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TEN-MEMBERED CYCLOTRIPEPTIDES: INFLUENCE OF THE RING-FLEXIBILITY ON INTRAMOLECULAR REACTIONS

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Summary: Cyclization of different models of linear precursors of 10-membered cyclotripeptides is described. Macrocyclic compounds, aza-cyclols, N-acyl-diketopiperazines and acylamidines were isolated and their formation related to the flexibility of the ring.

Several investigations have been devoted to 9-membered cyclotripeptides and to the transannular reaction between amide groups which, in these systems, leads to tetrahedral intermediates (aza-cyclols) stable enough to be isolated.[1] 10-Membered rings containing three amide bonds, have received comparatively little attention. They were obtained by incorporating a ß-aminoacid residue into a diketopiperazine, with the intermediate formation of unstable aza-cyclols containing two fused 6-membered rings.[2] Relative to 9-membered cyclotripeptides, 10-membered analogs allow less strained conformations with reduced tendency toward transannular interactions between amide groups. Accordingly, aza-cyclols tautomeric with 10-membered cyclotripeptides have not been isolated up to now.

As a prosecution of our studies in this field,[3] we report here on the cyclization in mild alkaline aqueous medium of p-nitrophenyl esters of linear peptides of the type ßX-Phe-Pro and on the successful isolation of a related aza-cyclol. ß-Alanine and anthranilic acid (Ant) derivatives were used as N-terminal residue, since they represent two extreme cases of high and low conformational flexibility; the sequence X-Phe-Pro, on the other hand, has been found in a great number of stable peptidic aza-cyclols.[4]

Cyclization was performed by treating the active esters at room temperature with aqueous alkaline buffer (1.0 mmol in 30 ml of dioxane, 15 ml of 0.1 M Na_2CO_3 and 15 ml of 0.1 M $NaHCO_3$). The solvent was removed under vacuum and the residue partitioned between water and chloroform. The organic layer was washed with saturated aqueous Na_2CO_3 and water. Drying and evaporation of the solvent followed by column chromatography on silica gel of the residue, gave the cycli-

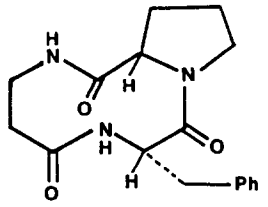
zation products.

ßAla-Phe-Pro-ONp (1)[5] was prepared starting from the corresponding Boc-tripeptide acid by activation with DCCI/pNO_2phenol and deprotection with TFA/CH_2Cl_2.

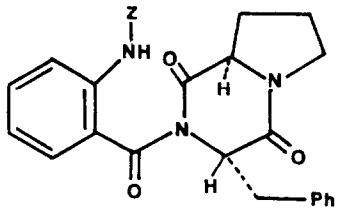
Treatment of isatoic anhydride or N-methyl-isatoic anhydride with Phe-Pro-OMe in the presence of 4-dimethylamino pyridine[6] gave anthranoyl and N-methyl-anthranoyl dipeptide methyl esters in good yields; these compounds were converted into the active esters (3) and (5) by aqueous alkaline hydrolysis followed by activation with DCCI/pNO_2phenol.

Z-Ant-Phe-Pro-ONp (4) was obtained by acylation of Ant-Phe-Pro with benzyl chloroformate and activation with DCCI/pNO_2phenol.

Cyclization of linear precursors (1) and (3), containing a primary ß-amino group, gave (2) and (6) respectively. Cyclotripeptide (2) was obtained in 30% yield; mp 210-11°C (AcOEt); $[\alpha]_D^{20}$-35° (c 1.0 $CHCl_3$). Chemical and spectroscopic properties are in accordance with the assigned structure: IR(KBr) spectrum shows bands at 3290, 3205, 1670, 1655, 1640 and 1530 cm^{-1}, and in ^{1}H-NMR ($CDCl_3$)



(2)



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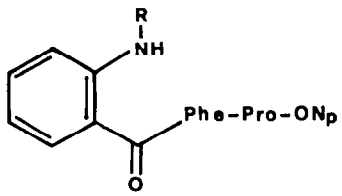
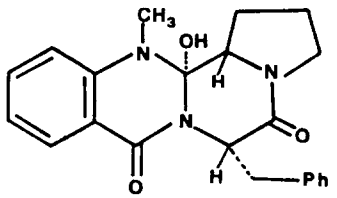


(3) R = H
(4) R = $PhCH_2OCO$ = Z
(5) R = CH_3



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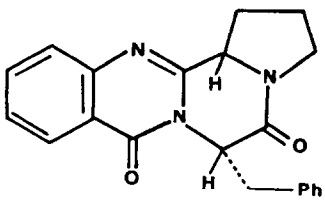
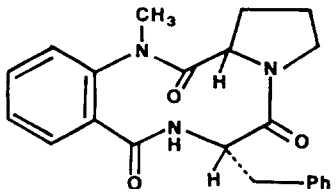


