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THE STEREOSPECIFIC CYCLOLIZATION OF N-(α-HYDROXYACYL)-PHENYLALANYL-PROLINE LACTAMS[1]

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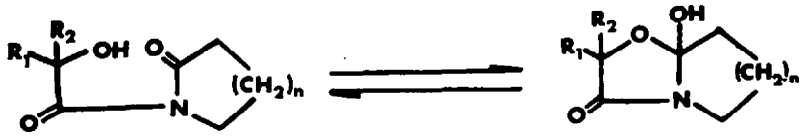
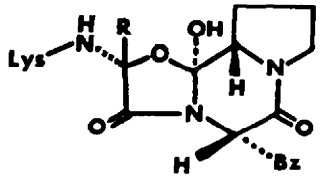
Abstract—The spontaneous ring closure of N-(α-hydroxyacyl)-phenylalanyl proline-lactams to cyclols was shown to take a stereospecific course. These facts have made an important contribution to the synthesis of ergotamine.

INTRAMOLECULAR ring formation between the alcoholic hydroxyl and the lactam carbonyl group in N-(α-hydroxyacyl)-lactams (I) leads to bicyclic compounds of type (II), the so-called cyclols. The tendency of these compounds to exist either in the open (I) or closed (II) form depends on various electronic and structural factors.[2]



I II

In order to expand our knowledge of cyclol formation, and with a possible synthesis of the peptide part of the ergot alkaloid ergotamine[3] (IIIa) in mind, we have prepared a number of tricyclic cyclols by our previously reported[2] synthetic route, where the simple lactams (α-pyrrolidone, α-piperidone, caprolactam) have been replaced by optically active diketopiperazines. In this work the question of the stereochemical course of cyclolization was studied in detail.



IIIa R=CH_3 Ergotamin
IIIb R=$CH(CH_3)_2$ Ergocristin

Bz=Benzyl

Lys=Lysergyl

[1] This publication represents the detailed description of experiments which have been presented in a paper at the 4*th European Symposium on Peptides* in Moscow, August (1961) by A. Hofmann, A. J. Frey, H. Ott, and J. Rutschmann, *Zh. Vsesoyuz. Khim Obshestva Im. R. I. Mendeleeva* **7**, 466 (1962).

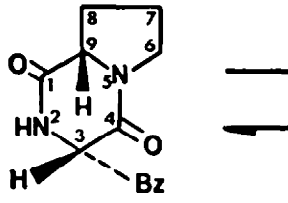
[2] See first publication of this series: R. Griot and A. J. Frey, *Tetrahedron* **19**, 1661 (1963).

[3] A. Hofmann, A. J. Frey and H. Ott, *Experientia* **17**, 206 (1961).

As our model diketopiperazine we selected S-phenylalanyl-S-proline-lactam[4] (IV) [the basic component of the peptide part of the ergot alkaloids ergotamine (IIIa) and ergocristine (IIIb)] and also S-phenylalanyl-R-proline-lactam (V).

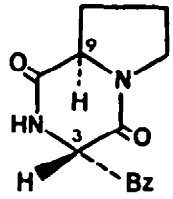
The best procedure for preparing (IV) consists in the coupling of N-carbobenzoxy-S-proline and S-phenylalanine methyl ester to S-(N-carbobenzoxy)-prolyl-S-phenylalanine methyl ester followed by removal of the carbobenzoxy group by catalytic hydrogenation whereby ring formation occurs giving (IV).

S-Phenylalanyl-R-proline lactam (V) was prepared in excellent yield by treating (IV) with dilute sodium hydroxide[5] at room temperature for a short time. Both diastereoisomers (IV) and (V) form an equilibrium mixture in alkaline solution that lies 90–95 per cent on the side of the R-proline form (V). From inspection of Dreiding stereo-models the greater thermodynamic stability of (V) is plausible since this molecule shows less steric interactions than (IV).



