Peptidic Cyclols. Synthesis, and Crystal and Molecular Structure of a Tricyclic Thia-cyclol [1]

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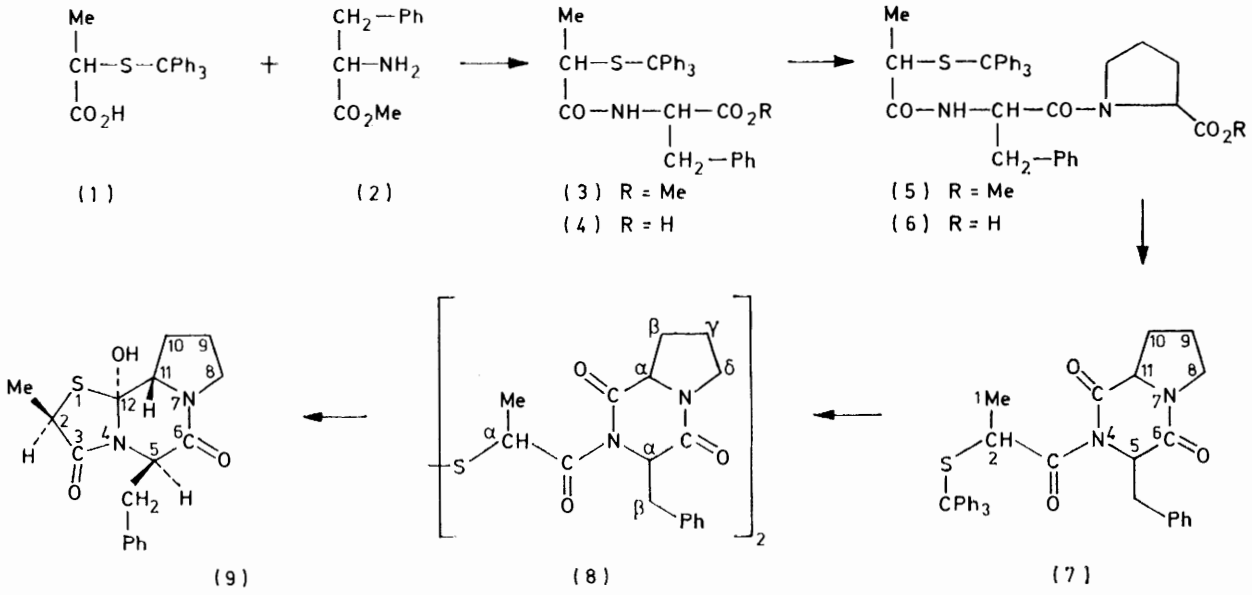
By following a three-step procedure, the linear peptide [(*RS*)-2-tritylthiopropionyl]-L-phenylalanyl-L-proline (6) has been converted into the cyclic derivative (9). On the basis of spectroscopic data and *X*-ray crystallographic analysis, compound (9) is shown to be a thia-cyclol whose tricyclic system is related to the peptidic portion of the ergot alkaloids. Properties of the new compound are compared to those of previously studied peptidic aza- and oxa-cyclols. The thiazolidinone ring of (9) adopts in the crystal an approximate envelope conformation, whereas the pyrrolidine ring assumes a half-chair conformation. The benzylic side chain of the phenylalanine residue adopts in the crystal a folded conformation which seems to be preferred even in chloroform solution.

For many years our laboratories have been engaged in the synthesis and structural studies of stable peptidic cyclols.[2] These compounds, which are characterized by the presence of a free hydroxy group, represent an uncommon example of the stable tetrahedral addition intermediates deriving from intramolecular attack of OH, NH, or SH groups on amide carbonyls. The chemistry of cyclo-oligopeptides, which are increasingly considered as valuable model systems for the elucidation of protein structure,[3] is strictly related to cyclol formation [4,5] and cyclolic tautomers are apparently stable from intramolecular addition of SH groups to amide carbonyls, is at present unknown. We report here the synthesis, the properties and the *X*-ray crystallographic analysis of the first peptidic thia-cyclol whose structure is related to the peptidic portion of the ergot alkaloids.

RESULTS AND DISCUSSION

To achieve such a synthesis two different procedures were considered: (*i*) treatment in mild alkaline aqueous medium of the *p*-nitrophenyl ester of the linear peptide 2-mercaptopropionylphenylalanylproline. This method



Scheme Only the 2*R*,5*R*,11*S*,12*S* enamtiomer of (9) is shown

forms of the nine-membered cyclo-tripeptides and cyclo-depsitripeptides in which a residue of a primary α-amino-acid is contained.

Several cyclolic cyclo-tripeptides derived from intramolecular interaction of OH or NH groups with amide carbonyls are at the present known and their structure and properties have been extensively studied.[2,6] Although aromatic thia-cyclols, obtained from *N*-(*o*-mercaptobenzoyl)lactams, have been synthesized and studied,[7] the entire class of peptidic thia-cyclols, derived was successfully employed for the synthesis of aza-cyclols [2] and oxa-cyclols,[8] starting from *Z*-Ala-Phe-Pro-ONp and from *N*-[(2*R*)-2-ethoxycarbonyl-2-hydroxypropionyl]-Phe-Pro-ONp respectively; (*ii*) synthesis of the *S*-protected *N*-(2-mercaptopropionyl)-*cyclo*-(Phe-D-Pro). Deprotection should be followed by intramolecular addition of the SH group to the imidic carbonyl to give the desired thia-cyclol or the tautomeric cyclo-thiodepsipeptide.

As a useful starting material to explore both pro-

cedures we prepared by conventional methods [(*RS*)-2-tritylthiopropionyl]-L-phenylalanyl-L-proline. Treatment of this compound with dicyclohexylcarbodi-imide and *p*-nitrophenol gave the *S*-protected active ester. Although sulphur detritylation in the presence of activated carboxy functions is known, no satisfactory results have been obtained in the detritylation of the active ester with trifluoroacetic acid in different conditions. This difficulty should be at least in part connected with the cyclization tendency of the detritylated compound.