(6)



(9)

spectrum one of the two exchangeable protons appears as doublet coupled to Phe-αCH; as expected (2) is unaffected by treatment with methanolic $NH_2NH_2.H_2O$. Acylamidine (6) was isolated as an oil in 33% yield; $[\alpha]_D^{20}$ +430° (c 0.5 $CHCl_3$). In the ^{1}H-NMR ($CDCl_3$) spectrum no exchangeable protons are present and the Pro-αCH appears at 4.3 δ in accordance with a trans-arrangement of this hydrogen with the benzylic side chain. ^{13}C-NMR ($CDCl_3$) reveals two carbonyl singlets at 162.8 and 160.4 δ and in the mass spectrum peaks at 345 (M^+,41%) and 254 (M^+-91, 100%) m/z are present.

Thus, with the sequence ßAla-Phe-Pro, the macrocyclic tautomer (2) is the only isolable form. This result is in accordance with the data already known concerning 10-membered systems obtained by ß-aminoacyl insertion.[2] The formation of acylamidine (6), on the other hand,is analogous to that found by Liberek and Zarebski[7] during cyclization of peptides containing anthranilic acid as C-terminal residue and confirms i) the tendency of the rigid residue of the anthranilic acid to favour transannular interactions and ii) the instability of aza-cyclols containing two fused 6-membered rings, despite the mild reaction conditions and the presence of the Phe-Pro sequence.

Cyclization of the linear precursors (4) and (5) was then examined; in these systems the conversion of intermediate aza-cyclols into acylamidines is prevented by the presence of the substituent R on the nitrogen. Cyclization of (4)gave the N-acyl trans-diketopiperazine (7) as an oil in 44% yield; $[\alpha]_D^{20}$ +40° (c 0.5 $CHCl_3$); in the ^{1}H-NMR ($CDCl_3$) spectrum the NH signal appears as a singlet (8.7δ) and in the ^{13}C-NMR ($CDCl_3$) four amide carbonyl singlets are present (153.4, 164.6, 169.3 and 172.3δ). Treatment of (7) with methanolic $NH_2NH_2\cdot H_2O$ gave cyclo(-Phe-D-Pro-), 3,4-dihydro-1H-1,3,4-benzotriazepine-2,5-dione and benzyl alcohol.

Cyclization of (5) gave a mixture of the two isomeric compounds (8) and (9). Cyclol (8) could be isolated in 33% yield; mp 140-42°C (AcOEt-hexane), $[\alpha]_D^{20}$ +322° (c 0.5 $CHCl_3$); IR($CHCl_3$) 3480, 3250, 1650, 1610 and 1490 cm^{-1}; in the ^{1}H-NMR (DMSO) the OH signal appears at 8.0 δ as a sharp doublet (J 1.5 Hz) long range coupled to Pro-αCH (3.9δ); in ^{13}C-NMR($CDCl_3$) spectrum the singlets of cyclolic carbon and of the two carbonyl carbons are found at 94.1, 161.9 and 167.3 δ respectively. 10-Membered cyclopeptide (9) was isolated in 17% yield; mp 185-87°C (AcOEt); $[\alpha]_D^{20}$ +96° (c 0.5 $CHCl_3$); IR ($CHCl_3$) 3350, 1660 br., 1595 and 1450 cm^{-1}. ^{1}H-NMR (DMSO) spectrum shows the NH signal as a large doublet (J 12 Hz) at 8.65 δ coupled to Phe-αCH; the aromatic H-atom ortho to the anthranilic CO is found at high field (5.2 δ) as a consequence of the anisotropic shielding effect of the phenylalanine aromatic ring.[8] In the ^{13}C-NMR ($CDCl_3$) spectrum three

carbonyl signals are present at 171.7, 163.9 and 167.2δ . Compounds (8) and (9) are stable in the solid state and in chloroform solution; slow tautomerization into cyclol (8) is observed when cyclopeptide (9) is dissolved in hydroxylated solvents.

Thus, the presence of the rigid skeleton of the aromatic ring makes 10-membered tripeptidic aza-cyclols such as (8) stable enough to be isolated. However, in contrast to the findings concerning 9-membered cyclotripeptides,[9] no aza-cyclols involving the urethane NH group could be isolated; the N-acyl diketopiperazine (7) was in fact the only isolable form. This result, together with the easy dehydration leading to (6), reflects the influence of electronic and steric factors on the stability on tetrahedral intermediates.

References and Notes

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8. Details on the conformation in solution and the solid state of (9) will be reported elsewhere.
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