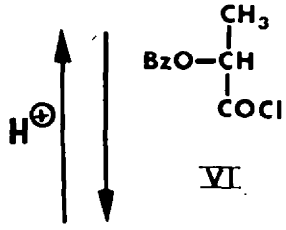
(3S:9S)

IV

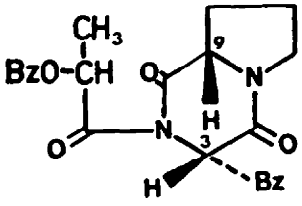


(3S:9R)

V

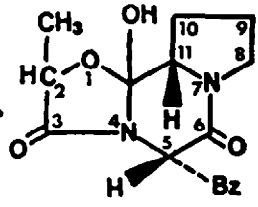


VI

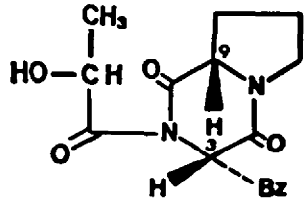


(3S:9S)

VII



VIII



(3S:9S)

IX

[4] This compound was first prepared in pure form by M. Bergmann and J. Tutzmann, *J. Biol. Chem.* **155,** 535 (1944).

[5] The easy racemization or isomerization respectively of diketopiperazines in alkaline solution is well known. See: M. Bevarnick and H. T. Clarke, *J. Amer. Chem. Soc.* **60,** 2426 (1938); P. A. Levene and M. H. Pfaltz, *J. Biol. Chem.* **63,** 661 (1925); M. Bergmann, L. Zervas and H. Koester, *Ber. Dtsch. Chem. Ges.* **62,** 1901 (1929).

A mixture of (3S:9S)-diketopiperazine (IV) and racemic α-benzyloxypropionyl chloride (VI) when heated for several hours in an inert organic solvent in the presence of the theoretical amount of pyridine lead to the oily N-acyl-diketopiperazine (VII). The unchanged (3S:9S)-configuration of this product could easily be shown by mild acid hydrolysis to *the starting diketopiperazine* (IV). Hydrogenolytic cleavage of the benzyl ether group gave a crystalline substance (m.p. 193–194°, $[\alpha]_D^{20} = -23{\cdot}5°$) in 71 per cent overall yield. The chemical properties of this compound gave strong evidence for the cyclol structure (VIII).

The slightly water soluble substance dissolved readily in the calculated amount of 0·1N sodium hydroxide. The *pK* in water was 9·07. After 24 hours standing at room temperature in the presence of excess 1N sodium hydroxide, the starting material could be quantitatively regenerated by the addition of the calculated amount of hydrochloric acid to the alkaline solution. This fact proves that in aqueous solution no equilibrium between the open form (IX) and the cyclol form (VIII) is established.[6] The presence of even a very small portion of the open form (IX) would result in a partial hydrolytic decomposition of the starting material because of the high instability of N-acyl-lactams[2] and N-acyl-diketopiperazines (e.g. (VII) or (XV)) toward alkali.

When (VIII) was treated with an alcohol–water solution of hydrochloric acid at room temperature for 2 hours it was recovered unchanged, whereas the N-acyl-diketopiperazine (VII) was hydrolyzed under these reaction conditions to the corresponding acid and the diketopiperazine (IV).

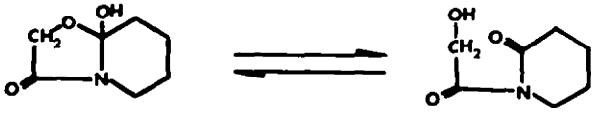
Compound (VIII) was very stable toward oxidation with chromic acid in acetone. If the open form (IX) was present rapid formation of N-pyruvoyl-S-phenylalanyl-S-proline-lactam would have been expected.[7]

The hydroxyl group behaved like a typical tertiary hydroxyl since it failed to give simple ester derivatives such as the acetate, tosylate, etc.

A negative iodoform test ruled out the presence of a CH_3-CHOH-group and therefore the structure (IX).

The reaction of the optically active diketopiperazine (IV) with the racemic acid chloride (VI) gave rise to a mixture of two diastereoisomeric acyl-diketopiperazines (VII). The removal of the protecting benzyl group was followed by spontaneous ring closure to (VIII), resulting in the formation of a new asymmetric center in position 12. Theoretically four stereoisomeric forms are possible. However, on thin layer plates separation into only two spots could be observed. In the NMR spectrum of compound (VIII) the C-methyl region contains two doublets of equal intensity ($\delta_1 = 83$ c.p.s., $J_1 = 7$ c.p.s.; $\delta_2 = 88$ c.p.s. $J_2 = 7$ c.p.s.) unambiguously revealing the presence of

[6] As mentioned in the previous communication of R. Griot and A. J. Frey (i.e. by dissolving cyclol (i) in water) an equilibrium mixture between (i) and the open form (ii) in the ratio of approximately 7:1 formed.



i *ii*

[7] N-Lactyl anilide as a model substance was easily oxidized under the same conditions to N-pyruvoyl anilide.

two stereoisomeric cyclols (labelled as A and B respectively). This result proves that the spontaneous cyclolization takes place in a stereospecific way with respect to the newly formed asymmetric center 12.

