SYNTHESIS AND SPECTRAL PROPERTIES OF CYCLOTRIPEPTIDES CONTAINING 2-AZETIDINECARBOXYLIC ACID OR PROLINE

J.Vičar[a], P.Maloň[b], A.Trka[b], J.Smolíková[b], I.Frič[b] and K.Bláha[b]

[a] *Institute of Medicinal Chemistry, Palacký University, 775 15 Olomouc and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

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The synthesis of cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*XV*) and of the analogous cyclotripeptide *VII* derived from L-proline is reported. The mass, IR, UV, and CD spectra of *VII* and *XV* are given. In both instances, the results appear to indicate the occurrence of a single conformation in solution, with a C_3 symmetry. The mutual position of amide groups creating a homoconjugated chromophoric system makes possible electronic interactions manifesting themselves in CD spectra. The possible occurrence of nonplanar amide groups is discussed.

The cyclotripeptides are interesting objects particularly from the standpoint of their unique stereochemistry. Thus, all the amide groups are necessarily *cis* and most probably nonplanar[1]. It has been shown that only those cyclotripeptides can be synthesised all the peptide bonds of which exhibit the character of tertiary amides[2], *i.e.*, contain residues of proline, 4-hydroxyproline and/or sarcosine in various combinations[3-5]. Providing that the particular cyclotripeptide should contain a secondary amide group, cyclic dimerisation takes place with the formation of a cyclohexapeptide or cyclol structures are produced[2]. The synthesis of sarcosine-containing cyclotripeptides was observed to be accompanied by a side reaction consisting in the formation of a cyclodipeptide by removal of one amino acid residue in the course of the cyclisation[5]. The stereochemistry has been discussed only with cyclo(L-prolyl-L-prolyl-L-prolyl) (*VII*) in connection with empirical calculations[1], CD and ^{1}H-NMR spectral measurements[6-8], and X-ray structure determination[9,10].

The object of the present work is the synthesis of cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*XV*), a cyclotripeptide derived from the lower homologue of proline, *i.e.*, 2-azetidinecarboxylic acid. The four-membered ring of the starting amino acid should introduce into the polycyclic system a steric strain which could manifest itself by a changed behaviour of the cyclotripeptide

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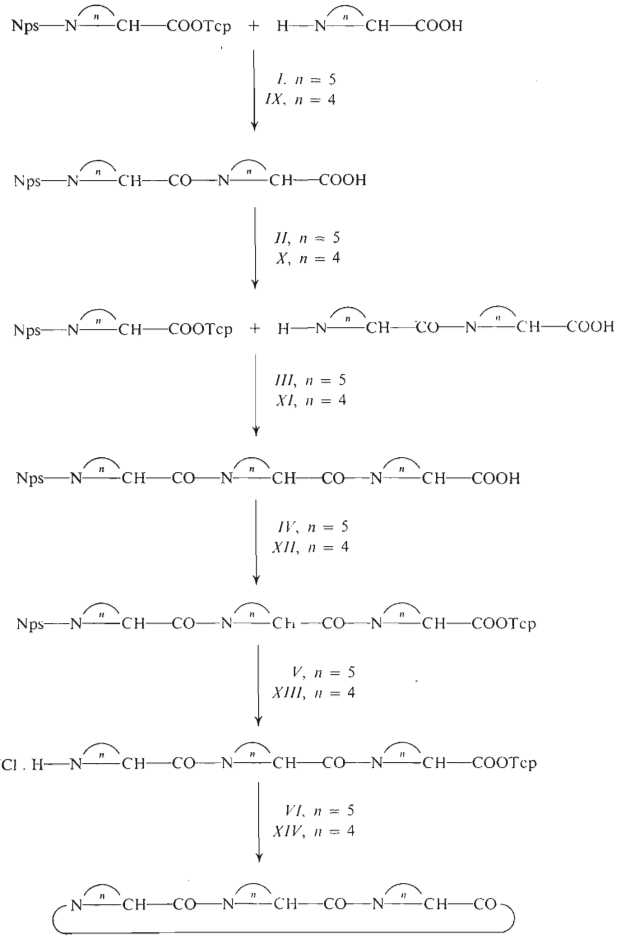

Nps—N(n)CH—COOTcp + H—N(n)CH—COOH
I, n = 5
IX, n = 4
Nps—N(n)CH—CO—N(n)CH—COOH
II, n = 5
X, n = 4
Nps—N(n)CH—COOTcp + H—N(n)CH—CO—N(n)CH—COOH
III, n = 5
XI, n = 4
Nps—N(n)CH—CO—N(n)CH—CO—N(n)CH—COOH
IV, n = 5
XII, n = 4
Nps—N(n)CH—CO—N(n)CH—CO—N(n)CH—COOTcp
V, n = 5
XIII, n = 4
HCl . H—N(n)CH—CO—N(n)CH—CO—N(n)CH—COOTcp
VI, n = 5
XIV, n = 4
N(n)CH—CO—N(n)CH—CO—N(n)CH—CO
VII, n = 5
XV, n = 4

XV with respect to the proline-containing cyclotripeptide *VII*. The comparison was effected with the use of circular dichroism, mass spectrometry, and IR spectroscopy.

The synthesis of the two cyclotripeptides *VII* and *XV* was performed according to a uniform scheme with the use of the 2-nitrobenzenesulfenyl group in N-protection and of trichlorophenyl esters in the formation of peptide bonds, see Scheme 1. The final cyclisation was carried out with satisfactory yields according to Rothe and coworkers[11,12] in pyridine. In the proline series, the synthesis was effected with the L-enantiomer of the amino acid while in the 2-azetidinecarboxylic acid series the D-enantiomer was used for technical reasons. Both syntheses yielded homogeneous crystalline cyclotripeptides which were shown by gas chromatography to lack any cyclodipeptides as admixture (the cyclodipeptides could arise by cleavage of the chain during the cyclisation[5]).

