there are two different $v(C=O)$ vibrations suggesting an asymmetric deformation of the piperazinedione ring. This shows a difference from previously measured compounds with one 5-membered and one 6-membered ring (*cis*- and *trans*-c(Pro-Pip)) where the single $v(C=O)$ vibration under the same conditions suggests a symmetrical deformation of the 2,5-piperazinedione ring. This observation is in agreement with X-ray determination[12] of the structure of *cis*-c(L-Pro-L-Leu) where a symmetric deformation of the piperazinedione ring was found despite the difference in the substituents. The replacement of a 5-membered ring in c(Pro-Pip) by a 4-membered (*Ia, Ib*) changes the wavenumber of the stretching vibration of the carbonyl in the amide group, the nitrogen atom of which is part of the 6-membered ring, to only a slight degree ($v(C=O)$ in *trans*-c(D-Pro-L-Pip) $1669{\cdot}0$ cm^{-1},* in *cis*-

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*   Values of $v(C=O)$ wavenumber are given in the text for all tricyclic 2,5-piperazinediones as calculated by band separation (Table V).

TABLE IV

Wavenumbers of Bands (cm^{-1}) with Largest Contribution of $\nu(C_{(o)}-N)$ Vibration

Substance^a	CCl$_4$	CHCl$_3$	Substance	CCl$_4$	CHCl$_3$
Ia	1402·2 1408·8	1419·1	c(Pro-Gly)	1426·4 1442·4	1433·0 1450·0
Ib	1430·7 1463·6	1443·2 1467·2	cis-c(Pro-Leu)	1408·3	1416·3
IIa	1398·0 1407·0	1416·0	trans-c(Pro-Leu)	1427·7 1431·7	1434·1
IIIa	1396·8	1415·4	VIIa	b	1417·5
			VIIb		1437·6
c(Pip-Gly)	1455·2 1462·1	1459·1 1465·1	c(Aze-Gly)	1412·8 1433·7	1425·7 1438·7
cis-c(Pip-Leu)	1433·9 1443·9	1434·9 1445·4	Va	1386·2	1400·8
trans-c(Pip-Leu)			Vb	1407·3	1423·5
VIa	1436·6 b	1440·3	VIIIa	b	1405·5
		1438·7	VIIIb		1429·6
VIb	1435·6 1441·4	1439·0 (1445·8)			

TABLE V

Wavenumbers (cm^{-1}) and Intensities of $\nu(C=O)$ Bands after Computer Separation, Measured in Tetrachloromethane

Compound^a	$\nu(C=O)$	B	$\sum B$	Compound^a	$\nu(C=O)$	B	$\sum B$
cis-c(Pip-Pip)	1663·4	45 524·7	45 524·7	trans-c(Pip-Pip)	1662·4	45 069·2	45 069·2
cis-c(Pro-Pro)	1676·4	45 108·3	45 633·6	trans-c(Pro-Pro)	1668·8	43 419·6	44 104·8
	1665·3	525·3			1661·9	685·2	
IIIa^b	1690·0	40 124·4	46 566·2	—			—
	1668·0	6 441·8					
cis-c(Pip-Pro)	1668·3	48 584·6	48 584·6	trans-c(Pip-Pro)	1673·1	37 240·8	48 840·4
					1667·3	11 639·6	
Ia	1693·4	34 382·9	54 349·5	Ib	1689·0	34 227·9	58 713·9
	1670·6	19 966·6			1667·8	24 486·0	
IIa	1696·0	34 608·1	60 464·9	—			—
	1671·4	25 856·8					

