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SYNTHESIS AND X-RAY CRYSTAL STRUCTURE OF A TRIPEPTIDIC CYCLOL

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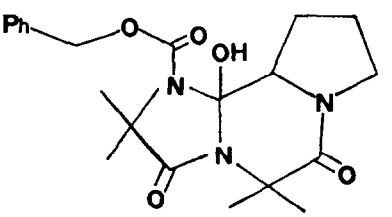
Properties, spectral data and X-ray crystallographic analysis of a tetracyclic tripeptidic aza-cyclol, obtained by cyclization of CF_3COOH·Pro-Phe-Pro-CNp in aqueous medium, are reported.

The importance of tetrahedral intermediates in a wide variety of enzymatic and non enzymatic reactions is well documented and is acquiring increasing attention.[1] We reported previously[2] the isolation of the first N-protected peptidic tetrahedral intermediates of type 1 (aza-cyclols) by cyclization of the corresponding linear tripeptides. Investigations of the factors determining the stability and tautomerism of peptidic aza- and oxa-cyclols showed that replacement of the proline residue by azetidine-2-carboxylic acid[2b] gives more stable systems, whereas replacement by pipecolic acid[3] or by sarcosine[2b,3] gives less stable compounds. These data suggest that increasing conformational rigidity imposed on the system by the annelation with 5- or 4-membered cyclic α-iminoacids, favourably affects cyclols stability. In accordance with this observation and in order to synthesize peptidic aza-cyclols free from protecting groups, we started to investigate cyclization of activated linear tripeptides containing two proline residues.



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A very recent report by M. Rothe and M. Fähnle[4], describing the first example of isolation of a tripeptidic cyclol, prompts us to disclose our results in this field.

As suitable models for initial experiments, Phe-Pro-Pro-CNp 2 and Pro-Phe-Pro-CNp 3 were selected; the great majority of stable peptidic cyclols contains in fact the sequence Phe-Pro. Cyclization of both tripeptides can give a cyclol through an intermediate 9-membered cyclo-tripeptide; in the case of Pro-Phe-Pro-CNp on the other hand, an intermediate N(α-aminoacyl)-diketopi-

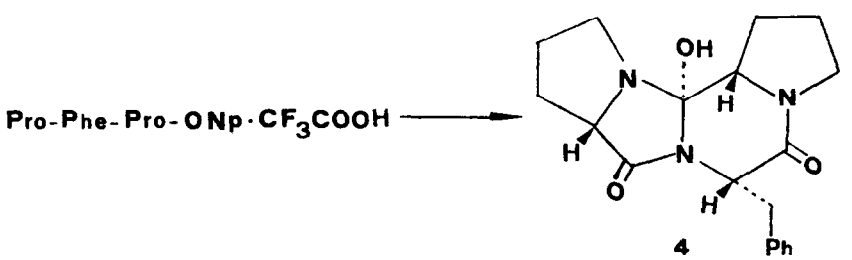
perazine, namely prolyl-cyclo(-Phe-Pro-), can also be involved.

Cyclization was performed by treating trifluoroacetates of active esters 2 and 3[5] for three hours at room temperature with aqueous alkaline buffer (1.0 mmol in 100 ml of dioxane, 50 ml 0.1 M $NaHCO_3$ and 50 ml 0.1 M Na_2CO_3). The solvents were removed under vacuum and the residue taken up with water. The mixture was extracted with ethyl acetate and washed with 0.1 M Na_2CO_3, 2 M $KHSO_4$ and saturated NaCl solution. Drying and evaporation of ethyl acetate afforded a residue which was used to isolate cyclization products. An analogous procedure was previously employed for the cyclization of Z-Ala-Phe-Pro-ONp to the corresponding cyclol 1[2a].

Accurate column chromatography of the residue obtained from active ester 2 (5-8% of the starting material) did not reveal the presence of cyclol 4 or of diketopiperazines. In the adopted conditions the prevailing reaction is the hydrolysis of the active ester.

In the case of active ester 3, tetracyclic azacyclol 4 was the main component of the residue (250 mg from 2.30 g of starting material). Chromatography through silica gel (25 g, eluant ethyl acetate - methanol 8-2) gave 180 mg of a foam which was crystallised from ethyl acetate; 120 mg of 4, $C_{19}H_{23}N_3O_3 \cdot H_2O$ (8.5% yield) were obtained[6], mp 95-7°C, $[\alpha]_D^{20}$ -58° (c 0.7 MeOH). 4 is a fairly stable monohydrate crystalline compound which slowly decomposes on standing in solution at room temperature. Spectral properties follow: IR($CHCl_3$) 3515 (sharp), 3400(broad), 1700, 1640, 1450, 1400 cm^{-1}; mass spectrum (70 eV), m/e (relative intensity, %) 341(2.6,M^+), 323(43), 250(1.4), 243(0.7), 204 (100, M^+-18-91-28), 153(5.5), 135(8.6), 91(12), 70(32); ^{13}C-NMR($CDCl_3$, 25°C, δppm from TMS, Bruker WH90, 22.63 MHz) 22.19(t)C9, 26.21^x(t)C10, 26.40^x(t)C14, 29.38^x(t)C15, 34.49(t)C16, 46.66^{xx}(t)C13, 46.99^{xx}(t)C8, 56.11(d)C5, 65.56$^+$(d) C11, 66.79$^+$(d)C2, 96.37(s)C12, 127.44(d)C20, 128.67(d)C19,C21, 130.42(d)C18, C22, 138.06(s)C17, 164.53^x(s)C6, 173.01^x(s)C3.[7]