To examine procedure (*ii*) we treated the linear peptide (6) with acetic anhydride and sodium acetate. It is known that analogous *N*-protected linear tripeptides, containing proline as the *C*-terminal residue, can react in these conditions to give the corresponding *N*-acyl-*trans*-diketopiperazines [9] [starting from *N*-benzyloxycarbonyl-L-alanyl-L-phenylalanyl-L-proline, the *N*-(*N*-benzyloxycarbonyl-L-alanyl)-*cyclo*-(L-phenylalanyl-D-prolyl) was obtained [2c]]. Probably due to the steric hindrance of the *S*-trityl group at the α-position of the first residue, cyclization of (6) was found to be slower than in the above-mentioned cases. To achieve satisfactory yields, heating at 100 °C for 5 h was necessary. In these conditions partial racemization of the phenylalanine asymmetric centre took place. As expected, the reaction mixture contained only the two partially racemic diastereoisomeric *N*-acyldiketopiperazines [namely (2*S*,5*S*,11*R*) and (2*R*,5*S*,11*R*)] having differing configurations at C-5 and C-11.[10,11] Treatment of the diastereoisomers isolated from the reaction mixture with hydrazine hydrate,[2c,12] in controlled conditions, did not in fact reveal the presence of *cis*-diketopiperazine; only *cyclo*-(Phe-D-Pro), containing *ca.* 35% of *cyclo*-(D-Phe-Pro), could be isolated.

Removal in a single step of the *S*-trityl group from (7) was found to be unsatisfactory due to simultaneous fission of the imidic bonds. Since a system formed by two acyldiketopiperazines joined by a disulphide bridge appeared to be a suitable precursor of the thia-cyclol, we treated the *S*-trityl-acyl-diketopiperazine (7) according to the method of Kamber and Rittel.[13] This procedure allows the one-step conversion of the *S*-tritylcysteine-containing protected peptides into cystine peptides, by treatment with iodine in methanol. In the case of the *S*-trityl-acyl-diketopiperazine (7) Kamber–Rittel oxidation gave, at room temperature, the desired disulphide (8). Crystallization of this product from methanol afforded almost pure crystals of the most abundant diastereoisomer. Reaction with hydrazine on this compound gave racemic *trans*-diketopiperazine; as could be deduced on the basis of the subsequent results, compound (8) corresponded to the (2*S*,5*S*,11*R*)-diastereoisomer and to its enantiomer.

The structure of the disulphide (8) was in accordance with the spectroscopic properties. The i.r. spectrum showed three carbonyl bands at 1 715, 1 690, and 1 670 cm^{-1}. In the ^1H n.m.r. spectrum the C_α-H protons of the phenylalanine and 2-mercaptopropionic acid residues appeared at low field (δ 4.80 and 5.40, respectively) as expected for *N*-acyldiketopiperazines.[2d,11] *pro*-C^α-H, on the other hand, was found at δ 2.45; the upfield shift of this proton is related to the magnetic shielding effect of the benzylic side-chain located on the same side of the diketopiperazine ring.[2c,14] In the ^{13}C n.m.r. spectrum three carbonyl singlets were present and both *pro*-C_β and *pro*-C_γ signals showed values at relatively high field as found in other proline-containing diketopiperazines.[3c,3e,15]

To attain the experimental conditions favourable to the conversion of the disulphide (8) into the thia-cyclol, the use of a mild, basic reducing agent in a dipolar aprotic solvent was advisable. Such conditions should provide general base-catalysis promoting the attack of the thiol group on the imidic carbonyl, and avoiding solvolysis of the imidic bonds. When $NaBH_4$ in dimethylformamide was employed, rapid disappearance of the starting disulphide was observed at 0 °C and from the reaction mixture the racemic thia-cyclol (9) could be isolated in good yields. Although dimethylformamide

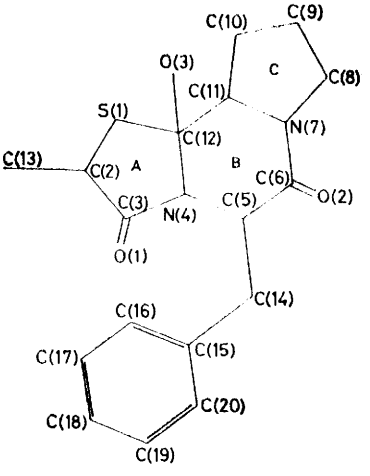


FIGURE 1 The numbering scheme of the thia-cyclol (9)

has proven very useful as the solvent in certain $NaBH_4$ reductions,[16] as far as we know its use in disulphide reduction has not been reported previously.

The thia-cyclol (9) is a fairly stable compound which can be crystallized from ethyl acetate and stored at room temperature for months without alteration. There is no evidence for the existence of tautomeric structures [corresponding to the *N*-(2-mercaptopropionyl)-diketopiperazine or to the *cyclo*-thiodepsipeptide] as found in the case of aromatic thia-cyclols.[7] It seems then that the proline-containing tricyclic system, analogous to that present in the ergot alkaloids, is particularly efficient in stabilizing cyclol forms. This effect is probably related to the conformational rigidity associated to the fusion of the three rings.

The cyclol structure assigned to (9) is based on spectroscopic data and an *X*-ray crystallographic analysis. The crystallographic numbering scheme is shown in Figure 1 and is adopted throughout the work.

The i.r. spectrum (KBr) showed a broad OH absorp-

tion centred at 3 150 cm^{-1} and two strong carbonyl bands at 1 630 and 1 695 cm^{-1}; no absorptions were observed in the ranges 1 470—1 600 and 2 500—2 700 cm^{-1} (amide second band and sulphur–hydrogen bond).