EXPERIMENTAL

Melting points (uncorrected) were taken on a heated microscope stage (Kofler block). Thin-layer chromatography was performed on ready-for-use Silufol (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_1, chloroform–methanol (98 : 2); S_2, chloroform–methanol (96 : 4); and S_3, chloroform–methanol (92 : 8). Analytical samples were dried at room temperature and 1 Torr for 8—16 h. Optical rotations were measured on a Perkin–Elmer 141 apparatus in the concentration of 0·50—0·55 g of the substance per 100 ml of methanol. Mass spectra were recorded on a double focussing mass spectrometer AEI MS 902 (A.E.I., Manchester, U.K.) with the use of a direct inlet at the ion source temperature of 160—180°C and ionisation energy 70 eV. In high resolution measurements, the resolving power 15000 was used; the accuracy was ±3 ppm. The CD spectra were measured on a Roussel-Jouan Dichrographe 185 Model II in cells of optical paths 1·0, 0·1, and 0·02 cm (concentration 0·2—0·3 mg/ml) and expressed in $[\theta]$ (deg cm^2 dmol^{-1}). The temperature dependence of CD curves was measured in 1 : 1 methanol–ethanol at −160°C to +40°C. The UV spectra were taken on a Cary 14 apparatus in acetonitrile and 2,2,2-trifluoroethanol; concentration 0·5 mg/ml; ε in l mol^{-1} cm^{-1}. The IR spectra were recorded on a Model 621 Perkin-Elmer apparatus. The spectra in KBr micropellets were measured in the range from 4000 to 400 cm^{-1}. Owing to a low solubility, the spectra in tetrachloromethane were measured in saturated solutions in an 1 cm cell in the range of 1750 to 1630 cm^{-1} and 1460—1285 cm^{-1}. The spectra in tribromomethane were measured in the range of 1500—1180 cm^{-1}. The temperature dependence was examined in about 0·05M tribromomethane solutions of cyclotripeptides *VII* and *XV* at 37—110°C. Solutions in chloroform were measured in the range of 4000—400 cm^{-1}. The apparatus was calibrated with steam.

o-Nitrobenzenesulfenyl-L-proline 2,4,5-Trichlorophenyl Ester (*I*)

A mixture of *o*-nitrobenzenesulfenyl-L-proline (1·43 g), 2,4,5-trichlorophenol (1·18 g), N,N′-dicyclohexylcarbodiimide (1·13 g), and chloroform (25 ml) was prepared at −15°C, stirred at −10°C for 1 h, kept at 0°C overnight, and evaporated. Ether was added to the residue and the

SCHEME 1 $n = 5$ for L-proline series,
$n = 4$ for D-2-azetidinecarboxylic acid series.

N,N'-dicyclohexylurea filtered off. The filtrate was evaporated and the residue triturated with light petroleum to afford 1·82 g (83%) of the ester *I*, m.p. 89—93°C (m.p. 93—96°C after recrystallisation from ethanol); $[\alpha]_D^{25}$ −94·2°. For $C_{17}H_{13}Cl_3N_2O_4S$ (447·7) calculated: 45·61% C, 2·93% H, 6·26% N; found: 45·72% C, 2·81% H, 6·40% N.

o-Nitrobenzenesulfenyl-L-prolyl-L-proline (*II*)

L-Proline (0·575 g) and N-ethylpiperidine (0·685 ml) were dissolved in water (2 ml) and a solution of compound *I* (2·13 g) in dimethylformamide (10 ml) was added with stirring. The mixture was stirred at room temperature for 2 h, kept at the same temperature for 2 days, and evaporated. The residue was dissolved in 5% aqueous ammonia and the solution washed with ethyl acetate. The aqueous layer was acidified with 0·5M-H_2SO_4, extracted with ethyl acetate, the extract dried over anhydrous sodium sulfate, evaporated, and the residue triturated with light petroleum to afford 1·33 g (77%) of compound *II*, m.p. 137—142°C, containing an admixture of *o*-nitrobenzenesulfenyl-L-proline as determined by thin-layer chromatography in the solvent system S_1. Recrystallisation from ethyl acetate–light petroleum yielded 1·05 g (61%) of chromatographically homogeneous *II*, m.p. 152—154°C (m.p. 154·5—155°C after a further recrystallisation); $[\alpha]_D^{25}$ −176·0°. For $C_{16}H_{19}N_3O_5S$ (365·4) calculated: 52·59% C, 5·24% H, 11·50% N; found: 52·71% C, 5·19% H, 11·30% N.

L-Prolyl-L-proline (*III*)

To a stirred solution of compound *II* (0·95 g) in acetone (15 ml) and ether (25 ml), 1·2M hydrogen chloride in tetrahydrofuran (4 ml) was added and, after 15 min, the solution decanted. The mass adhering to walls of the vessel was triturated with ether and dissolved in 50% aqueous methanol. The solution was applied to a column (12 ml) of Dowex 50 ion exchange resin in the H^+ form and the column was washed with 50% aqueous methanol. Elution with a 9 : 1 mixture (80 ml) of 50% aqueous methanol with pyridine and evaporation of the effluent yielded 0·52 g (87%) of compound *III*, m.p. 144—146°C (reported[11], m.p. 144—145°C). The yield refers to *III* containing one molecule of water.

o-Nitrobenzenesulfenyl-L-prolyl-L-prolyl-L-proline (*IV*)

To a stirred solution of compound *III* (300 mg) and N-ethylpiperidine (0·195 ml) in water (3 ml), a solution of compound *I* (690 mg) in dimethylformamide (14 ml) was added. The mixture was stirred at room temperature for 24 h, kept at the same temperature for 12 h, and evaporated. The residue was dissolved in 5% aqueous ammonia and the solution washed with ethyl acetate. The aqueous layer was acidified with 0·5M-H_2SO_4, extracted with ethyl acetate, the extract dried over anhydrous sodium sulfate, and evaporated to afford 680 mg of a material containing as admixture a faster travelling contaminant (thin-layer chromatography in S_2). The crude product was dissolved in chloroform and the solution applied to a column of silica gel (particle size 30—60 μ; 50 g). The column was washed with chloroform (100 ml) and then eluted with chloroform–methanol (97 : 3). Evaporation of the eluate yielded 400 mg (64%) of compound *IV* as a yellow foam; $[\alpha]_D^{25}$ −211·5°. For $C_{21}H_{26}N_4O_6S$ (462·5) calculated: 54·53% C, 5·67% H, 12·11% N; found: 54·66% C, 5·81% H, 11·84% N.