-c(L-Pro-L-Pip) 1 668·3 cm^{-1}, in *trans*-c(L-Aze-D-Pip) *(Ib)* 1 667·8 cm^{-1}, in *cis*-
-c(D-Aze-D-Pip) *(Ia)* 1 670·6 cm^{-1}). This suggests a maintenance of geometric para-
meters in that part of the ring in comparison with c(Pro-Pip). The wavenumber
of the ν(C=O) band of the amide group the nitrogen atom in which is part of the
4-membered ring is increased in both diastereoisomers of c(Aze-Pip). This increase
is attributed mainly to an increase in non-planarity of the amide group in this half
of the 2,5-piperazinedione ring. In *cis*-c(Aze-Pro) *(IIa)* the wavenumber of the
ν(C=O) band of amide group the nitrogen atom of which is part of the 5-membered
ring does not basically differ from values for this vibration in both isomers c(Pro-Pip)
(ν(C=O) in *cis*-c(Aze-Pro) *(IIa)* 1 671·4 cm^{-1}, in *cis*-c(Pro-Pip) 1 668·3 cm^{-1}, in
trans-c(Pro-Pip) 1 669·0 cm^{-1}). This suggests a similar geometry of part of the
2,5-piperazinedione system in the neighbourhood of the amide bond arising from
the 5-membered ring, as in c(Pro-Pip). The wavenumber of the stretching vibration
band of the carbonyl group in the amide bond arising from the 4-membered ring
has the highest value of the entire series (1 696·0 cm^{-1}). We consider this again
a manifestation of non-planarity of the amide grouping.

In *cis*-c(Aze-Aze) *(IIIa)* one ν(C=O) vibration exists for both carbonyls of the
2,5-piperazinedione ring (in the spectrum Fermi resonance splitting can still be ob-
served, as has been discussed). The value of the ν(C=O) wavenumber read off
directly from the spectrum (1 690·0 cm^{-1}) is quite high, as a result of amide group
non-planarity.

Annelation of one 5- or 6-membered ring to the piperazinedione ring substituted
with benzyl side chain (compounds *VIa, VIb, VIIa, VIIb*) gives rise to two amide
systems, secondary and tertiary. In the spectra this is manifest by two bands of ν(C=O)
vibrations. The higher wavenumber band belongs to the secondary amide system.
This was suggested by measurements in associated and nonassociated states. Only
the larger wavenumber band is sensitive to a change in concentration and therefore

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Notes to Table IV and V.

^{a} For measurements see Table II. Directly read values. Concentration of tetrachloromethane
solutions: *I, IIa, Vb* 0·07M (cell 0·1 mm); *IIIa*, c(Pip-Gly) saturated solution (1 mm cell);
c(Pro-Gly), *IV*, saturated solution (10 mm cell); remainder 0·015M (0·5 mm cell). Concentration
of chloroform solutions: c(Pip-Gly), c(Pro-Gly) 0·05M (0·1 mm cell); *IV* 0·08M (0·1 mm cell);
remainder 0·07M (0·1 mm cell). ^{b} Insoluble.

^{a} For measurements see Table II. Bands were separated on computers Elliott 503 and Tesla 200.
Concentrations for *Ia, Ib, IIa, IIb* 0·07M (0·05 mm cell), for *IIIa* 0·0033M (1 mm cell), for remainder
0·015M (2 mm cell) in tetrachloromethane; B integrated intensity (in units 1 mol^{-1} cm^{-2}) of
separate bands; $\sum$B sum of intensities of both bands. ^{b} Wavenumber value corrected for Fermi
resonance 1 687·0 cm^{-1}.

is an attribute of the vibration of the secondary amide bond. Angular effects of annelation of the side-ring are manifest in both bands of $\nu(C{=}O)$ by an increase in wavenumber. At the same time the wavenumber of the $\nu(C{=}O)$ vibration of the tertiary amide bond increased with decreasing size of the anneled ring much more rapidly than the wavenumber of the $\nu(C{=}O)$ vibration of the secondary amide bond (nonplanarity of the amide bond may play a role here). As a matter of fact in c(Aze-Phe) (*VIIIa, VIIIb*) both $\nu(C{=}O)$ vibration bands completely overlap. Cyclodipeptides containing leucine or glycine instead of phenylalanine show parallel properties to the above series of phenylalanine derivatives. In all three series there is a superposition of both $\nu(C{=}O)$ vibrations of the two carbonyl groups in compounds with anneled 4-membered rings.