TABLE 1

^1H N.m.r.[a] and ^{13}C n.m.r.[a,b] data of compounds (8) and (9)

		N-Acyldiketopiperazine (8)		Thia-cyclol (9)	
Residue		^1H N.m.r.	^{13}C N.m.r.	^1H N.m.r.	^{13}C N.m.r.
2-Mercaptopropionic acid	Me	1.58d (7.0)	17.19q	1.58d (7.5)	18.74q
	$C_\alpha H$	4.80q (7.0)	48.51d	4.28q (7.5)	41.80d
	C=O		173.2s		173.7s
	Ph	7.32m	135.7 (C-1); 130.5 (C-2 and -6); 128.9 (C-3 and -5); 127.9 (C-4)	7.10; 7.26m	135.7 (C-1); 130.3 (C-2 and -6); 127.9 (C-3 and -5); 127.4 (C-4)
Phenylalanine	$C_\beta H_2$	3.0—3.5m	38.03t	3.12dd (3.0 and 13.5) 3.90dd (5.4 and 13.5)	35.02t
	$C_\alpha H$	5.40m	58.97 *d	4.40dd	58.90d
	C=O		164.4s		167.2s
Pyrrolidine	$C_\delta H_2$	3.40m	44.86t	3.50m	46.30t
	$C_\gamma H_2$		22.04t	1.2—2.1m	22.36t
	$C_\beta H_2$	1.5—2.1m	29.16t		26.50t
	$C_\alpha H$	2.45m	60.49 *d	2.26t	64.50d
	C=O (or C–OH)		169.7s	6.70br s [7.60s, $(CD_3)_2SO$]	91.40s

[a] Chemical shifts in δ from $SiMe_4$, in $CDCl_3$ solution; *J*/Hz in parentheses. [b] Asterisked values may be interchanged.

Characteristic features of the ^1H n.m.r. spectrum (Table 1) were: (*i*) the upfield shift of the C_α-H protons at C(2) and C(5) as compared with the corresponding values found for the *N*-acyl-diketopiperazines; (*ii*) the OH signal occurring as a sharp singlet at δ 7.60 in $(CD_3)_2SO$; and (*iii*) the upfield shift of the proton at C(11) (found at δ 2.26) as compared to the values (δ 3.6—3.7) [1,17] found for the corresponding proton in aza- and oxa-cyclols possessing a *cis* arrangement of the hydrogen atoms at C(5) and C(11). This upfield shift (*ca.* 1.40 p.p.m.) is analogous to that observed (1.20 p.p.m.) for the Pro-C_α-H in *cyclo*-(Phe-D-Pro) as compared to *cyclo*-(Phe-Pro),[3e] and is consistent with ring-current shielding by the benzylic side chain at C(5), located *cis* to H(11). The ^{13}C n.m.r. spectrum showed only two carbonyl signals (δ 173.7 and 167.2) and a singlet at δ 91.4, consistent with the presence of a carbon atom bonded to three hetero-atoms.[2b] The mass spectrum showed significant peaks at *m/e* 332 (M^+) and 314 ($M^+ - 18$); the fragmentation pattern was very similar to that of the acyldiketopiperazine (8), with the most relevant peaks related to the diketopiperazine moiety. The base peak in the spectra of both (8) and (9) was found at *m/e* 244, a value which corresponds to the molecular ion in the mass spectrum of *cyclo*-(Phe-Pro).

TABLE 2

Final fractional co-ordinates of the non-hydrogen atoms with e.s.d.s in parentheses

	x	*y*	*z*
S(1)	0.032 4(1)	0.419 3(1)	0.370 3(0)
C(2)	0.183 0(3)	0.405 8(2)	0.375 0(2)
C(3)	0.231 2(3)	0.547 5(2)	0.379 7(2)
N(4)	0.152 0(2)	0.641 6(2)	0.380 1(1)
C(5)	0.187 1(3)	0.784 8(2)	0.391 2(2)
C(6)	0.077 6(3)	0.873 7(2)	0.380 7(2)
N(7)	−0.007 5(2)	0.818 4(2)	0.392 4(1)
C(8)	−0.125 2(3)	0.893 6(3)	0.375 1(2)
C(9)	−0.182 8(3)	0.793 6(3)	0.404 4(2)
C(10)	−0.158 9(3)	0.656 9(3)	0.377 5(2)
C(11)	−0.012 1(2)	0.674 4(2)	0.407 2(2)
C(12)	0.013 8(2)	0.599 8(2)	0.347 2(2)
C(13)	0.304 0(3)	0.321 2(3)	0.461 4(3)
C(14)	0.337 8(3)	0.815 6(3)	0.490 4(2)
C(15)	0.358 9(3)	0.763 8(3)	0.576 5(2)
C(16)	0.309 9(3)	0.835 1(3)	0.615 1(2)
C(17)	0.321 1(4)	0.783 3(4)	0.690 6(2)
C(18)	0.383 4(4)	0.659 2(4)	0.728 8(2)
C(19)	0.434 8(4)	0.589 1(3)	0.692 3(3)
C(20)	0.424 0(3)	0.640 9(3)	0.617 1(2)
O(1)	0.331 8(2)	0.573 5(2)	0.384 3(2)
O(2)	0.073 4(2)	0.995 7(2)	0.364 9(1)
O(3)	−0.098 1(2)	0.633 0(2)	0.249 6(1)

TABLE 3

Fractional co-ordinates of the hydrogen atoms * from the final Fourier-difference

	x	*y*	*z*
H(2)	0.151	0.354	0.310
H(5)	0.186	0.806	0.331
H(8a)	−0.206	0.922	0.299
H(8b)	−0.084	0.983	0.421
H(9a)	−0.123	0.799	0.483
H(9b)	−0.297	0.812	0.365
H(10a)	−0.160	0.575	0.415
H(10b)	−0.241	0.640	0.299
H(11)	0.069	0.635	0.482
H(13a)	0.265	0.220	0.455
H(13b)	0.393	0.317	0.463
H(13c)	0.341	0.367	0.529
H(14a)	0.419	0.773	0.491
H(14b)	0.352	0.925	0.498
H(16)	0.262	0.934	0.585
H(17)	0.279	0.840	0.718
H(18)	0.391	0.616	0.787
H(19)	0.486	0.492	0.723
H(20)	0.467	0.585	0.589
H(O3)	0.910	0.590	0.215

* Hydrogen atoms are numbered according to the numbers of the carbon atoms to which they are bonded; when more than one hydrogen atom is bonded, the letters a, b, c, are used.