o-Nitrobenzenesulfenyl-L-prolyl-L-prolyl-L-proline 2,4,5-Trichlorophenyl Ester (*V*)

At −15°C, a mixture of compound *IV* (340 mg), 2,4,5-trichlorophenol (205 mg), N,N'-dicyclohexylcarbodiimide (200 mg), and ethyl acetate (5 ml) was prepared, kept in a refrigerator for

2 days, and at room temperature for 1 day, and evaporated. An 1 : 1 mixture (15 ml) of ethyl acetate and ether was then added and the N,N'-dicyclohexylurea was filtered off. The filtrate was evaporated and the residue triturated with light petroleum to afford 360 mg (71%) of a yellow foam of compound *V*, $[\alpha]_D^{25}$ −197·0°. For $C_{27}H_{27}Cl_3N_4O_6S$ (642·0) calculated: 50·52% C, 4·24% H, 8·73% N; found: 50·96% C, 4·68% H, 8·54% N.

L-Prolyl-L-prolyl-L-proline 2,4,5-Trichlorophenyl Ester Hydrochloride (*VI*)

Compound *V* (300 mg) was dissolved in a mixture of ethyl acetate (1 ml) and ether (20 ml) and then 1·3M hydrogen chloride in tetrahydrofuran (0·8 ml) was added with stirring. The precipitate *VI* was collected with suction and washed with ether. Yield, 180 mg (75%); $[\alpha]_D^{25}$ −160·6°. For $C_{21}H_{25}Cl_4N_3O_4$ (525·3) calculated: 48·02% C, 4·80% H, 8·00% N; found: 47·90% C, 5·13% H, 8·39% N.

Cyclo(L-prolyl-L-prolyl-L-prolyl) (*VII*)

At 105°C, a solution of compound *VI* (160 mg) in dimethylformamide (5 ml) was added with stirring in 0·5 ml portions and in 20—30 min intervals into pyridine (150 ml). When the addition was completed, the mixture was stirred at 105°C for 8 h, kept at room temperature for 1 day, and evaporated. To the residue, acetone (3 ml) and ethyl acetate (3 ml) was added. The crystals were collected with suction and washed with ethyl acetate. Yield, 66 mg (74%) of compound *VII*; sublimation at 250°C and 0·2 Torr afforded 54·5 mg (61%) of purified *VII*, decomposing above 350°C, $[\alpha]_D^{25}$ +46·2° (reported[12], m.p. 338°C (decomp.) and $[\alpha]_D^{25}$ +48·5°). High resolution mass spectrum (*m/e*, composition): 291 (M), $C_{15}H_{21}N_3O_3$; 222, $C_{11}H_{14}N_2O_3$; 194, $C_{10}H_{14}N_2O_2$; 166, $C_9H_{14}N_2O$; 138, $C_8H_{14}N_2$; C_4H_8N.

o-Nitrobenzenesulfenyl-D-2-azetidinecarboxylic Acid Dicyclohexylammonium Salt (*VIII*)

A mixture of D-2-azetidinecarboxylic acid (2·2 g), 2M-NaOH (11 ml), and dioxane (25 ml) was treated with stirring over 15 min alternately with *o*-nitrobenzenesulfenyl chloride (4·6 g) and 2M-NaOH (11 ml) in ten portions each. The solution was diluted with water (200 ml), filtered, the filtrate acidified with 0·5M-H_2SO_4, extracted with ethyl acetate, and the extract evaporated. The residue was dissolved in ether and the insoluble portion (0·6 g of a yellow solid, m.p. 131 to 138°C) was filtered off. Dicyclohexylamine (2·8 ml) was then added to the filtrate and the whole was kept in a refrigerator overnight to deposit 4·7 g (54%) of the salt *VIII*, m.p. 140—145°C. After two recrystallisations from ethyl acetate–light petroleum, the analytical sample of *VIII* melted at 143—148°C; $[\alpha]_D^{25}$ +28·7°. For $C_{22}H_{33}N_3O_4S$ (435·6) calculated: 60·66% C, 7·64% H, 9·65% N; found: 60·29% C, 7·68% H, 9·60% N.

o-Nitrobenzenesulfenyl-D-2-azetidinecarboxylic Acid 2,4,5-Trichlorophenyl Ester (*IX*)

The *o*-nitrobenzenesulfenyl-D-2-azetidinecarboxylic acid was liberated from the salt *VIII* (1·30 g) by means of 0·5M-H_2SO_4, extracted with ethyl acetate, the extract dried over anhydrous sodium sulfate, and evaporated. The residue was dissolved in chloroform (12 ml), the solution cooled down, and treated at −15°C with 2,4,5-trichlorophenol (0·72 g) and N,N'-dicyclohexylcarbodiimide (0·65 g). The mixture was stirred at −10°C for 2 h, kept in a refrigerator overnight, and evaporated. Ether was added to the residue and the insoluble N,N'-dicyclohexylurea filtered off. The filtrate was evaporated, the residue triturated with light petroleum and then with ethanol to solidify, the solid collected with suction, and washed with light petroleum. Yield, 1·05 g (81%)

of the ester *IX*, m.p. 103—109°. After two crystallisations from ethanol, the analytical sample melted at 110·5—112·5°C; $[\alpha]_D^{25}$ +110·4°. For $C_{16}H_{11}Cl_3N_2O_4S$ (433·5) calculated: 44·33% C, 2·62% H, 7·38% N; found: 44·76% C, 2·77% H, 7·18% N.

o-Nitrobenzenesulfenyl-D-2-azetidinecarbonyl-D-2-azetidinecarboxylic Acid (*X*)

Compound *X* was prepared analogously to compound *II* in 75% yield. M.p. 158—163°C. After recrystallisation from ethyl acetate–light petroleum, the analytical sample of *X* melted at 161 to 163°C; $[\alpha]_D^{25}$ +165·0°. For $C_{14}H_{15}N_3O_5S$. 1/2 H_2O (337·4) calculated: 48·54% C, 4·65% H, 12·13% N; found: 48·70% C, 4·48% H, 12·06% N.

