Tetrahedron Letters No. 11, pp 1009 - 1012, 1978. Pergamon Press. Printed in Great Britain.

CYCLIZATION OF ACTIVATED N-BENZYLOXYCARBONYL-TRIPEPTIDES

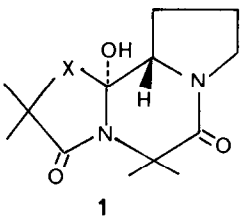
Gino Lucente[1a*], Francesco Pinnen[1b], and Giancarlo Zanotti[1c]

Istituto di Chimica Farmaceutica dell'Università di Catania
95125 - Catania (Italy)

Istituto di Chimica Farmaceutica dell'Università and Centro di Studio
per la Chimica del Farmaco del CNR - 00100 - Roma (Italy)

(Received in UK 5 January 1978; accepted for publication 23 January 1978)

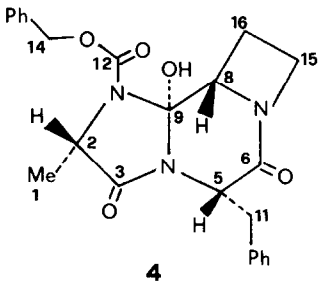
The theoretical interest of cyclols, particularly in the fields of peptides and cyclo-peptides, is well known. Though several cyclols stable enough to be isolated have been described to date, the interesting class of cyclolic peptides is at present limited to compounds possessing the same tricyclic framework 1 found in the peptidic moiety of the ergot alkaloids.[2] Attempted cyclization of linear peptides not containing proline has been reported not to give aza-[3] or oxa-cyclols.[4]



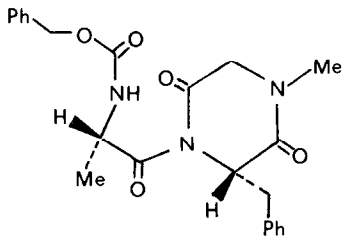
1

Oxa-cyclols X = O
Aza-cyclols X = NCOOR

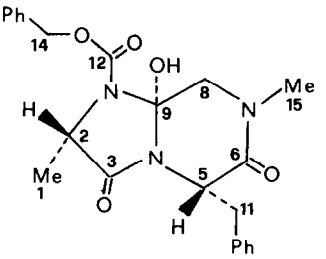
Since different factors attributable to the presence of the proline residue may play a role in the formation and stabilization of the ergot-like cyclolic peptides { i.e. N-C_α rigid bond, cis-allowed configuration of the amide group, and conformation of the intermediate N-acyl-cyclo(-X-Pro-) } it seemed interesting to examine the cyclization of linear tripeptides containing α-imino-acids different from proline. We report here the first results concerning L-azetidine-2-carboxylic acid and sarcosine. The following tripeptides have been selected as suitable models: Z-Ala-Phe-Aze-ONp 2 and Z-Ala-Phe-Sar-ONp 3. Compounds 2 and 3 were prepared starting from Z-Ala-Phe-N_3 and from the corresponding imino-acid in alkaline aqueous tetrahydrofuran; treatment of the resulting acid with di-p-nitrophenyl sulfite in pyridine and with p-nitro-phenol/dicyclohexylcarbodiimide gave 2 and 3, respectively. Cyclization was performed by treating the active esters for two hours at room temperature with alkaline buffer (10.0 mmol of ester in: 300 ml of dioxan, 150 ml 0.1 M $NaHCO_3$ and 150 ml 0.1 M Na_2CO_3).[5] The solvents were removed and the residue taken up with water. The mixture was extracted with $CHCl_3$ and washed with 0.1 M Na_2CO_3 and water. Drying and evaporation of the $CHCl_3$ afforded the residue (A) which was used to isolate the cyclization products.



4



6



7

In the case of active ester 2, compound 4 was the main component of residue A (45-35% of the starting material). One crystallization from AcOEt removed the impurity represented by 5-10% of the Z-tripeptide p-nitrophenyl ester. 4 {mp 185--6°; $[\alpha]D^{20}$+82° (c 2.5 $CHCl_3$)} was found to react with CH_2N_2 in EtOH {the methyl derivative melts at 138-9°; $[\alpha]D^{20}$+53° (c 1.5 $CHCl_3$); M^+ and M^+-CH_3OH at 449 and 417 m/e resp.)}. It is soluble in 1N NaOH (from which it can be recovered unchanged upon acidification) and was found to be exceptionally stable when treated (1 mmol) at room temperature (12 h) with $NH_2NH_2{\cdot}H_2O$ (2 mmol) in MeOH solution (1%). IR($CHCl_3$) 3500-3300, 1715, 1670, 1445 cm^{-1}; no peaks in the amide II band region. The mass spectrum shows M^+ at 435 m/e and base peak at 56 m/e ($\overline{CH_2CH_2{=}NH^+}$); other significant peaks at m/e(%) 417(5), 391(3), 344(5), 300(8), 289(5), 245(15), 217(16), 189(8), 131(10), 91(90). The ^{1}H-NMR spectrum (DMSO-d_6) shows a sharp doublet at 7.88δ[6] (J 1.5Hz) coupled to AzeC_αH;[7] Ala and Phe C_αH appear as sharp signals. The ^{13}C-NMR shows three different C=O signals and a singlet at 91.0 ppm from TMS, consistent with a cyclolic carbon atom.[3]