Crystallization of the thia-cyclol (9) from ethyl acetate afforded suitable crystals for the *X*-ray crystallographic analysis. Since the molecule is chiral and

crystallizes in a centric space group ($P2_1/c$), both the enantiomers are present in the crystals. Table 2 gives the final co-ordinates of the non-hydrogen atoms and

TABLE 4

Bond distances (Å) with e.s.d.s in parentheses

S(1)–C(2)	1.814(4)	N(7)–C(11)	1.473(3)
S(1)–C(12)	1.832(2)	C(8)–C(9)	1.514(4)
C(2)–C(3)	1.517(4)	C(9)–C(10)	1.544(4)
C(2)–C(13)	1.529(8)	C(10)–C(11)	1.527(6)
C(3)–N(4)	1.362(4)	C(11)–C(12)	1.520(3)
C(3)–O(1)	1.219(4)	C(12)–O(3)	1.397(6)
N(4)–C(5)	1.471(3)	C(14)–C(15)	1.499(6)
N(4)–C(12)	1.454(5)	C(15)–C(16)	1.387(4)
C(5)–C(6)	1.524(5)	C(15)–C(20)	1.387(5)
C(5)–C(14)	1.553(8)	C(16)–C(17)	1.387(6)
C(6)–O(2)	1.246(3)	C(17)–C(18)	1.383(7)
C(6)–N(7)	1.324(4)	C(18)–C(19)	1.375(5)
N(7)–C(8)	1.477(5)	C(19)–C(20)	1.383(6)

Table 3 lists the fractional co-ordinates of all the hydrogen atoms found in the final Fourier-difference; bond distances and angles are reported in Tables 4 and 5. Stereochemical and conformational details can be

TABLE 5

Bond angles (°) with e.s.d.s in parentheses

C(2)–S(1)–C(12)	93.1(1)	C(8)–C(9)–C(10)	103.9(3)
S(1)–C(2)–C(3)	106.7(2)	C(9)–C(10)–C(11)	102.6(3)
S(1)–C(2)–C(13)	111.9(2)	N(7)–C(11)–C(10)	103.1(3)
C(3)–C(2)–C(13)	112.0(4)	N(7)–C(11)–C(12)	107.2(2)
C(2)–C(3)–N(4)	112.8(3)	C(10)–C(11)–C(12)	116.9(4)
C(2)–C(3)–O(1)	123.3(3)	S(1)–C(12)–N(4)	104.6(2)
N(4)–C(3)–O(1)	124.0(3)	S(1)–C(12)–C(11)	111.9(2)
C(3)–N(4)–C(5)	121.6(3)	S(1)–C(12)–O(3)	113.3(2)
C(3)–N(4)–C(12)	117.1(3)	N(4)–C(12)–C(11)	108.0(3)
C(5)–N(4)–C(12)	119.9(3)	N(4)–C(12)–O(3)	111.9(2)
N(4)–C(5)–C(6)	113.1(3)	C(11)–C(12)–O(3)	107.1(3)
N(4)–C(5)–C(14)	112.0(3)	C(5)–C(14)–C(15)	113.4(3)
C(6)–C(5)–C(14)	108.8(3)	C(14)–C(15)–C(16)	121.0(3)
C(5)–C(6)–N(7)	118.2(2)	C(14)–C(15)–C(20)	120.3(3)
C(5)–C(6)–O(2)	119.9(3)	C(16)–C(15)–C(20)	118.7(4)
N(7)–C(6)–O(2)	121.8(3)	C(15)–C(16)–C(17)	121.1(4)
C(6)–N(7)–C(8)	122.0(2)	C(16)–C(17)–C(18)	119.5(3)
C(6)–N(7)–C(11)	124.6(2)	C(17)–C(18)–C(19)	119.8(4)
C(8)–N(7)–C(11)	112.7(2)	C(18)–C(19)–C(20)	120.7(4)
N(7)–C(8)–C(9)	102.4(3)	C(19)–C(20)–C(15)	120.2(3)

obtained from Figure 2 where a general view of the molecule [(2*R*,5*R*,11*S*,12*S*)-enantiomer] is presented. The sums of the bond angles around the two nitrogen

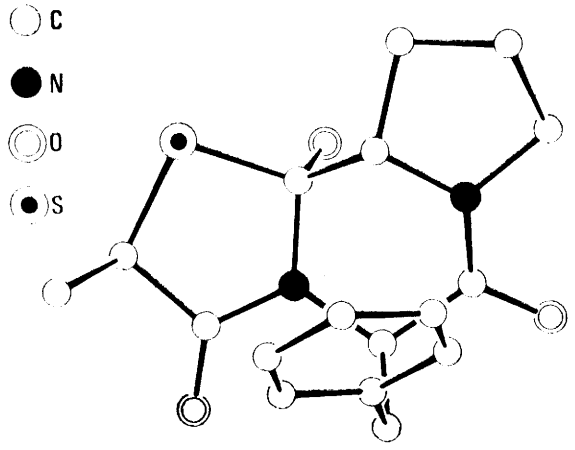


FIGURE 2 Molecular structure of the (2*R*,5*R*,11*S*,12*S*)-enantiomer of the thia-cyclol (9)

atoms N(4) and N(7) are 358.6 and 359.3°, respectively, indicating a fairly planar substitution at these centres. The relative configuration at the four asymmetric carbon atoms corresponds to an *anti* 11-H–12-OH arrangement, with C(2)-Me, the benzylic side-chain at C(5), and H(11) all on the same side of the tricyclic system. It is interesting to note that an identical arrangement of the same substituents has been found previously for an oxa-cyclol [10] and an aza-cyclol [2c] obtained by different methods.