D-2-Azetidinecarbonyl-D-2-azetidinecarboxylic Acid (*XI*)

To a solution of compound *X* (1·17 g) in methanol (25 ml), 1·1M hydrogen chloride in tetrahydrofuran (4 ml) was added, the mixture stirred at room temperature for 4 min, and evaporated. The residue was dissolved in 50% aqueous methanol and the solution applied to a column of Dowex 50 (H^+) ion exchange resin (9 ml). The column was washed with 50% aqueous methanol and then eluted with 3% of aqueous ammonia in 50% aqueous methanol. Evaporation of the eluate yielded 0·63 g (98%) of compound *XI*, m.p. 173—177°C. The analytical sample was recrystallised from methanol–ether–light petroleum; m.p. 174—178°C; $[\alpha]_D^{25}$ +244·8°. For $C_8H_{12}N_2O_3$ (184·2) calculated: 52·17% C, 6·57% H, 15·20% N; found: 52·82% C, 6·57% H, 15·35% N.

o-Nitrobenzenesulfenyl-D-2-azetidinecarbonyl-D-2-azetidinecarbonyl-D-2-azetidinecarboxylic Acid (*XII*)

To a solution of compound *XI* (263 mg) and N-ethylpiperidine (0·203 ml), a solution of compound *IX* (617 mg) in dimethylformamide (8 ml) was added with stirring. The mixture was stirred at room temperature for 24 h, kept overnight, and evaporated. The residue was dissolved in 5% aqueous ammonia and the solution washed with ethyl acetate. The aqueous layer was acidified with 0·5M-H_2SO_4, extracted with ethyl acetate, the extract dried over anhydrous sodium sulfate, and evaporated. Yield, 0·58 g (96%) of the acid *XII*, homogeneous on chromatography in S_3; $[\alpha]_D^{25}$ +205·7°. For $C_{18}H_{20}N_4O_6S$. 1/2 H_2O (429·5) calculated: 50·34% C, 4·92% H, 13·04% N; found: 50·06% C, 4·84% H, 12·54% N.

D-2-Azetidinecarbonyl-D-2-azetidinecarbonyl-D-2-azetidinecarboxylic Acid 2,4,5-Trichlorophenyl Ester Hydrochloride (*XIV*)

Compound *XIV* was prepared analogously to compound *VI* without isolation of the intermediate *XIII*. At −15°C, a mixture of compound *XII* (247 mg), 2,4,5-trichlorophenol (190 mg), N,N′-dicyclohexylcarbodiimide (220 mg), and chloroform (7 ml) was prepared. After the evaporation of chloroform, the residue was dissolved in a mixture of ethyl acetate and ether and the insoluble N,N′-dicyclohexylurea was filtered off. The filtrate was evaporated, the residue dissolved in ethyl acetate (2 ml), and the solution stirred with ether (10 ml) and 1·28M hydrogen chloride in tetrahydrofuran (1 ml) for 15 min. The precipitate was collected with suction and washed with ether. The hydrochloride *XIV*, $[\alpha]_D^{25}$ +157·2°. For $C_{18}H_{29}Cl_4N_3O_4$ (483·2) calculated: 44·74% C, 3·96% H, 8·70% N; found: 44·19% C, 4·34% H, 8·77% N.

Cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*XV*)

Compound *XV* was prepared from compound *XIV* in 38% yield analogously to compound *VII*. Compound *XV* decomposes above 350°C and sublimes at 250°C and 1 Torr. For $C_{12}H_{15}N_3O_3$ (249·3) calculated: 57·82% C, 6·07% H, 16·86% N; found: 58·07% C, 6·22% H, 16·76% N. High resolution mass spectrum (*m/e*, composition; with multiplets, percentage of ions of the same nominal mass is given in parentheses): 249 (M), $C_{12}H_{15}N_3O_3$; 221, $C_{10}H_{11}N_3O_3$ (80%); 221, $C_{11}H_{15}N_3O_2$ (20%); 194, $C_9H_{10}N_2O_3$ (60%); 194, $C_{10}H_{14}N_2O_2$ (40%); 193, $C_{10}H_{13}N_2O_2$; 166, $C_8H_{10}N_2O_2$ (60%); 166, $C_7H_6N_2O_3$ (40%); 138, $C_5H_4N_3O_2$ (45%), 138, $C_6H_6N_2O_2$ (45%); 138, $C_7H_{10}N_2O$ (10%); 110, C_6H_8NO; 56, C_3H_6N. $[\alpha]_D$ + 4·9°, $[\alpha]_{300}$ + 52·4° (c 0·12, methanol).

RESULTS AND DISCUSSION

In order to confirm the structure of cyclotripeptides *VII* and *XV*, their mass spectra were examined in detail. Furthermore, it was of interest to compare fragmentation of known cyclic peptides with that of the present two cyclotripeptides. The fragmentation of the molecular ion of the proline cyclotripeptide *VII* (Fig. 1) is initiated by elimination of a pyrroline molecule and continues (apparently without ring-opening) by a successive expulsion of three molecules of carbon monoxide (Scheme 2). The spectrum exhibits all the corresponding metastable peaks: m^+ (291 − 222) = 169·36; m^+ (222 − 194) = 169·53; m^+ (166 − 138) = 114·7. The peak at *m/e* 194 is de facto the molecular ion of the cyclo(prolyl-prolyl) cyclodipeptide. The mass spectrum of the cyclotripeptide *VII* from the peak at *m/e* 194 downwards is almost identical with that of the authentic[13,14] cyclo(prolyl-prolyl) except for a higher intensity of the peak at *m/e* 166 in the spectrum of the cyclotripeptide *VII* and insignificant