The integrated intensity was determined only for bands of $\nu(C{=}O)$ vibrations of compounds with two anneled rings. Determination of the integrated intensity of the bands of $\nu(C_{(0)}{-}N)$ vibrations would require a very complex separation of a number of overlapping bands. The integrated intensities presented in Table V are summarized values (with respet to Fermi resonance) for all bands found in the carbonyl region of the spectrum measured in tetrachloromethane. From the presented data it would appear that annelation of smaller and smaller rings, with the resulting angular distortion of the 2,5-piperazinedione ring and with non-planarity at the $C_{(0)}{-}N$ bond, has no effect on the intensity of the $\nu(C{=}O)$ band in that case in which the 2,5-piperazinedione ring is symmetrically substituted. With asymmetric substitution the integrated intensity increases slightly with decreasing size of the side-ring.

The $\nu(C_{(0)}{-}N)$ region: Bands corresponding to $\nu(C_{(0)}{-}N)$ vibrations were, as previously reported[2], detected by means of solvent shift. In previous observations there were reported changes in band position of the $\nu(C_{(0)}{-}N)$ vibration in relation to side-ring annelation where in cyclodipeptides containing proline there was a basic difference between *cis* and *trans* diastereoisomers. In *cis* isomers c(Pro-Pro) and c(Pro-Pip) the wavenumber of the $\nu(C_{(0)}{-}N)$ vibration was $1420-1425\ \mathrm{cm}^{-1}$. Due to the large shift with solvent change this vibration was characteristic as opposed to the situation with *trans* isomers $\nu(C_{(0)}{-}N)$ (about $1450\ \mathrm{cm}^{-1}$, small solvent shift). The low value and increase in characteristicity of this vibration was interpreted as a reflection of a boat conformation of the 2,5-piperazinedione ring and non-planarity of the amide bond. *cis*-2,5-Piperazinediones with a 4-membered ring show the following trend: with decreasing size of the anneled ring there is a decrease in the wavenumber of the $\nu(C_{(0)}{-}N)$ vibration arising from the 4-membered ring and the characteristicity of this vibration increases, as is seen from the increasing solvent shift. This trend documents the deepening of the boat conformation of the 2,5-piperazinedione ring as a result of annelation of smaller and smaller rings. The wavenumber of the $\nu(C_{(0)}{-}N)$ vibration from 6- (*Ia*) and 5-membered (*IIa*) rings is also decreased as a result of a marked boat conformation of the 2,5-piperazinedione ring. This wavenumber shifts in the same direction with increasing non-planarity of the amide

group. Differences in non-planarity at this site in substance *Ia* and *IIa* are manifest in differences in the wavenumbers of both $C_{(0)}$—N bonds (Table IV). In *trans*-c(Aze-Pip) (*Ib*) we assume an asymmetric deformation of the 2,5-piperazinedione ring. One half of this ring, part of the 6-membered ring and the amide bond arising from it, is planar and the $\nu(C_{(0)}$—N) vibration of this bond is manifest by several bands slightly sensitive to a solvent change (the most sensitive is at $1463 \cdot 6 \text{ cm}^{-1}$). The second half, corresponding to a 4-membered ring with an amide bond arising from it, is not planar and in the infrared spectrum shows a band at $1430 \cdot 7 \text{ cm}^{-1}$ with a greater solvent shift.

In the series of bicyclic cyclodipeptides substituted with an acyclic side chain, *i.e.* substances containing phenylalanine or leucine residues, there is the same behaviour in dependance on both structural parameters — relative configuration of substituents and size of the anneled ring. Here we must take into account the fact that in the region 1390 to 1500 cm^{-1} $\nu(C_{(0)}$—N) bands of the tertiary amide bond and amide-II bands of secondary *cis*-amide groups are located. (The latter bands have a contribution of the deformation vibration of the N—H bond.) Because of the low transmittance of solvents in this region we had to use such high concentrations of solute in tetrachloromethane solution that there was a marked degree of association. Association was only slight in chloroform solution. One must also consider association effects on the shift of the amide-II band. Increased association causes a band shift to greater wavenumbers (in tetrachloromethane) or it may give rise to a doubling of the bands. In chloroform the change in the mesomeric state of the $C_{(0)}$—N bond produces a band shift