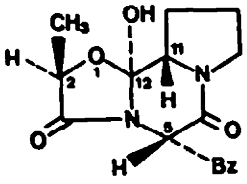
The successful separation of the cyclol mixture (VIII) into the pure components A and B was accomplished by chromatography on silica-gel. As cyclol A and B differ only slightly in their m.p. and specific rotation (see Table 1) their purity can best be detected by N.M.R. Pure A and B respectively show one neat and sharp doublet for the C_2-Me group centered at 88 c.p.s. and 83 c.p.s. respectively.

TABLE 1

Compound	m.p.	$[\alpha]_D^{20}$	NMR(C_2-Me)	abs. configuration	stereoformula
(VIII) = A + B	193–197°	−23·5°	83/88 cps	(2R,S:5S:11S:12S)	
A	188–189°	−23°	88 cps	(2R:5S:11S:12S)	X
B	197–201°	−24°	83 cps	(2S:5S:11S:12S)	XI

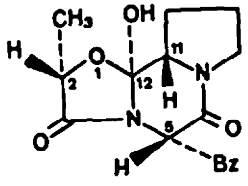
A consideration of the Dreiding stereo models revealed that the 11,12-anti-arrangement of H and OH is sterically much more favorable than the alternative 11,12-syn-configuration. The N.M.R. spectra of the pure cyclols A and B show a

VIII



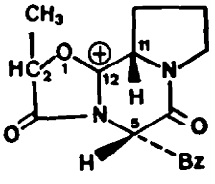
(2R:5S:11S:12S)

Cyclol A



(2S:5S:11S:12S)

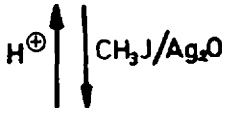
Cyclol B



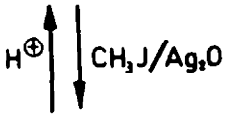
(5S:11S)

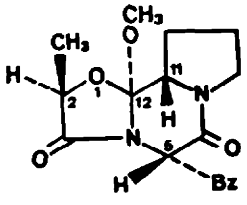
XII

X



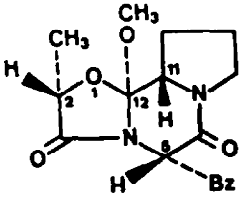
XI





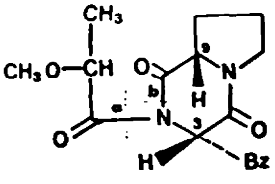
(2R:5S:11S:12S)

XIII



(2S:5S:11S:12S)

XIV



(3S:9S)

XV

sharp doublet (J = 2 c.p.s.) for the hydroxyl proton, which can only be the result of a spin–spin interaction with the adjacent proton at C_{11}.[8] Since the same coupling occurs in both cyclols it indicates that the 11,12-anti-configuration is present in both compounds. The chemical proof of the correctness of this assignment will be described in our detailed publication on the total synthesis of ergotamine.[9]

In order to assign the absolute configuration of cyclol A and B respectively in position 2 we then synthesized the (2S:5S:11S:12S)-cyclol starting with S-phenylalanyl-S-proline-lactam (IV) and S(—)-α-benzyloxy-propionylchloride.[10] The S(—)-configuration of this acid chloride was proven by hydrogenolytic cleavage of (—)-α-benzyloxy-propionic acid ($[\alpha]_D^{20} = -92°$ in ethanol) to give S(+)-lactic acid isolated as its crystalline zinc salt. The acylation product, N-(S-α-benzyloxypropionyl)-S-phenylalanyl-S-proline-lactam (m.p. 137°, $[\alpha]_D^{20} = -149°$) yielded 81% of the crystalline (2S:5S:11S:12S)-cyclol on hydrogenation. This compound was identical in m.p. and N.M.R. spectrum with cyclol B. The exact stereoformula of cyclol A is therefore (X) and cyclol B is (XI).

The cyclol hydroxyl group could readily be methylated with methyl iodide and silver oxide. Compounds (X) and (XI) gave the cyclol methyl ethers (XIII) (m.p. 108°; $[\alpha]_D^{20} = +45°$) and (XIV) (m.p. 114–116°; $[\alpha]_D^{20} = +43°$) respectively. According to the N.M.R. spectra both substances were sterically pure.