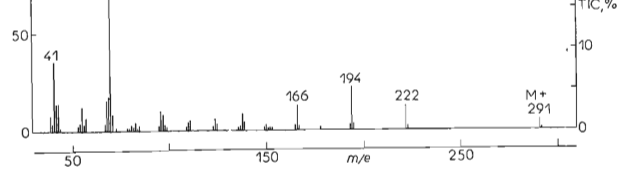
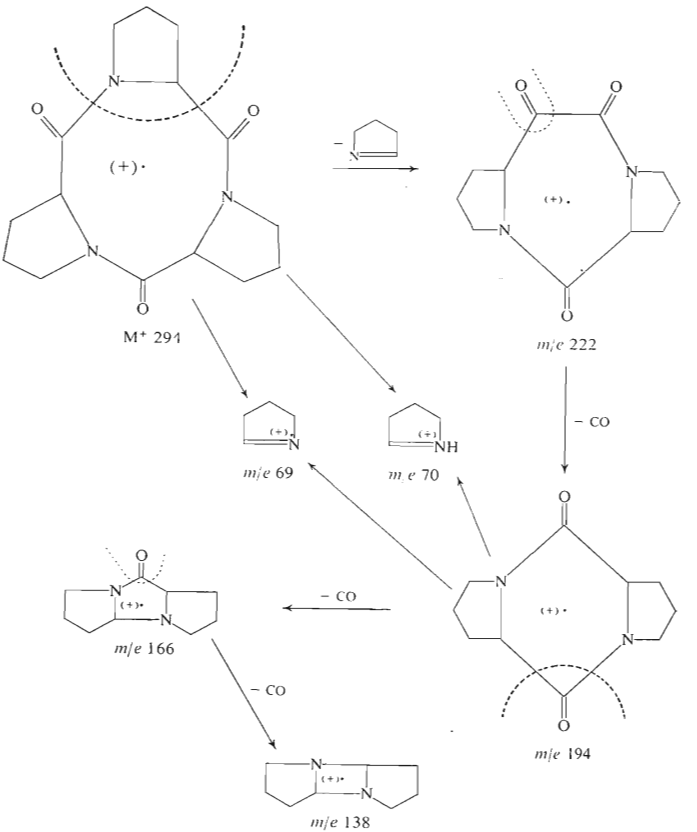


FIG. 1

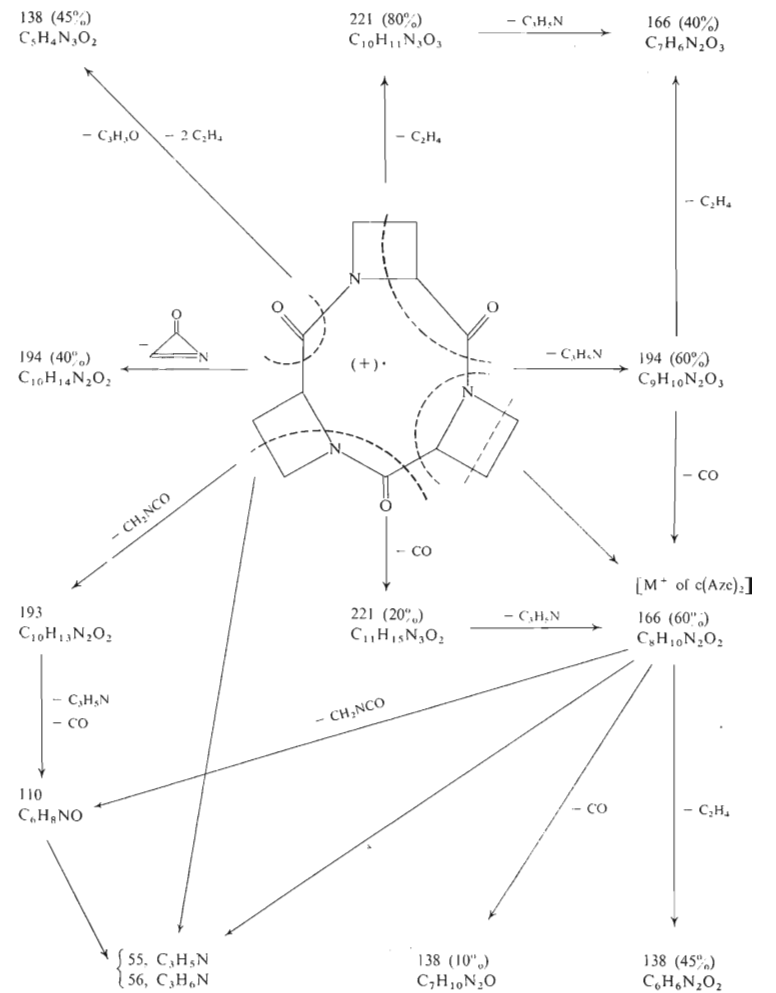
Electron Impact Mass Spectrum of Cyclo(L-prolyl-L-prolyl-L-prolyl) (*VII*)

Direct inlet, temperature of the ion source 160°C, ionization energy 70 eV, TIC total ion current, b.p. base peak.



SCHEME 2

deviations in intensities of some further peaks. In both cases, the base peak is formed by the pyrrolidinium ion at *m/e* 70. As it may be seen from Scheme 3, the fragmentation of the 2-azetidinecarboxylic tripeptide *XV* (Fig. 2) is considerably more complex.