TABLE VI
ν(N—H) Wavenumbers (cm^{-1})

| Compound[a]	CCl$_4$		CHCl$_3$	
	ν(N—H) free	ν(N—H) bound	ν(N—H) free	ν(N—H) bound
IV	3 423·6	—	3 411·5	—
Va	3 413·0	—	3 403·8	—
Vb	3 414·6	—	3 403·8	—
VIa	3 398·5 (95%)	3 382·8 (5%)	3 387·5	[b]
VIb	3 398·5 (60%)	3 384·6 (40%)	3 385·5	3 374·6
VIIa	3 407·2 (5%)	3 391·2 (95%)	[c]	3 378·9
VIIb	3 408·3 (100%)	—	3 397·9	[b]
VIIIa	3 410·5 (5%)	3 389·8 (95%)	3 400·1	3 375·6
VIIIb	3 407·2 (100%)	—	3 396·7	[b]

[a] For measurements see Table II. Direct values. Concentrations in tetrachloromethane: *IV*, *VIIIb* saturated solution (10 cm cell); *Va*, *Vb* $5 \cdot 10^{-4}$M (10 cm cell); remainder $3 \cdot 10^{-4}$M (10 cm cell). Concentration of chloroform solutions $2 \cdot 10^{-3}$M (2 cm cell). [b] Asymmetry of the band at lower wavenumbers. [c] Asymmetry of the band at higher wavenumbers.

also to higher wavenumbers, but the simultaneous decrease in association tends to shift the band to lower wavenumbers. This has been tested by measuring the temperature dependence of *cis*-c(Pip-Leu) in tetrachloroethylene. With increasing temperature, as the band of associated $\nu(N—H)$ disappeared, one of the bands in the region $1400-1500$ cm^{-1} shifted to lower values. The band started at $1436\cdot5$ cm^{-1} at $50°C$ and shifted to $1432\cdot9$ cm^{-1} at $88\cdot8°C$. In bromoform, where there was no association at $50°C$, a further increase in temperature caused no spectral shifts. The band remained at $1436\cdot1$ cm^{-1}. This substance therefore showed a clear solvent shift only when measured in the given solvents in the nonassociated state. This effect may distort the amide-II band shift, particularly in substances with a planar or almost planar conformation of the 2,5-piperazinedione ring and with a small solvent shift in this band.

The conformational characteristics of the studied compounds presented here were derived on the basis of NMR studies[8]. IR spectroscopy, however, made possible greater detail in our concepts of some geometric parameters. A decrease in size of the anneled ring is manifest by a sharper angle ϱ, which in azetidine derivatives is quite marked in degree. Undoubtedly, annelation of a smaller ring also results in a deviation of the amide bond from planarity, *i.e.* that bond arising from the smaller ring. The concept appears plausible that the cause of this distortion is an increase in the pyramidal arrangement at the nitrogen atom which is more suitable for the small internal bond angle of the anneled ring. Other changes in the spectroscopic characteristics in relation to the size of the anneled ring are in full agreement with the concept of a deepening of the boat conformation of the 2,5-piperazinedione ring with annelation of smaller and smaller rings. *trans*-Dianneled compounds generally tend to have a planar central ring. In the case of annelation of a 4-membered ring planarity of the central ring, and planar arrangement of the amide bonds, can only be attained by severe distortion of the bond angles. The latter deformation is obviously too great an energetic requirement, so that planar (or partially planar) arrangement arises only if the second ring is sufficiently large, *i.e.* if it behaves essentially like an acyclic substituent.

The $\nu(N—H)$ region: In the region of the $\nu(N—H)$ stretching vibrations a decrease in the wavenumber of free $\nu(N—H)$ band can be observed with 2,5-piperazinediones substituted with isobutyl $(-10$ cm$^{-1})$ or benzyl groups $(-15$ cm$^{-1})$ in comparison with nonsubstituted (glycine) derivatives. This shift can be considered as an effect of increased bulk. In compounds *VIa, VIb, VIIa, VIIIa* two bands were found in this region which were assigned to stretching vibrations of free N—H groups and of N—H groups attached by hydrogen bonds to π-electrons of the aromatic nucleus (Table V). From the ratio of intensities of both $\nu(N—H)$ bands we estimated the population of conformers in which such hydrogen bonds can be realised: *VIa* 5%, *VIb* 40%, *VIIa* 95%, *VIIb* 0%, *VIIIa* 95%, *VIIIb* 0%. In *trans* isomers *VIIb, VIIIb*, the benzyl substituent is oriented over the 2,5-piperazinedione ring (folded conforma-