With the exchange of the cyclol hydroxyl group by a methoxyl group the alkali solubility (preceding from the acid character of the cyclol hydroxyl group) obviously is lost. The methyl ethers (XIII) and (XIV) were very stable toward alkali and when heated for several hours with dilute sodium hydroxide could be recovered unchanged in excellent yield. In contrast the N-(α-methoxypropionyl)-S-phenylalanyl-S-proline lactams (XV), prepared by acylation of S-phenylalanyl-S-proline lactam (IV) with α-methoxypropionyl chloride[11] were extremely sensitive to alkali and could be hydrolyzed with 0·1N sodium hydroxide at 0° by direct titration of the imide group. It was found that part of the molecule (XV) was split at the amide bond a and part at the amide bond b. The enormous difference between the methylated products (XIII or XIV) and the N-acyl-diketopiperazines (XV) when treated with alkali is excellent evidence for the existence of the cyclol structures in the aforementioned compounds.

In this connection it is worth mentioning, that substance (XV) was hydrolyzed by dilute ammonia in a few minutes or standing in a sodium hydrogen carbonate solution for several hours to α-methoxypropionic acid and S-phenylalanyl-R-proline lactam (V). Under the same conditions S-phenylalanyl-S-proline lactam remained completely unchanged. From this result it can be concluded that in N-acylated diketopiperazines of this type the base-catalyzed isomerization of the S-proline configuration to the R-form takes place even more rapidly than the above-mentioned isomerization of S-phenylalanyl-S-prolinelactam (IV) to S-phenylalanyl-R-proline lactam (V). This fact can be explained on the basis of the increased electrophilicity of the amide carbonyl group in position 1.

[8] This is an interesting and unusual coupling between a hydroxylic proton and another proton separated by four bonds. To our knowledge, no similar case has ever been described in the literature.

[9] *Helv. Chim. Acta.* In press.

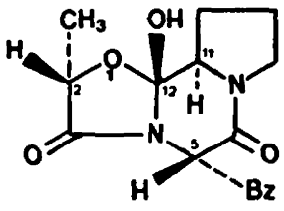
[10] The resolution of racemic α-benzyloxypropionic acid by fractional crystallization of the cinchonidine salts has been described by P. A. Levene, *J. Biol. Chem.* **113**, 153 (1936).

[11] The use of racemic α-methoxypropionyl chloride in this acylation lead again to a mixture of two diastereoisomers which could be separated into the pure forms by fractional crystallization.

The cyclol methyl ethers (XIII) and (XIV) revert back to the starting cyclols (X and XI) respectively when heated for 1 hour in dilute acetic acid. This substitution of the methoxyl group by the hydroxyl group no doubt occurs by an S_N-reaction passing through the carbonium ion (XII), which is stabilized by resonance with the adjacent oxygen and nitrogen atoms.

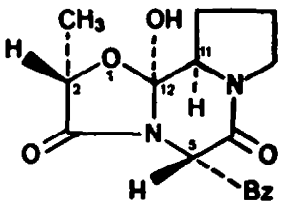
The acylation of S-phenylalanyl-R-proline lactam (V) with S(−)-α-benzyloxy-propionyl chloride followed by reductive cleavage of the protecting benzyl ether group gave a 69% over-all yield of a substance of m.p. 175–179° and a specific rotation $[\alpha]_D^{20}$ +108°. This substance can be represented by the structural and stereo-formula (XVI). The cyclol character of this compound was established in a way similar to that described for cyclol (VIII). An equilibrium between the open form and the cyclol form in aqueous solution can again be excluded because of its stability to alkali at room temperature. Evidence for the steric homogeneity of this (2S:5S:11R)-cyclol (XVI) appears in the N.M.R. spectrum where the C_2-methyl group is a sharp doublet centered at 85 c.p.s. (J = 7 c.p.s.).