138 (45%)
$C_5H_4N_3O_2$
221 (80%)
$C_{10}H_{11}N_3O_3$
$-C_3H_5N$
166 (40%)
$C_7H_6N_2O_3$
$-C_3H_3O$
$-2C_2H_4$
$-C_2H_4$
$-C_2H_4$
194 (40%)
$C_{10}H_{14}N_2O_2$
(+)·
$-C_3H_5N$
194 (60%)
$C_9H_{10}N_2O_3$
$-CO$
$-CH_2NCO$
$-CO$
[M+ of c(Aze)2]
193
$C_{10}H_{13}N_2O_2$
221 (20%)
$C_{11}H_{15}N_3O_2$
$-C_3H_5N$
166 (60%)
$C_8H_{10}N_2O_2$
$-C_3H_5N$
$-CO$
$-CH_2NCO$
110
C_6H_8NO
$-CO$
$-C_2H_4$
55, C_3H_5N
56, C_3H_6N
138 (10%)
$C_7H_{10}N_2O$
138 (45%)
$C_6H_6N_2O_2$

SCHEME 3

Except for the elimination of the molecule of CO and C_3H_5N and formation of $C_3H_5N^{(+)\cdot}$ and $C_3H_6N^{(+)\cdot}$ ions, all the remaining fragmentation processes of this sterically strained system consist in cleavage of the four-membered ring in the 2-azetidine-carboxylic acid residue, the particles C_2H_4, CH_2NCO, C_2HNO, and C_3H_3O being eliminated in various combinations. From the peak at *m/e* 166 downwards, the spectrum of the cyclotripeptide *XV* is very similar to that of the cyclo(2-azetidinecarbonyl-2-azetidinecarbonyl) cyclodipeptide[14] (*cf.* the above spectral resemblance of *VII* to the corresponding cyclodipeptide). The higher intensity of the peak at *m/e* 138 in the spectrum of the cyclotripeptide *XV* (28% relative intensity *versus* 6·7% relative intensity in the spectrum of the cyclodipeptide) is particularly due to the contribution of the $C_5H_4N_3O_2^{(+)\cdot}$ which is directly formed from the molecular ion of the cyclotripeptide (Scheme 4). In the further fragmentation of the $C_8H_{10}N_2 . O_2^{(+)\cdot}$ ion, *i.e.*, the quasimolecular ion of cyclo(2-azetidinecarbonyl-2-azetidinecarbonyl), elimination of C_2H_4 is preferred to that of CO (in the ratio of 4·5 : 1) while in the fragmentation of the virtual molecular ion of this cyclodipeptide[14], the elimination of CO predominates (in the ratio of 6·4 : 1).

As indicated by mass spectral analysis of compounds *VII* and *XV*, the fragmentation of cyclotripeptides (contrary to that of cyclohexapeptides) consists exclusively in elimination of small particles without any previous opening of the cyclic molecular ion. Such a fragmentation makes possible the formation of entities of the cyclodipeptide character. This formation is probably aided by *a*) the forced *cis*-configuration of peptide bonds, *b*) the relative proximity of the nitrogen atom in residue 1 to the carbonyl carbon atom in residue 2 (0·22 nm in the crystal conformation[10]) resulting in superimposed carbonyl π-orbital and the unshared electron pair orbital

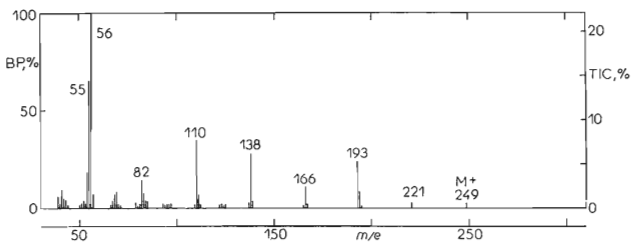
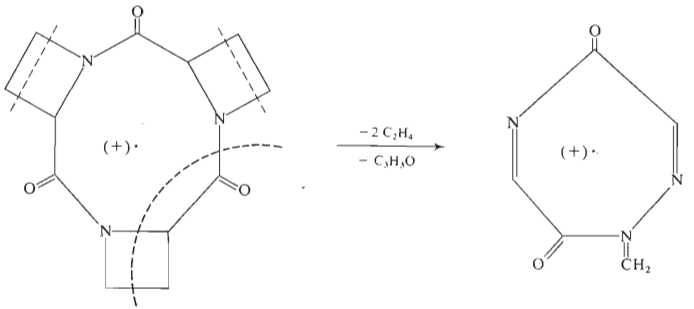


FIG. 2

Electron Impact Mass Spectrum of Cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl-D-2--azetidinecarbonyl) (*XV*)

For the conditions see Fig. 1.



SCHEME 4

on the nitrogen atom and facilitating the transanular interaction, and *c*) the nonplanarity of amide bonds (as inferred from conformation in the crystalline state[10]) which should increase the basicity of the nitrogen atom.

In addition to ^{1}H-NMR spectra[15], ^{13}C-NMR spectra[16,17], and X-ray diffraction[9,10], some contributions to the knowledge on the conformation of the present peptides can also be inferred from IR spectra (Table I) particularly regarding the differences in conformation in the crystalline state and in solutions. With the proline cyclotripeptide, the spectral differences between the crystal and solution are concentrated in a very small range below 1 500 cm^{-1} (disappearance of the band at 900 cm^{-1} and a change of the relative intensity of bands at 1 310 and 1 295 cm^{-1}). Furthermore, the comparison does not show any significant shift of the stretching vibration band of the C'—N bond. In the crystalline state, the proline cyclotripeptide exhibits two equally intensive bands of the ν(C'=O) vibration while only a single band can be observed in solutions. As shown by the crystallographic examination of cyclo(L-Pro)$_3$ (*VII*), two conformers are present in the unit cell and differ particularly in the degree of nonplanarity of peptide groups. It could be thus anticipated that the multiplicity of the ν(C'=O) band is due to this circumstance. More likely however, this multiplicity is caused by intermolecular interactions in the crystal lattice where the oxygen atoms are exposed to an intermolecular contact. The spectrum of cyclo(L-Pro)$_3$ (*VII*) in solution indicates the presence of a conformation with nondistinguishable geometry of amide groups because of the C_3 symmetry of this conformation or because of a rapid displacement (pseudorotation[18,19]) of the three equivalent conformers corresponding to one of the conformations in the crystalline state.