As can be seen from the data of Tables 6 and 7, the thiazolidinone ring adopts an envelope C_s–C(12) con-

TABLE 6

Relevant torsion angles (°) with e.s.d.s in parentheses.[a] In the second column are reported the calculated values for the hexa-atomic ring of the oxa-cyclol (11) [b]

Ring A		
C(12)–S(1)–C(2)–C(3)	11.8(2)	
S(1)–C(2)–C(3)–N(4)	0.7(3)	
C(2)–C(3)–N(4)–C(12)	−18.0(3)	
C(3)–N(4)–C(12)–S(1)	25.8(3)	
N(4)–C(12)–S(1)–C(2)	−20.3(2)	
Ring B		
C(12)–N(4)–C(5)–C(6)	8.9(4)	14.4
N(4)–C(5)–C(6)–N(7)	19.8(3)	13.3
C(5)–C(6)–N(7)–C(11)	−5.1(3)	−1.5
C(6)–N(7)–C(11)–C(12)	−35.1(2)	−32.8
N(7)–C(11)–C(12)–N(4)	58.6(2)	52.9
C(11)–C(12)–N(4)–C(5)	−48.4(3)	−48.1
Ring C		
N(7)–C(8)–C(9)–C(10)	−32.3(2)	
C(8)–C(9)–C(10)–C(11)	39.7(3)	
C(9)–C(10)–C(11)–N(7)	−30.7(3)	
C(10)–C(11)–N(7)–C(8)	11.3(3)	
C(11)–N(7)–C(8)–C(9)	13.3(3)	
Phenyl residue		
N(4)–C(5)–C(14)–C(15)	−53.4(4)	
C(6)–C(5)–C(14)–C(15)	72.3(3)	
C(5)–C(14)–C(15)–C(16)	−82.3(3)	
C(5)–C(14)–C(15)–C(20)	95.6(3)	
Peptide groups		
C(12)–N(4)–C(3)–O(1)	163.2(3)	
C(5)–N(4)–C(3)–O(1)	−3.1(4)	
C(5)–N(4)–C(3)–C(2)	175.7(2)	
C(8)–N(7)–C(6)–O(2)	8.0(3)	
C(11)–N(7)–C(6)–O(2)	177.4(2)	
C(8)–N(7)–C(6)–C(5)	−174.5(2)	

[a] Computed according to W. Klyne and V. Prelog, *Experientia*, 1960, **16**, 521. [b] Ref. 19.

formation, with C(12) displaced out of the plane of the other ring atoms by 0.386 Å on the opposite side of the methyl group.

In view of the increasing interest concerning the conformation of the pyrrolidine ring in proline-containing peptides and cyclo-peptides,[3c,18] it seemed interesting to compare the conformational data of the thia-cyclol to those of the other cyclols and related cyclo-dipeptides. In the aza-cyclols (10) [2e] and the oxa-cyclol (11),[19] both having a *cis*-arrangement of H(5) and H(11), the pyrrolidine ring adopts an approximate C_s–C(10)$_{endo}$ conformation, analogous to the C_s–C^{β}_{endo} symmetry found in the case of proline containing LL-cyclic dipeptides.[3c,18]

In the pyrrolidine ring of the thia-cyclol (9), which

has a *trans*-arrangement of H(5) and H(11), there is no planar grouping of four atoms, and the simplest description of the conformation corresponds to C_2–$C(10)_{endo}$–$C(9)_{exo}$ half-chair symmetry, with C(9) and

TABLE 7

Displacements from least-squares planes (Å). Equations of least-squares planes in the form $Ax + By + Cz + D = 0$ are in the crystal system and x, y, and z are the fractional coordinates

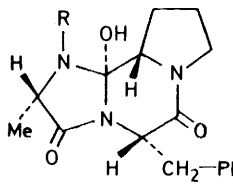
Plane 1: $0.4432x + 0.3680y - 14.1243z + 5.0628 = 0$
Plane 2: $0.0172x - 1.8239y - 13.5016z + 6.7333 = 0$
Plane 3: $1.7804x + 0.7223y - 15.2517z + 4.9847 = 0$
Plane 4: $1.1595x - 1.5450y - 14.6066z + 7.0044 = 0$

	Plane 1	Plane 2	Plane 3	Plane 4
S(1)	0.002			
C(2)	−0.003		−0.116	
C(3)	0.004		0.001	
N(4)	−0.002	0.434 *	−0.079	
C(5)		0.023	−0.082	
C(6)		0.001		
N(7)		−0.057		0.000
C(8)		0.037		0.000
C(9)				−0.341 *
C(10)		0.435 *		0.291 *
C(11)		0.005		0.000
C(12)	0.386 *	0.952 *	0.148	
O(1)			0.128	
O(2)		−0.009		

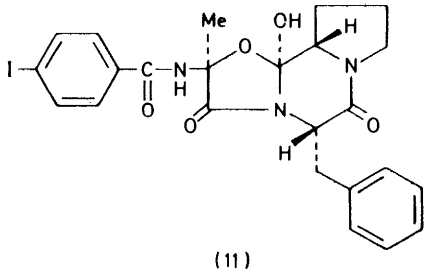
* Indicates atom not included in the calculation of the plane.