Residue A from 3 was 30% of the starting material. By PLC fractionation the three components 5, 6, and 7 were isolated, together with traces of starting active ester. 5 {15% of A; mp 184-5°; $[\alpha]D^{20}$+39° (c 2.3 MeOH)} was identified as cyclo(-Phe-Sar-). An authentic specimen prepared by hydrogenolysis of Z-Phe-Sar-OMe {$[\alpha]D^{20}$-13° (c 2.0 MeOH)} showed $[\alpha]D^{20}$+47.5° (c 2.3 MeOH). Chemical and spectroscopic properties of 6 {30% of A; mp 147-49°; $[\alpha]D^{20}$+78° (c 1.0, $CHCl_3$)} are consistent with the structure of N-acyl-diketopiperazine. This is rapidly hydrolyzed by aqueous NaOH. Treatment with methanolic $NH_2NH_2{\cdot}H_2O$ gives Z-Ala-NH-NH_2 and cyclo(-Phe-Sar-). IR($CHCl_3$) 3430, 1715, 1670, 1515 cm^{-1}. In the ^{1}H-NMR spectrum, α-protons of Ala and Phe are found shifted downfield, as expected for an acyl-diketopiperazine;[8] slow exchange with D_2O is observed for the NH signal, which appears as a doublet coupled to AlaC_αH. In the mass spectrum peaks corresponding to M, M-18, and M-91 are present; below 218m/e, the fragmentation follows the pattern observed for diketopiperazine 5. To the third component of the mixture {45% of residue A; mp 145-7°; $[\alpha]D^{20}$+16.5° (c 0.3, $CHCl_3$)} cyclol

structure 7 was assigned. This component was found to be soluble in 1N NaOH; solution was - however - accompanied by tautomerization to acyl-diketopiperazine and hydrolysis. Methanolic hydrazine gave quantitatively Z-Ala-NH-NH_2 and cyclo(-Phe-Sar-); after PLC separation the two compounds were found to have high optical purity.[9] IR($CHCl_3$) showed no amide II band (absorptions at 3550-3200, 1715, 1650 cm^{-1}). The mass fragmentation pattern was practically identical to

Table 1. ^{1}H-NMR Chemical shifts δ ppm (J,Hz). Varian EM-390, 90 MHz (DMSO-d_6- TMS)

	4	7	6	5
Ala $C_\alpha H$	4.10 q (6.5)	3.97 q (6.5)	5.33 m[a)]	
Ala $C_\beta H_3$	1.36 d (6.5)	1.26 d (6.5)	1.22 d (7.2)	
Phe $C_\alpha H$	4.48 ABX	4.49 ABX	5.05 m	4.20 m[b)]
Phe $C_\beta H_2$	2.86, 3.30 ABX (Jvic 7.0, 6.0; Jgem13.5)	3.02, 3.28 ABX (Jvic 9.5, 5.0; Jgem 13.0)	3.10 m	2.89, 3.16 ABX (Jvic 4.5, 4.5: Jgem 13.5)
Aze or Sar	$C_\alpha H$ 4.72 t $C_\beta H_2$ 2.1-2.8 m $C_\gamma H_2$ 3.8 m	CH_2 3.80, 4.13 AB q (12) NCH_3 2.97 s	CH_2 2.40, 3.70 AB q(18.5) NCH_3 2.68 s	CH_2 2.68, 3.48 AB q(17.5) NCH_3 2.65 s
OH or NH	7.88 d (1.5)[c)]	7.88 s[d)]	7.82 d (7.5)	8.28[e)]
$PhCH_2O$	5.12 s	5.18 AB q	5.10 s	
Ph	7.5 - 7.1 m	7.6 - 7.1 m	7.5 - 7.0 m	7.5 - 7.0

[a] Quartet after D_2O exchange. [b] Three lines after D_2O exchange. [c] 3.2 broad singlet in $CDCl_3$. [d] 4.1 broad signal in $CDCl_3$. [e] Unresolved doublet.