tion). Here, there are favourable geometric conditions for interaction of the aromatic system and dipoles of amide groups however not for hydrogen bonding starting from N—H group. The IR spectroscopy cannot evidence a preference for any of three rotamers *S*, *T*, *U* (Scheme 1). However, NMR spectroscopy shows for compounds *VIIb* and *VIIIb* the population of 60% of the rotamer *S*. In *cis* isomers *VIIa*, *VIIIa*, the local arrangement which would enable the same interaction as in *trans* isomers *VIIb*, *VIIIb* is geometrically excluded and the molecule takes up one of the conformations *P*, *Q*, *R*. According to the inspection of the space-filling models, a hydrogen bond between the N—H group and aromatic ring can occur in conformers *Q* and *P*. From NMR data the preference (75%) of extended conformations *Q* or *R* is obvious. So we can conclude that in *VIIa* and *VIIIa* approximately 75% of molecules assume a conformation like *Q*, the rest a conformation like *P* with intramolecular hydrogen bonding in both conformations. With diastereoisomeric pipecolic acid derivatives (*VIa* and *VIb*) a greater intramolecular hydrogen bonding was observed with *trans* than with *cis* isomer. The reason for this different configurational influence is to be seen in a different geometrical shape of the 2,5-piperazinedione ring. In all cyclodipeptides containing proline or 2-azetidinecarboxylic acid residues the 2,5-piperazinedione ring assumed a more or less deep boat conformation. However in the *cis*-isomer *VIa*, the piperazinedione ring is nearly planar as evidenced by NMR spectroscopy In this situation there is still a good possibility for an interaction between the aromatic ring and dipoles of amide groups. The aromatic ring is located over the 2,5-piperazine-
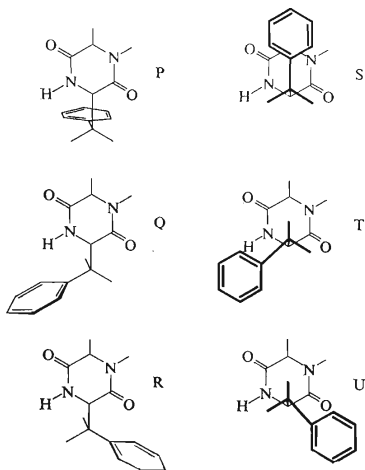
Scheme 1

dione ring and intramolecular hydrogen bonding is effectively excluded. In the corresponding *trans* isomer *VIb* a very flattened boat conformation was found, probably with reversed chirality than is the chirality of the boat-shaped 2,5-piperazinedione ring in *trans*-c(Pro-Phe) (*VIIb*). Non-strained six-membered ring behaves in some respect like an acyclic side chain. A competition of both side chains could be expected; no one of them could be conformationally dominating. We have to admit for compound *VIb* a mixture of several thermodynamically similar conformations with different physical properties. In agreement with this assumption we found slightly more than 50% population of folded conformers by means of NMR spectroscopy and 60% population of hydrogen bonded species by means of IR spectroscopy with compound *VIb*.

Comparing properties of the prolyl derivatives and corresponding derivatives of the 2-azetidinecarboxylic acid we can resume that the annelation of a four-membered ring to the 2,5-piperazinedione ring makes all peculiar properties of prolyl cyclodipeptides more pronouced: *a*) The boat conformation of the 2,5-piperazinedione ring is deepened, *b*) The change in bond angles is very large. *c*) The local conformation on the bridgehead nitrogen atom (azetidine–piperazine ring fusion) is evidently changed into a pyramidal one causing the non-planarity of the amide grouping. The conformational transmission of these effects on the other amide group is significant in all compounds with the exception of pipecolic acid derivatives. Pipecolic acid moieties of the 2,5-piperazinedione ring in various molecules remarkably preserve a planar (or nearly planar) arrangement.

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