A comparison of the Dreiding stereo-models, assuming that the amide bonds possess double bond character, revealed in the 11,12-syn form (XVII) a very strong 1,3 non-



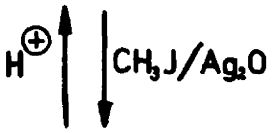
(2S:5S:11R:12R)

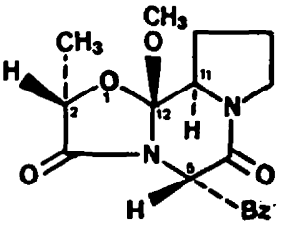
XVI



(2S:5S:11R:12S)

XVII





(2S:5S:11R:12R)

XVIII

bonded interaction between the axial 12-hydroxyl group and the axial 5-benzyl group. The considerably smaller steric compression in the 11,12-anti-configuration[12] must

[12] Unfortunately, the N.M.R. cannot be used as evidence for the 11,12-antiorientation of the H and OH since the hydroxyl proton did not appear as a sharp doublet but as a broad singlet.

be the reason for the stereospecific ring-closure to the (2S:5S:11R:12R)-cyclol (XVI).

The cyclolmethyl ether (XVIII), prepared by methylation of cyclol (XVI) with methyl iodide and silver oxide is sterically pure (N.M.R. spectrum: C_2-methyl doublet centered at 86 c.p.s. (J = 7 c.p.s.); O-methyl singlet at 195 c.p.s.) and showed exactly the same chemical behavior as the cyclol methyl ethers (XIII and XIV).

The findings described in this paper gave the key to the stereospecific synthesis of the peptide part of the ergot alkaloids and in addition valuable information with respect to the stereochemistry of this heterocyclic system.

EXPERIMENTAL*

S-*Phenylalanyl*-S-*proline lactam* (IV)

To a vigorously stirred solution of 2·34 moles S-(N-carbobenzoxy)-proline and 2·34 moles S-phenylalanine methyl ester in 6 l. methylene chloride, 2·7 moles dicyclohexylcarbodiimide were added in small portions within 20 min. After standing for 1 hr at room temp 150 ml glacial acetic acid was added and the precipitated dicyclohexyl urea filtered off and the filtrate concentrated *in vacuo*. Upon dilution of the oily residue with ether some additional urea could be removed by filtration. Evaporation of the organic solvent yielded S-(N-carbobenzoxy)-pro-S-pha-methyl ester (1015 g) as a viscous oil. This crude dipeptide was used in the next step without further purification.

S-(N-Carbobenzoxy)-pro-S-pha-methyl ester (200 g, crude) was dissolved in 2 l. glacial acetic acid 50 g 5% palladium on alumina added and the mixture was hydrogenated on a shaking apparatus. In approximately 2 hr slightly less than 1 mole hydrogen had been taken up. The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo*. The oily residue was heated for 30 min in order to complete ring closure of the dipeptide methyl ester to the diketopiperazine. By crystallization from ethyl acetate–ether (1:20) 89 g (corresponding to 80% overall yield) of S-pha-S-prolactam (IV) was obtained as prisms, m.p. 130–132°. For analysis it was recrystallized from ethyl acetate–ether; m.p. 133° $[\alpha]_D^{20}$ −83° (*c* = 0·2 in water). (Found: C, 68·7; H, 6·7; N, 11·7; O, 12·9. $C_{41}H_{16}N_2O_2$ requires: C, 68·8; H, 6·6; N, 11·5; O, 13·1%).

S-*Phenylalanyl*-R-*proline lactam* (V)

S-Pha-S-prolactam (60 g) was dissolved in 700 ml of 0·5 N sodium hydroxide and kept for 15 min at room temp. A slight excess of 15% hydrochloric acid was then added, the aqueous solution saturated with sodium chloride and extracted with methylene chloride. After drying and evaporating, the oily residue was crystallized from ethyl acetate in prisms (47·5 g, 79% yield) m.p. 146–149°. For analysis the S-pha-R-prolactam (V) was recrystallized from ethyl acetate; m.p. 148–150°; $[\alpha]_D^{20}$ +92° (*c* = 0·2 in water). (Found: C, 68·8; H, 6·7; N, 11·4; O, 13·0. $C_{14}H_{16}N_2O_2$ requires: C, 68·8; H, 6·6; N, 11·5; O, 13·1%).