On the contrary, cyclo(D-Aze)$_3$ (*XV*) exhibits a sole band of the ν(C′=O) vibration both in the crystalline state and in solutions. The spectral region below 1 500 cm^{-1} is very complex in the crystalline state of compound *XV* while in solutions a considerable number of bands disappears. Moreover, a marked shift (+33 cm^{-1}) of the band due to the stretching vibration of the C′—N bond may be observed in the chloroform solution with respect to the crystalline state. With cyclo(D-Aze)$_3$ (*XV*), the three-dimensional structure of which is not known, a single conformation with a significant nonplanarity can be expected in the crystalline state while in solutions, the conformation exhibits more planar (averaged) amide bonds. The difference in conformations of cyclotripeptides *VII* and *XV* can be *inter alia* ascribed to the difference in valence angles due to the presence of fused four-membered rings in compound *XV*; thus for example, the lower value of the internal valence angle of the carbonyl group in compound *XV* manifests itself by a shift of the ν(C′=O) band by 6·7 cm^{-1} upwards. Differences in nonplanarity of the amide group in compounds *VII* and *XV* may also play an important role. Let us compare in this connection the IR spectrum of the cyclotripeptides *VII* and *XV* with those of cyclo(L-Pro-D-Pro), ν(C′—N) 1 450 cm^{-1} in tetrachloromethane (an almost planar 2,5-piperazinedione ring[20]) and of cyclo(L-Pro-L-Pro), ν(C′—N) 1 420 cm^{-1} in tetrachloromethane (boat conformation of the 2,5-piperazinedione ring[20]). The ν(C′—N) value of cyclo(L-Pro)$_3$

TABLE I

Wavenumbers of IR Bands of Cyclo(L-prolyl-L-prolyl-L-prolyl) (*VII*) and Cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*XV*) in the Region of Stretching Vibrations of C′=O and C′—N Bonds

Solvent	ν(C′=O), cm^{-1}	ν(C′—N), cm^{-1}
	cyclotripeptide *VII*	
KBr	1 650 sh, 1 645 vs, 1 628 sh, 1 620 vs	1 440 s
CCl_4	1 660 w, 1 651 vs,	1 434 s
$CHCl_3$	1 655 sh, 1 645 vs	1 442 s
$CHBr_3$	1 638 vs	1 439·5 s
	cyclotripeptide *XV*	
KBr	1 649 vs, 1 630 sh, 1 618 sh	1 438 m
CCl_4	1 657·5 vs	1 450·5 m
$CHCl_3$	1 653 sh, 1 640 vs	1 457 m
$CHBr_3$	1 654 sh, 1 640 vs	1 456·2 m

(*VII*) lies exactly in the middle between the two cyclodipeptide values. On the other hand, the ν(C′—N) value of cyclo(D-Aze)$_3$ (*XV*) is situated at unusually high wavenumbers, contrary to the extraordinarily low ν(C′—N) value in the case of cyclo(D-Aze-D-Aze), 1 396·8 cm^{-1} in tetrachloromethane[21]. The shift of ν(C′—N) due to the change of solvents (tetrachloromethane–chloroform) is the same with compounds *VII* and *XV* and as indicated by comparison with the pair of cyclodipeptides, the nonplanarity of amide bonds does not appear to be extremely high.

The IR spectra of cyclo(L-Pro)$_3$ (*VII*) are more temperature-dependent in accord with the expected higher flexibility. With increasing temperature, the intensity of the ν(C′—N) bands considerably decreases and the wavenumbers shift to lower values (by about -3 cm^{-1}) in the temperature range between 37°C and 110°C. The observed spectral changes are reversible and are most likely due to the influence of temperature on solvation of the molecule, *i.e.*, weakening of hydrogen bonds between $CHBr_3$ and amide groups at an elevated temperature.

From the standpoint of stereochemistry, valuable information could be supplied by measurements of chiroptical properties. Satisfactory experimental CD and UV data were therefore collected as a basis of further theoretical considerations and confrontation with semiempirical calculations.

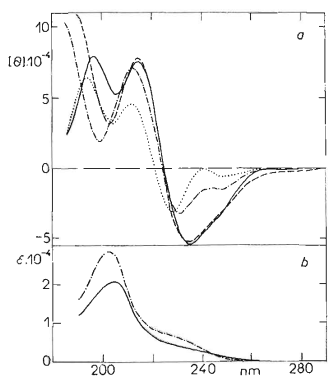


FIG. 3

CD (*a*) and UV (*b*) Spectra of Cyclo(L-prolyl-L-prolyl-L-prolyl) (*VII*) in Various Solvents ——— in acetonitrile, – – – in ethanol, ·—·—· in water, ····· in 2,2,2-trifluoroethanol.

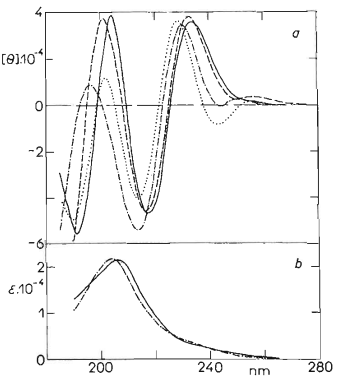


FIG. 4

CD (*a*) and UV (*b*) Spectra of Cyclo(D-2-azetidinecarbonyl-D-2-azetidinecarbonyl-D-2-azetidinecarbonyl) (*XV*) in Various Solvents For the designation of curves see Fig. 3.

The course of UV absorption curves of compounds *VII* and *XV* in a poorly polar solvent is very similar (Figs 3 and 4). We may observe a region of a weak absorption at 250–265 nm ($\varepsilon \leq 1000$), a distinct shoulder at 220–250 nm ($\varepsilon \sim 4000$), and a shortwavelength absorption band at 190–220 nm (maximum at 205 nm, $\varepsilon \sim 20000$). Contrary to common amides and small peptides, the spectrum is bathochromically shifted and the extinction coefficient of the $\pi - \pi^*$ transition is relatively low. Furthermore, the existence of a shoulder at about 230 nm with a high molar extinction coefficient is not usual. In polar media, the UV spectrum of the proline derivative *VII* exhibits an increase of intensity by about 20–30% while the absorption of the 2-azetidinecarboxylic derivative *XV* does not depend on the polarity of the solvent.