Table 2. ^{13}C-NMR Chemical shifts (δppm from TMS) Bruker WH90, 22.63MHz ($CDCl_3$)[a)]

Cpd	Number of carbon (off resonance)											
	1(q)	2(d)	3(s)	11(t)	5(d)	6(s)	15	16(t)	8	9(s)	12(s)	14(t)
4	18.9	55.3	166.4^x	33.9	55.3	169.3^x	49.1(t)	23.4	70.7(d)	91.0	152.7	67.7
7	18.7	54.5^x	165.8^{xx}	38.0	54.9^x	168.2^{xx}	35.5(q)		58.7(t)	94.6	153.1	67.7

[a] Starred values may be reversed.

that of 6 (M^+, 423m/e). ^{13}C-NMR revealed three carbonyls and the singlet at 94.6 ppm. In the ^{1}H-NMR the exchangeable proton appears as a sharp singlet at 7.88δ. The downfield shift observed for Sar CH_2 and Me protons[10] as compared to the corresponding signals shown by 5 and 6, as well as the values of the vicinal H_α-H_β coupling constants relative to Phe residues[11] in 7 and 5, clearly show

that the folded conformation of the benzylic side chain, adopted by the acyl-diketopiperazine 6 and by the diketopiperazine 5,[10] is not retained in cyclol 7, for which the conformation extended toward the nitrogen,[11,12] as found in the proline-containing aza-cyclol,[13] seems to be preferred.

The reasons why aza- and oxa-cyclols are preferred over the isomeric cyclo-peptides or acyl-diketopiperazine forms, are only just emerging in the literature and are related to the favoured conformations adopted by these systems.[14] The isolation of 7, the first example of a bicyclic peptidic cyclol, suggests that the rigid ring of a cyclic imino-acid is not essential for cyclol formation. On the other hand, cyclization of tripeptides containing Pro [15] or Aze as C-terminal residue, leads to tricyclic systems which show reduced propensity to equilibrate with the less stable acyl-diketopiperazine forms,[16] probably because of the increasing conformational rigidity.

References and notes

1. (a) Università di Catania, (b) Università di Roma; (c) Centro CNR, Roma.
2. See P.A. Stadler and P. Stutz in "The Alkaloids", R.H.F. Manske Editor, Academic Press, New York, 1975, p 1.
3. F.Conti, G.Lucente, A.Romeo and G.Zanotti, Int.J.Peptide Prot.Res., 5, 353 (1973).
4. P. Stutz and P.A. Stadler, Monatsh. Chem., 107 763 (1976).
5. Conditions adopted in the synthesis of proline-containing cyclol (See ref. 15); all new compounds gave correct elemental analyses.
6. In the ^{1}H-NMR spectra of thia-cyclols, the CH signal (DMSO-d_6) is found as strong singlet at 7.2δ : M. Rothe and R. Steinberger, Tetrahedron Lett., 649 (1970).
7. Analogous coupling between ProC_αH and CH is found in ^{1}H-NMR of oxa-cyclols, see: H. Hott, A.J. Frey and A. Hofmann, Tetrahedron, 19, 1675 (1963).
8. P. Stutz, R. Brunner and P.A. Stadler, Experientia, 936 (1973).
9. Only one of the two diastereomeric cyclols is isolated from the cyclization of 2 and 3. As in the case of proline-containing oxa- and aza-cyclols, the reaction then follows a stereoselective course. This finding, together with the similarity in the spectroscopic data with that for the latter, suggests that the arrangement between the CH and the Phe benzylic side chain is cis, as indicated in formulae 4 and 7.
10. G. Gawne, G.W. Kenner, N.H. Rogers, R.C. Sheppard and K. Titlestad in "Peptides 1968", Bricas Editor, North Holland Publishing Company, Amsterdam, 1968, p 28.
11. J. Vicar, J. Smolikova, and K. Blaha, Collect.Czech.Chem.Commun., 38, 1957 (1972).
12. P.E. Young, V. Madison, and E.R. Blout, J.Amer.Chem.Soc., 98, 5365 (1976).
13. S. Cerrini, W. Fedeli,and F. Mazza, Chem.Commun., 1607 (1971).
14. P.G. Sammes in "Fortschr.Chem.Organ.Naturstoffe", Springer Verlag, Vol. 32, Wien 1975, p 51-118.
15. G. Lucente and A. Romeo, Chem.Commun., 1605 (1971).
16. G. Lucente, A. Romeo, and G. Zanotti, Experientia, 31, 17 (1975).