N-(α-*Benzyloxypropionyl*)-S-*phenylalanyl*-S-*proline lactam* (VII)

S-Pha-S-prolactam (24·4 g, 0·1 mole) was dissolved in 300 ml absolute dioxane, 11·3 ml (0·14 mole) dry pyridine and 27·8 g (0·14 mole) racemic α-benzyloxypropionyl chloride was added and the reaction mixture heated to 80° for 5 hr in an oil bath. The solution was then concentrated to a viscous oil under vacuum at 60°, diluted with 200 ml ether and 160 ml water and vigorously shaken for 30 min in order to hydrolyze the unreacted acid chloride. The etheral solution was washed with dil hydrochloric acid and sodium hydrogen carbonate, dried over anhydrous sodium sulfate and evaporated to dryness. The resulting N-(α-benzyloxypropionyl)-S-pha-S-proline lactam was an oil (34·5 g) which was hydrogenated without further purification. By extraction of the combined aqueous phases with methylene chloride 8·0 g (32·8%) of the unreacted S-pha-S-prolactam was recovered.

Mild acid hydrolysis. Acyl-diketopiperazine (VII, 100 mg) was dissolved in 1 ml methanol and 1 ml 5N hydrochloric acid. After standing for 4 hr at room temp water was added and the solution extracted first with ether (to remove the starting material) and then with methylene chloride. The

* All m.p. were corrected. The N.M.R. spectra were taken on a Varian A-60 spectrometer. Deuterochloroform was used as a solvent, and chemical shifts as well as coupling constants were given in c.p.s.-units relative to tetramethyl silane as the internal reference standard.

methylene chloride solution was dried over sodium sulfate and evaporated to dryness *in vacuo*. The crystalline residue (30 mg) was shown to be practically pure S-pha-S-prolactam by thin-layer chromatography.

Cyclol mixture (VIII)

N-Acyl-diketopiperazine (34·4 g crude) dissolved in 400 ml glacial acetic acid was hydrogenated in the presence of 40 g 5% Pd on alumina within 5 hr. The catalyst was filtered off and the filtrate evaporated thoroughly *in vacuo* to give a crystalline residue. By recrystallization from ethyl acetate 15·0 g (71%) of cyclol (VIII) was obtained; m.p. 193–197°; $[\alpha]_D^{20}$ −23·5° (c = 0·2 in ethanol); N.M.R. spectrum: C_2-Me doublets at 83 c.p.s. and 88 c.p.s. respectively. (Found: C, 64·5; H, 6·4; N, 8·8; O, 20·6. $C_{17}H_{20}N_2O_4$ requires: C, 64·5; H, 6·4; N, 8·9; O, 20·3%).

Chromatographic separation of the cyclol-mixture (VIII) *into the diastereoisomeric cyclols* A (X) *and* B (XI)

Cyclol (VIII, 2 g) was adsorbed onto a column of 75 g silica gel. The column was eluted with chloroform containing 0·75% ethanol and 40 fractions (25 ml) were collected. The first fractions (1 through 12) consisted of pure cyclol A (X) since only one spot was observed on thin layer plates. Combinations of these fractions and crystallization from ethyl acetate yielded 182 mg of pure cyclol A; m.p. 188–189°, $[\alpha]_D^{20}$ −23° (c = 0·2 in ethanol); N.M.R. spectrum: C_2-Me doublet centered at 88 c.p.s. (J = 7 c.p.s.), OH doublet at 164 c.p.s. (J = 2 c.p.s.). (Found: C, 64·3; H, 6·5; N, 9·1; O, 20·2. $C_{17}H_{20}N_2O_4$ requires: C, 64·5; H, 6·4; N, 8·9; O, 20·3%).

From the last fractions (31 through 40) 235 mg of crystalline cyclol B (XI) were obtained as prisms, m.p. 197–201°; $[\alpha]_D^{20}$ −24° (c = 0·2 in ethanol). N.M.R. spectrum: C_2-Me doublet centered at 83 c.p.s. (J = 7 c.p.s.); OH doublet at 140 c.p.s. (J = 2 c.p.s.). (Found: C, 64·5; H, 6·5; N, 9·0; O, 20·1. $C_{17}H_{20}N_2O_4$ requires: C, 64·5; H, 6·4; N, 8·9; O, 20·3%).