The CD curves of the cyclotripeptides *VII* and *XV* are shown on Figs 3 and 4. At first sight, the complex shape is surprising. In polar solvents, 2,2,2-trifluoroethanol and water, six dichroic bands can be identified: two weak bands at about 250 and 240 nm and four very strong bands with extrema at 230, 215, 200 and 190 nm (the last extremum cannot be observed in 2,2,2-trifluoroethanol). In the less polar acetonitrile and in ethanol, the three Cotton effects at longest wavelengths coalesce into a broad band with a maximum at 230–235 nm and a shoulder in the region of longer wavelengths. The reversed absolute configurations of substances *VII* and *XV* manifest themselves with a great fidelity in enantiomeric shapes of the appropriate CD spectra recorded under comparable conditions. Compound *XV* exhibits a relatively higher intensity of the Cotton effect at 200 nm; in compound *VII*, the amplitudes of CD bands are more sensitive to the change of solvents, particularly in the region of longer wavelengths. The temperature-dependence of the two CD curves was also measured (Fig. 5). At a low temperature, the enantiomeric shape of the two curves

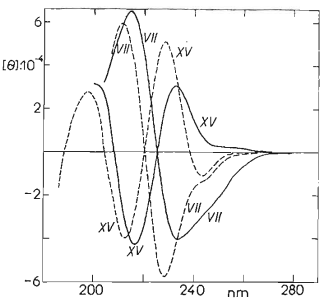


FIG. 5

Comparison of CD Spectra of Compounds *VII* and *XV* at +40°C (———) and at −160°C (– – –) in an 1 : 1 Methanol–Ethanol Mixture

is violated: the CD spectral course in the 240–250 nm region is almost identical with the two compounds of a reversed absolute configuration.

The first interpretation of the chiroptical properties of the proline cyclotripeptide *VII* was attempted by Deber and coworkers[8]. The CD spectrum of compound *VII* was examined in methanol and the region of three transitions at longest wavelengths coalescing into a single broad band in this solvent was ascribed[8] to a "strong $n - \pi^*$ transition" with a maximum at 233 nm. A comparison was also attempted with the CD spectra of cyclo(L-Pro)$_2$ and cyclo(Gly-L-Pro-Gly)$_2$ which, however, are of a quite different character. Additional experimental CD data presented in this paper and the reported[10] findings on the X-ray structure make now possible a more detailed interpretation.

The occurrence of six dichroic bands in the present CD spectra could suggest the presence of several conformers in solution or a different conformational state of the particular amide groups. However, as shown by measurement of IR spectra, the presence of several various conformers is improbable. Furthermore, measurement of CD spectra in a wide range of temperatures (from +40°C to −160°C) does not indicate changes of the conformational character at least in the overall arrangement of the molecule. The unusually high intensity of dichroic bands in the 210–240 nm region does not support the idea that the ordinary $n - \pi^*$ bands of the particular amide groups are involved in the present case. The presence of six intensive Cotton effects in CD spectra and of shoulder in the long wavelength region of UV spectra could be due to fixation of amide groups in such a mutual orientation which would favour their electronic interaction (*cf.* p. 2716). The planar amide group has the C_s symmetry and exhibits two singlet-singlet transitions in the accessible part of the electronic spectrum: the $n - \pi^*$ transition characterised by a small electric transition moment perpendicular to the plane of symmetry and a great magnetic transition moment lying in this plane; on the other hand, the $\pi - \pi^*$ transition is electrically strongly polarised in the plane of the amide group and its small magnetic moment is perpendicular to this plane. The formation of very strong Cotton effects could be *inter alia* due to a interaction between the electric transition moment of the $\pi - \pi^*$ transition of one amide group and the magnetic moment of the $n - \pi^*$ transition of another group. As indicated by symmetrical properties of both transitions, such an interaction cannot occur if the amide groups are coplanar (the participating monoexcited configurations have a different symmetry and cannot interact with each other) or perpendicular (the monoexcited configurations are in a strong interaction because of the equal symmetries but do not contribute to the optical rotatory strength since the magnetic moment of the $n - \pi^*$ transition and the electric moment of the $\pi - \pi^*$ transition are perpendicular to each other. Extreme values of the optical rotatory strength can be thus expected when the angle between normals of the amide group planes is approximately 45°. It may be inferred from the analysis of molecular models and from the X-ray analysis[10] of compound *VII* that the angles between the amide

groups (their planes) differ from 0°C and 90°C, the distances between these groups being small. Compounds *VII* and *XV* are thus capable of realising condition for the formation of a significant dichroism and band splitting in a wide coalescing range of $\pi - \pi^*$ and $n - \pi^*$ transitions.

The question whether the amide groups in the examined substances exhibit in solutions deviations from the planar arrangement, cannot be reliably answered under the present state of knowledge. The empirical calculations of Venkatachalam[1] give for *VII* the torsion $\Delta\omega = 25°$ as a condition for the closure of the ring system without bringing the H^{α} atoms into inacceptable close contact distances (1·4 Å). The results of the ^{1}H-NMR analysis are at variance[15] with considerations of Venkatachalam but the accuracy of the determination of dihedral angles from coupling constants can be insufficient for conclusions in this field. The nonplanarity of the amide groups of compound *VII* in the crystalline state was unambiguously determined.[22] The particular amide groups considerably differ with respect to the both parameters of nonplanarity ($\Delta\omega$, Θ_N; *cf.*[22]) but none of them is quite planar. It may be inferred from our calculations of electronic spectra of simple amides that the change from the planar to the nonplanar amide group is accompanied by a bathochromic shift of the $n - \pi^*$ transition. A distinct dichroic absorption in the region of relatively long wavelengths in spectra of cyclotripeptides *VII* and *XV* indicates that nonplanar amide groups assert themselves even in this case. Dichroic properties of the long-wavelength spectral region could depend on the "averaged" nonplanar amide bond in the molecule with a virtual C_3 symmetry as discussed in connection with IR spectra. It should be also emphasised that the longest-wavelength dichroic bands of compounds *VII* and *XV* measured at a low temperature, exhibit an enantiomeric character when referred to the same absolute configuration. This effect could suggest a reversed nonplanarity (*i.e.*, a reversed inherent chirality) of amide groups in compounds *VII* and *XV* analogously to the cyclodipeptide pair cyclo(L-Pro-L-Pro) and cyclo(L-Aze-L-Aze), *cf.*[23].

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