C(10) displaced by −0.341 and 0.291 Å, respectively from the plane passing through N(7), C(8), and C(11). No correspondence is then found in this case with the conformation of the related *cyclo*-(Phe-D-Pro) which adopts an approximate C_2–C^{α}_{exo}–C^{β}_{endo} symmetry.[18]

As can be deduced from the internal torsion angles



(10a) R = Ph—CH_2O—CO—
(10b) R = *p*-BrC_6H_4—CH_2O—CO—



(11)

compared in Table 6, the 2-oxopiperazine ring adopts in the thia-cyclol (9) a conformation very similar to that observed for the same ring in the oxa-cyclol (11).[19] The atoms N(4) and C(12) are displaced by 0.434 and 0.952 Å, respectively, out of the plane passing through C(5), C(6), N(7), C(8), N(11), and O(2), both on the opposite side from H(11). This conformation is different from that found for the corresponding ring in both the aza-cyclols (10), where only the C(12) atom deviates from the same plane. The difference between the two internal torsion angles around the bonds C(5)–C(6) and N(4)–C(5) in the thia-cyclol (9) is 10.9°, indicating the quasi-isoclinal orientation of the phenylalanine C_{α}–C_{β} bond. The difference between the internal torsion angles around the bonds N(4)–C(12) and C(12)–C(11) is −107.0° and that between the angles around the bonds C(12)–C(11) and C(11)–N(7) is 93.7°; these values reveal the axial orientation of the cyclol 12-OH and of H(11).

The benzylic side-chain of the phenylalanine residue adopts in the crystal of the thia-cyclol (9) a folded conformation with the aromatic ring facing H(11). An analysis of the relative position of H(11) and of the aromatic ring shows that in the crystal the hydrogen atom falls in the shielding region of the phenyl group, with the half-angle of the cone being 12.9°, and is located 3.01 Å away from the centre of the aromatic ring. The above-mentioned high value of the upfield shift found for H(11) in the ^{1}H n.m.r. spectrum, as well as the small value of both the Phe-H_{α}-H_{β} vicinal coupling constants (Table 1), are consistent with a significant contribution in chloroform solution from the same folded rotamer found in the crystal. Thus the thia-cyclol (9) adopts a conformation of the benzylic side chain, analogous to that preferred by *cyclo*-(Phe-D-Pro).[3e,20] Cyclols (10a), (10b), and (11), all with the same stereochemistry at C(5), C(11), and C(12), adopt in the solid state different

TABLE 8

Relevant intermolecular contacts (Å) between non-hydrogen atoms

N(4) · · · C(15)	2.97	C(6) · · · C(16)	3.24
C(5) · · · C(16)	3.32	O(2) · · · O(3 I)	2.67
C(6) · · · C(15)	3.12		

I: $-x, \frac{1}{2} + y, \frac{1}{2} - z$.

conformations of the phenylalanine side-chain. In particular, both the aza-cyclols (10a) and (10b) adopt a configuration extended toward the nitrogen.[2e] The oxa-cyclol (11) [19] presents, on the other hand, a folded conformation stabilized by an intramolecular hydrogen bond between the aromatic ring and the cyclol hydroxy.

Relevant intermolecular contacts are listed in Table 8. Figure 3 shows the crystal packing of the thia-cyclol (9). The carbonyl oxygen O(2) forms a hydrogen bond with the hydroxy O(3) of a screw-related molecule. The distance O(2) · · · O(3) is 2.67 Å while that between O(2) and the hydrogen atom bound to O(3) is 1.85 Å; the angles O(2) · · · H–O(3), C(6)–O(2) · · · H, and C(6)–O(2) · · · O(3) are 178.8, 132.0, and 132.3°, respectively; the torsion angle C(6)–O(2)–H–O(3) is −148.3°.

EXPERIMENTAL

M.p.s were determined with a Buchi oil-bath apparatus. I.r. spectra were recorded with a Perkin-Elmer 521 spectrophotometer. ^{1}H N.m.r. spectra were recorded on a Varian EM-390 spectrometer ($SiMe_4$ as internal standard). ^{13}C

N.m.r. spectra were recorded on a Bruker WH-90 spectrometer operating at 22.63 MHz, for solutions in $CDCl_3$; chemical shifts are from $SiMe_4$ as internal standard. Mass spectra were determined with a Hewlett-Packard 5 982 A spectrometer, operating at 70 eV.

(RS)-2-*Tritylthiopropionic Acid* (1).—A mixture of (*RS*)-2-mercaptopropionic acid (2.6 g, 25 mmol) and trityl chloride (10.5 g, 37.5 mmol) in dry dimethylformamide (DMF) (15 ml) was stirred at room temperature for 48 h. The solid was filtered off, dissolved in benzene, and washed repeatedly with water. The solution was dried (Na_2SO_4) and evaporated under vacuum. Crystallization of the residue from ether gave (1) (5.5 g, 65%), m.p. 141—142 °C; δ 1.20 (3 H, d, *J* 7.5 Hz, Me), 3.02 (1 H, q, *J* 7.5 Hz, CH), 7.1—7.6 (15 H, m, CPh_3), and 10.7 (1 H, br s, OH) (Found:

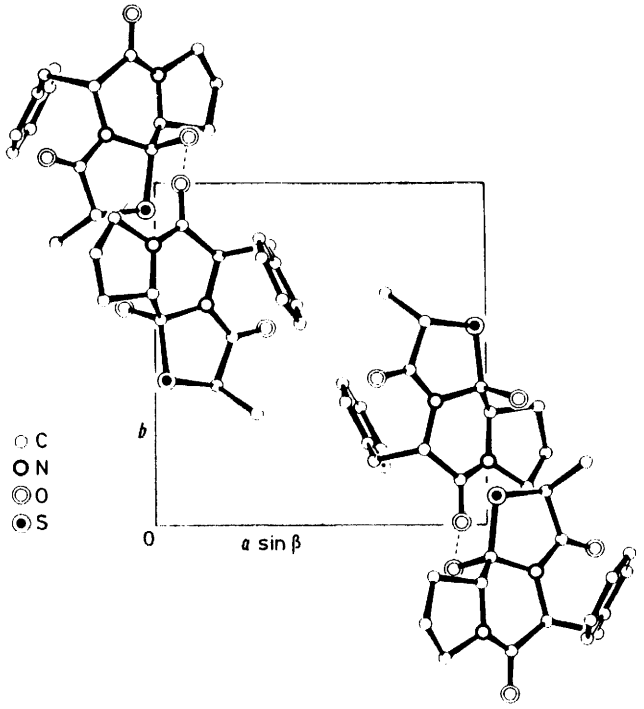


FIGURE 3 Molecular packing of thia-cyclol (9) projected on the plane perpendicular to the *c* axis. Dashed lines indicate hydrogen bonds connecting couples of the same enantiomer

C, 75.7; H, 5.75; S, 9.3. $C_{22}H_{20}O_2S$ requires C, 75.83; H, 5.79; S, 9.20%).