N-(S-α-*Benzyloxypropionyl*)-S-*phenylalanyl*-S-*proline lactam* (VII)

The mixture of 4·4 g (18 moles) of S-pha-S-prolactam, 2 g (25 mmoles) of pyridine and 5 g (25 mmoles) of S(−)-α-benzyloxypropionyl chloride in 60 ml absolute dioxane was heated for 5 hr at 80°. The solution was concentrated to a viscous oil *in vacuo* and then ether and water was added and the mixture shaken at room temp for 30 min, in order to hydrolyze the excess of acid chloride. The ether layer was extracted with hydrochloric acid and ice-cold saturated sodium hydrogen carbonate solution, dried over sodium sulfate and evaporated *in vacuo*. From the oily residue (5·7 g) 3·21 g (44%) of the title compound (VII) were obtained as prisms from ether, m.p. 135–136°, $[\alpha]_D^{20}$ −149° (c = 0·2 in ethanol). The recrystallized product gave m.p. 137°. (Found: C, 71·0; N, 6·6; O, 16·1. $C_{24}H_{26}N_2O_4$ requires: C, 70·9; H, 6·5; N, 6·9; O, 15·8%).

Cyclol (XI)

Acyl-diketopiperazine (VII; 2·26 g, 5·6 mmoles) was hydrogenated in 25 ml glacial acetic acid in the presence of 1 g 5% Pd on alumina. The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo* at 60°. Crystallization of the residue from ethyl acetate yielded 1·43 g (81%) of cyclol (XI) m.p. 198°, $[\alpha]_D^{20}$ −24·5 (c = 0·2 in ethanol); N.M.R. spectrum: C_2-Me doublet centered at 83 c.p.s. (J = 7 c.p.s.). The spectrum was identical with that of the aforementioned cyclol B. (Found: 64·3; H, 6·3; $C_{17}H_{20}N_2O_4$ requires: C, 64·5; H, 6·4%).

Hydrogenation of (−)-α-*benzyloxypropionic acid to* S-*lactic acid*

(−)-α-Benzyloxypropionic acid (2·6 g, $[\alpha]_D^{20}$ −92° in ethanol) was hydrogenated in 20 ml glacial acetic acid in the presence of 2 g 5% Pd on alumina. The catalyst was filtered off, the filtrate concentrated *in vacuo*, the residue diluted with water and the aqueous solution extracted with ether to remove any starting material. An excess of zinc carbonate was added to the water layer and evaporated to dryness. The residue was treated with 10 ml hot water, filtered and then the filtrate treated with ethanol. The zinc salt of S-lactic acid was obtained as a white powder (670 mg), $[\alpha]_D^{20}$ −9·2° (c = 1 in water).

Cyclol methyl ether (XIII)

Cyclol (X; 147 mg) dissolved in 6 ml chloroform was treated with 6 ml methyl iodide and 380 mg freshly prepared silver oxide, then stirred for 3 hr at room temp. After filtration and evaporation

of the solvents 160 mg of an oily residue was obtained. Crystallization from ether gave 120 mg (78%) of cyclol methyl ether (XIII) as small prisms, m.p. 108°, $[\alpha]_D^{20}$ +45° (c = 0·2 in ethanol); N.M.R. spectrum: C_2-Me doublet at 84 c.p.s. (J = 7 c.p.s.); OMe singlet at 181 c.p.s. (Found: C, 65·2; H, 6·5; N, 8·7; O, 19·5; $C_{16}H_{22}N_2O_4$ requires: C, 65·5; H, 6·7; N, 8·5; O, 19·4%).

Behavior against alkali. The cyclol methyl ether (XIII; 5 mg) was dissolved in 0·3 ml ethanol and 0·3 ml 1N sodium hydroxide solution and heated for 2 hr. On thin layer plates only unreacted starting material could be detected and 3 mg of the crystalline starting material could be isolated.

Behavior against acid. A solution of 54 mg cyclol methyl ether (XIII) in 5 ml 10% acetic acid was heated for 1 hr on a water bath. The solution was made alkaline with sodium bicarbonate and extracted with methylene chloride. Drying and evaporating of the solvent, followed by crystallization of the residue (51 mg) from ethyl acetate gave 42 mg (81%) of the cyclol (X) which had identical m.p. and N.M.R. spectrum as authentic (X).

Cyclol methyl ether (XIV)

This compound was prepared in exactly the same manner as the cyclol methyl ether (XIII). Starting with 91 mg cyclol (XI) 65 mg (68%) pure cyclol methyl ether (XIV) was obtained by crystallization from ether as prisms, m.p. 114–116°, $[\alpha]_D^{20}$ +43° (c = 0·2 in ethanol); N.M.R. spectrum: C_2-Me doublet at 86 c.p.s. (J = 7 c.p.s.); OMe singlet at 188 c.p.s. (Found: C, 65·6; H, 6·8; N, 8·6; O, 19·3. $C_{16}H_{22}N_2O_4$ requires: C, 65·5; H, 6·7; N, 8·5; O, 19·4%).