The presence in the ^{13}C-NMR spectrum of only two carbonyl signals and of a singlet at 96.37 ppm indicate a cyclolic structure[8]. It is interesting to



note that aza-cyclol 4, unlike N-protected analogous, does not show tautomerism with the aminoacyl-diketopiperazine form. Accordingly treatment with methanolic hydrazine [9,10] does not yield cyclo(-Phe-Pro-) and in the mass spectrum peaks at 243-245 and 125 m/e are very weak. Failure of 2 to give the cyclol, confirms on the other hand the intermediacy of an α-aminoacyl-diketopiperazine in the formation of 4.

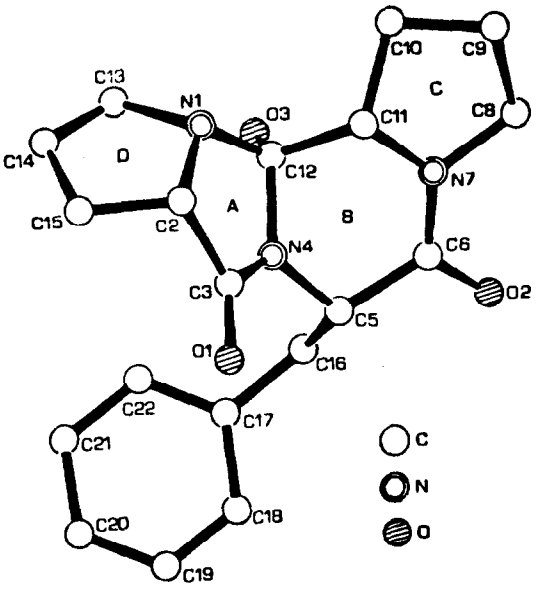


Fig. 1

In order to confirm structure and stereochemistry of 4 and to correlate its conformation to that of related cyclols, an X-ray structure determination was attempted.

4 crystallises from ethyl acetate solution in colourless prisms; $C_{19}H_{23}N_3O_3 \cdot H_2O$, F.W.=359.43, orthorombic, space group $P2_12_12_1$, with a = 9.529(4), b = 10.198(2) and c = 18.732(5) Å, Z = 4, d_c = 1.31 g cm^{-3}; Mo - Kα radiation. The structure was solved by direct method using the MULTAN programme. At the present stage of the refinement (by 9 x 9 block-diagonal least-squares calculations) the R and Rw values are 8.1% and 11.0% for 2271 observed reflections and for the 26 non-hydrogen atoms[11].

Conformational and stereochemical features of 4 (Fig. 1) are very close to that observed for type 1 aza-cyclols[12] in the solid state: closure of ring A occurs stereospecifically leading to 11-12 anti-arrangement; ring B has an approximate mirror plane passing through C6 and C12, with cis-arrangement of H5 and H11; the benzylic side chain assumes an extended conformation towards the nitrogen.

Rings A and C assume an envelope (C_s-C12) and half chair (C_2-C9-C10) conformation respectively. The pyrrolidine ring D has a rather low degree of puckering, with C_s-C13 symmetry; the nitrogen atom N1 has a pyramidal character, being 0.47 Å away from the plane of the substituents.

The oxygen O(W) of the water molecule interacts with three oxygen atoms of three different molecules at distances O(W)-O1 = 2.93 Å, O(W)-O2 = 2.75 Å, O(W)-O3 = 2.76 Å.

References and notes

1. See M.K. Kaloustian, M.I. Aguilar-Laurents de Gutierrez, R.B. Nader, J.Org.Chem., 44, 666 (1979) and references cited therein.
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3. See J. Rutschmann and P.A. Stadler in "Ergot Alkaloids and Related Compounds", B. Berde and H.O. Schild Editors, Springer Verlag, Berlin, 1978, p 29.
4. M. Rothe and M. Fähnle, Seventh American Peptide Symposium, University of Wisconsin-Madison, June 14-19, 1981.
5. Prepared by the corresponding 3,5-dimethoxy(α,α-dimethyl)benzyloxycarbonyl (Ddz) acids, by activation with DCCI/pNC_2phenol and deprotection with TFA/CH_2Cl_2.
6. Starting Ddz-tripeptides and aza-cyclol 4, gave correct elemental analyses.
7. Resonances were assigned by examining ^{1}H-coupled spectra and by comparison with spectra of related compounds. Starred values may be reversed.
8. F. Conti, G. Lucente, A. Romeo and G. Zanotti, Int.J.Peptide Prot.Res., 5, 353 (1973).
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11. The atomic co-ordinates are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EW.
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