[(RS)-2-*Tritylthiopropionyl*]-(S)-*phenylalanine* (4).—To a stirred solution at 0 °C of (*RS*)-2-tritylthiopropionic acid (5.11 g, 14.7 mmol) and of (*S*)-phenylalanine methyl ester (2.62 g, 14.7 mmol) in methylene chloride (50 ml), dicyclohexylcarbodi-imide (3.03 g, 14.7 mmol) was added. After stirring for 4 h at room temperature, the mixture was filtered and the solution washed with 2N-sodium hydroxide, 2N-hydrochloric acid, and water. The solvent was removed under reduced pressure and the oily residue was purified by column chromatography on silica gel (160 g). Elution with ether–hexane (7 : 3) gave the methyl ester of (4) (5.9 g) as an oil. T.l.c. showed the presence of the two diastereoisomers [silica gel; eluant ether–hexane (7 : 3); R_F 0.6 and 0.65)]. To a solution of the methyl ester (5.9 g, 11.6 mmol) was added 0.2N-NaOH (120 ml) in methanol (300 ml). After 8 h at room temperature the solution was evaporated under vacuum and the residue taken up in water. The aqueous alkaline solution was washed with ethyl acetate, acidified to pH 3 with 1N-hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to give *compound* (4) as an oil which was a mixture of the two diastereoisomers (4.6 g); δ 1.30 and 1.27 (2 Me, d, *J* 7.5 Hz), 3.0 (m, CH_2Ph), 3.6 (m, Me*CH*), 4.4 (m, *CH*–CH_2Ph), 6.6 and 6.7 (NH, 2 d), 7.0—7.5 (CPh_3 + CH_2*Ph*), and 9.2 (OH); (4) (200 mg) was carefully purified by t.l.c. and dried in high vacuum for elemental analysis (Found: C, 75.25; H, 5.9; N, 3.05; S, 6.4. $C_{31}H_{29}NO_3S$ requires C, 75.12; H, 5.9; N, 2.83; S, 6.48%).

[(RS)-2-*Tritylthiopropionyl*]-(S)-*phenylalanyl*-(S)-*proline Methyl Ester* (5).—To a solution of compound (4) (4.3 g, 8.68 mmol) in methylene chloride (70 ml) was added, with stirring at 0 °C, (*S*)-proline methyl ester hydrochloride (1.43 g, 8.68 mmol), *N*-methylmorpholine (0.88 g, 8.7 mmol), and dicyclohexylcarbodi-imide (1.79 g, 8.7 mmol). After stirring at 0 °C for 0.5 h, the mixture was left for 12 h at room temperature. The solid was removed by filtration, the solution washed with saturated aqueous $NaHCO_3$, 1N-hydrochloric acid, and water, and solvent evaporated to give (5) (6.0 g). Chromatography on silica (300 g), eluting with ether, gave *acyl-dipeptide ester* (5) (4.9 g) as an oil. T.l.c. examination showed the presence of the two diastereoisomers with very similar R_F values (*ca.* 0.8; silica, eluant ether); $\nu_{max.}$ ($CHCl_3$) 3 400, 1 740, 1 660—1 630, 1 500, and 1 440 cm^{-1}; δ 1.25 (C*Me*, d, *J* 7.5 Hz), 1.9 (m, CH_2N), 2.9 (m, CH_2Ph and CH_2N), 3.5 (m, *CH*Me), 3.6 and 3.7 (CO_2Me, 2 s), 4.5 (m, Pro-C_αH and Phe-C_αH), 6.8 (NH, 2 d), and 7.1—7.6 (CPh_3 + CH_2*Ph*) (Found: C, 73.0; H, 6.45; N, 4.8; S, 5.4. $C_{37}H_{38}N_2O_4S$ requires C, 73.24; H, 6.3; N, 4.6; S, 5.28%).

N-(2-*Tritylthiopropionyl*)-cyclo-(*phenylalanylprolyl*) (7).—By following the same procedure adopted for the alkaline hydrolysis of the methyl ester (3), compound (5) (4.7 g) was hydrolysed to give the corresponding acid (6) (4.2 g), which was used without further purification. Compound (6) (4.2 g, 7.1 mmol) was treated at 100 °C with acetic anhydride (140 ml) and sodium acetate (8.32 g) for 5 h. The mixture was evaporated to dryness under vacuum. Ethyl acetate was added and the solution was washed with aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (silica gel, 120 g). Elution with ether–light petroleum (8 : 2) gave a product (2.2 g), which was rechromatographed on a silica gel column (80 g of silica). By eluting with the same solvent mixture, *acyldiketopiperazine* (7) (1.7 g) as a mixture of the two partially racemic *trans*-diastereoisomers (2*S*,5*S*,11*R*) and (2*R*,5*S*,11*R*), was obtained; $\nu_{max.}$ ($CHCl_3$) 1 715—1 700, 1 670—1 650, 1 480, and 1 440 cm^{-1}; δ (more abundant isomer) 1.22 (3 H, d, *J* 7.5 Hz, Me), 1.3—2.2 (4 H, m, CH_2CH_2), 2.6 (1 H, m, Pro-C_αH), 3.15 (2 H, m, CH_2Ph), 3.5 (2 H, m, CH_2N), 4.32 (1 H, q, *J* 7.5 Hz, Me*CH*), 5.0 (1 H, three lines, Phe-C_αH), and 7.0—7.6 (m, CPh_3 + CH_2*Ph*) (Found: C, 75.45; H, 6.1; N, 4.65; S, 5.6. $C_{36}H_{34}N_2O_3S$ requires C, 75.23; H, 5.96; N, 4.9; S, 5.6%).