N-(α-*Methoxypropionyl*)-S-*phenylalanyl*-S-*proline lactam* (XV)

S-Pha-S-prolactam (976 mg, 4 mmoles) was dissolved in 10 ml dry pyridine. At −20° 980 mg (8 mmoles) racemic α-methoxypropionyl chloride was added and the reaction mixture kept for 3 hr in a refrigerator. The usual separation process resulted in 530 mg of a yellow oil consisting of a mixture of the two diastereoisomeric N-(α-methoxypropionyl)-S-pha-S-prolactams (XV). Separation was accomplished by fractional crystallization from ether. The less soluble compound (230 mg) crystallized in small prisms, m.p. 120–122°, $[\alpha]_D^{20}$ +138° (c = 0·13 in ethanol). (Found: C, 65·1; H, 6·6; N, 8·7; O, 19·6; OCH_3, 9·4. $C_{18}H_{22}N_2O_4$ requires: C, 65·4; H, 6·7; N, 8·5; O, 19·4; OCH_3, 9·4%).

The more soluble compound (197 mg) had m.p. 153–155°; $[\alpha]_D^{20}$ +101° (c = 0·2 in ethanol). (Found: C, 65·5; H, 6·6; N, 8·3; O, 19·5. $C_{18}H_{22}N_2O_4$ requires: C, 65·4; H, 6·7; N, 8·5; O, 19·4%).

Behavior toward alkali. Thirty-three mg (0·1 mmole) of each N-(α-methoxypropionyl)-S-pha-S-prolactam described above were dissolved in methanol and then 0·1N sodium hydroxide was added dropwise at 0° in the presence of phenolphthalein as an indicator. Immediate decolorization of the solution was observed until 0·9 ml (90% of the theory) of the sodium hydroxide solution had been added. By thin layer chromatography it was shown that part of the reaction products was the equilibrium mixture of the two diketopiperazines (IV and V).

The two N-acyl-diketopiperazines (XV) on standing in methanolic ammonia for 3 hr at room temp gave as the main product S-pha-R-prolactam. The same result was obtained on standing for 24 hr in an aqueous methanolic solution of sodium hydrogen carbonate.

Cyclol (XVI)

A mixture of 1·22 g (5 mmoles) of S-pha-R-prolactam, 0·52 ml (6·5 mmoles) of pyridine and 1·3 g (6·5 mmoles) of S(−)-α-benzyloxypropionyl chloride in 15 ml dry dioxane was heated for 14 hr at 80°. Ice-water was then added and the solution kept for 30 min at room temp in order to hydrolyze the excess of acid chloride. After diluting with ether the organic phase was extracted with 1N hydrochloric acid and with ice cold sodium sulfate and evaporated to give crude N-acyl-diketopiperazine as a viscous oil (1·9 g).

This crude material was hydrogenated in 30 ml glacial acetic acid in the presence of 500 mg palladium Mohr within 3·5 hr. The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo* at 60°. The crystalline residue (1·41 g) was recrystallized from a mixture of ethyl acetate, giving 1·09 g (69% over-all yield) of the (2S:5S:11R:12R)-cyclol (XVI) as prisms, m.p. 175–179°, $[\alpha]_D^{20}$ +110° (c = 0·2 in ethanol); N.M.R. spectrum: C_2-Me doublet at 85 c.p.s. (J = 7 c.p.s.). (Found: C, 64·7; H, 6·5; N, 9·1; O, 20·1. $C_{17}H_{20}N_2O_4$ requires: C, 64·5; H, 6·4; N, 8·9; O, 20·3%).

Cyclol methyl ether (XVIII)

Cyclol (XVI; 100 mg) dissolved in 45 ml chloroform was treated with 45 ml methyl iodide and 230 mg silver oxide and then the mixture was stirred for 2 hr at 20–30°. After filtrating, diluting with chloroform and extraction of the organic phase with 1N sodium hydroxide, the solvent was dried and evaporated *in vacuo*. The residue crystallized from ether in prisms (45 mg), m.p. 145°; N.M.R. spectrum: C_3-Me doublet at 86 c.p.s., OMe singlet at 195 c.p.s. (Found: C, 65·5; H, 6·6; N, 8·8; O, 19·2. $C_{18}H_{32}N_2O_6$ requires: C, 65·5; H, 6·7; N, 8·5; O, 19·4%).

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