Hydrazinolysis of (7). Hydrazine hydrate (2 mol) was added to a 1% methanolic solution of compound (7) (1 mol) and the reaction mixture was left for 18 h at room temperature. T.l.c. examination of the residue showed the absence of *cis*-prolyl-phenylalanyl-diketopiperazine, and p.l.c. on silica gel afforded the *trans*-isomer *cyclo*-(Phe-D-Pro) containing, on the basis of the optical purity, *ca.* 35% of *cyclo*-(D-Phe-Pro).

Detritylation of (7) *and Synthesis of the Disulphide* (8).—To a stirred solution of the tritylthio-acyl-diketopiperazine (7) (1.70 g, 2.96 mmol) in methanol (40 ml), iodine (760 mg, 3.0 mmol) was added at room temperature. After 1.5 h of stirring, 1N-$Na_2S_2O_3$ was added at 0 °C until decolourization occurred. The reaction product was precipitated by adding water, and the precipitate washed three times with water and taken up in benzene. The solution was repeatedly evaporated under vacuum by adding fresh benzene. The oily residue was dried under vacuum over P_2O_5, washed with ether–light petroleum (1 : 1), and then purified by column chromatography (80 g of silica for 1.03 g of product). Elution with ethyl acetate–ether (8 : 2) afforded *compound* (8) (0.6 g), which could be crystallized from methanol, m.p. 167—169 °C; $\nu_{max.}$ (KBr) 1 720, 1 695, 1 660—1 680, and 1 460 cm^{-1}; *m/e* 332 (12%), 314 (3), 299 (6), 245 (100), 153 (50), 125 (50), 91 (30), and 70 (40) (Found: C, 61.25; H, 5.8; N, 8.35; S, 9.5. $C_{34}H_{38}N_4O_6S_2$ requires C, 61.6; H, 5.8; N, 8.45; S, 9.67%).

Thia-cyclol (9).—To a stirred and cooled (−10 °C) solution of the disulphide (8) (500 mg, 0.76 mmol) in 30 ml of dry DMF, $NaBH_4$ (21 mg, 0.55 mmol) in DMF (5 ml) was added. After 0.5 h at −10 °C and 1.5 h at 0 °C, the solvent was removed under vacuum and the residue was chromatographed on a column of silica (100 g). Elution with ethyl acetate–ether (6 : 4) gave 250 mg of *thia-cyclol* (9); m.p. 170—171 °C (from ethyl acetate); $\nu_{max.}$ (KBr) 3 150 (broad), 1 695, 1 630, and 1 450 cm^{-1}; *m/e* 332 (45%), 314 (20), 299 (5), 245 (100), 153 (35), 125 (35), 91 (30), and 70 (60) (Found: C, 61.35; H, 6.1; N, 8.4; S, 9.8. $C_{17}H_{20}N_2O_3S$ requires C, 61.4; H, 6.06; N, 8.4; S, 9.64%).

Crystallographic Analysis.—Suitable single crystals of the thia-cyclol (9) were obtained from ethyl acetate. Approximate unit-cell parameters and the space group were determined from oscillation and Weissenberg photographs. Intensity data were collected on an automatic four-circle Syntex $P2_1$ diffractometer equipped with a graphite monochromator using Mo-K_α radiation. Refined unit-cell parameters were obtained by a least-squares fit of the θ angles of 15 high-order reflections widely separated in reciprocal space.

Crystal data. Thia-cyclol (9), $C_{17}H_{20}N_2O_3S$, M = 332.4. Monoclinic, a = 12.375(2), b = 9.996(4), c = 18.173(4) Å, β = 131.00(1)°, U = 1 696.6(8) Å^3, D_c = 1.30 g cm^{-3}, Z = 4, D_m = 1.30 g cm^{-3}. Mo-K_α radiation, λ = 0.710 7 Å, μ(Mo-K_α) = 0.87 cm^{-1}. Space group $P2_1/c$ from systematic absences. Intensities were collected up to 2θ 56.0° by the ω-scan technique using a scan speed within the interval 1.0—29.3 min^{-1} over a range of 1.5°. Background counts were taken for a time equal to that of the scan. Out of a total of *ca.* 4 000 independent recorded reflections, the intensities of three standard reflections monitored every 100 remained essentially constant throughout data collection. Lorentz and polarization factors were applied taking into account the monochromator crystal. No absorption or extinction corrections were applied.

Structure solution and refinement. The structure of the thia-cyclol (9) was solved by direct methods with the program MULTAN [21] employing the 300 reflections with $|E| > 1.74$. An E map computed with phases of the set with the highest figures-of-merit revealed all the non-hydrogen atoms, which were refined isotropically (4 × 4 blocks) and anisotropically (9 × 9 blocks) successively. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$ where $w = (a|+F_o| + c|F_o|^2)^{-1}$ with a and c of the order of $2F_{o(min.)}$ and $2/F_{o(max.)}$ respectively. A difference synthesis showed all the hydrogen atoms in stereochemically feasible positions; no residual significant peaks were present. These were included in the refinement with an overall isotropic B of 5.0 Å^2, keeping their positional parameters fixed. Scattering factors were taken from ref. 22. When the refinement was stopped the sum of the square of the ratios between the parameters shifts and the e.s.d.s was 0.05. The adequacy of the weighting scheme was checked by inspection of the mean of $w|\Delta F|^2$ as a function of the $|F_o|$ and sinθ/λ ranges: in both cases the function was nearly constant. The final R and R' were 0.048 and 0.073 respectively for all the observed reflections. All the calculations were carried out on the HP 21MX minicomputer of the CNR Research Area.[23] Observed and calculated structure factors together with anisotropic thermal parameters are listed in Supplementary Publication No. SUP 22745 (19 pp.).*

* For details see Notices to Authors, No. 7, *J.C.S. Perkin I*, 1979, Index issue.

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