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CYCLIZATION OF ACTIVATED TOSYL-PEPTIDES

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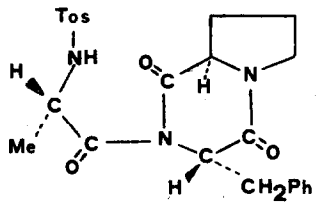
Side reactions occurring in the activation of carboxyl functions of peptides containing tosyl (Tos) as an N-blocking substituent have been described[1-2]. It was observed[2] that peptide bond and p-nitrophenyl ester formation fail in cases of compounds with the R-CH(NH-Tos)COOH structure, but are successful for compounds with the R-CH(NH-Tos)CONH-CH(R')COOH structure.

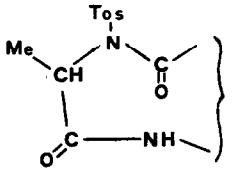
During the synthesis of Tos-L-Ala-L-Phe-$OC_6H_4NO_2$(p) from the corresponding acid I[3] using di-p-nitrophenyl sulphite in pyridine (room temperature, 2 hrs), we observed the formation of a by-product (5-10% yield) which was isolated and identified as 3(S)benzyl-6(S)methyl-1(N-tosyl)-piperazine-2,5-dione II, by the following spectral and chemical data: m/e(%) M^+372(2),308(25),253(5),217(14),198(5), 189(12),155(10),91(100); metastable peak for the transition $M^+ \rightarrow$ m/e 308; nmr (60 MHz, TMS, $CDCl_3$) δ ppm 1.11 (d, 3H, J=7.0Hz, $\underline{CH_3}$-CH-), 2.5 (s, 3H, $\underline{CH_3}$-C_6H_4-), 3.1 (d, 2H, J=5.5Hz, Ph-$\underline{CH_2}$-), 4.28 [multiplet (1H) which is simplified to a triplet (J=5.5Hz) by exchange with D_2O; -NH-$\underline{CH}$-CH_2], 4.82 (q, 1H, J=7.0Hz, CH_3-$\underline{CH}$-), 7.1 (1H, exchangeable with D_2O, superimposed on aromatic protons, -NH-), 7-7.5, 8 (m, 9H, aromatic protons); ir $\nu_{C=O}$(KBr) 1695, 1670 cm^{-1}. Hydrolysis of II (12 N HCl, 6 hrs reflux), gave L-phenylalanine and Tos-L-alanine(50% yield); tosyl-diketopiperazine II is then the cis-(L-L)isomer.

Investigation of the stability of Tos-L-Ala-L-Phe-$OC_6H_4NO_2$(p) showed that II is formed in good yield on allowing the active ester to stand in pyridine solution or by treating it for three hours at room temperature in an aqueous, mildly alkaline medium[4].

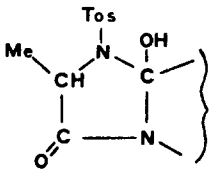
Cyclization of linear protected peptides under mild conditions is an unusual reaction in systems not containing proline residues. It seemed then interesting to examine the reactivity of the active esters of tosyl-tripeptides containing the sequence Ala-Phe-Pro. From these systems the formation of an acyl-diketopiperazine such as VII-A or of a cyclo-peptide VII-B was considered likely (diketopiperazine forming tendency of proline in the first case; tosyl-amino - p-nitrophenylester



VII-A



VII-B



VII-C

groups interaction in the second). Both VII-A and VII-B could lead to the cyclol form VII-C, particularly favoured in these systems[5].

Synthesis of Tos-L-Ala-L-Phe-L-Pro-$OC_6H_4NO_2$(p) was accomplished as follows: Tos-L-Ala-L-Phe-OCH_3 III was prepared with dicyclohexyl-carbodiimide from Tos-L--Ala and L-Phe-OCH_3. Tosyl dipeptide ester III, via the hydrazide IV and azide , gave Tos-L-Ala-L-Phe-L-Pro V; the tripeptide p-nitrophenyl ester was prepared as already reported for the dipeptide I. The tosyl-tripeptide p-nitrophenyl ester is more stable in dry pyridine as compared with the dipeptide active ester. In aqueous alkaline medium (0.1M $NaHCO_3$: 0.1M Na_2CO_3 : dioxane - 1:1:2) at room temperature for 1.5 hrs, the following products were isolated (yield): tripeptide acid V (25%); Tos-L-Ala (2-5%); starting p-nitrophenyl ester (10%); cyclo(L-phenylalanyl-D-prolyl)[6] (2-5%); a new compound $C_{24}H_{27}N_3O_5S$ VII (60%) and p-nitrophenol.

On the basis of chemical and spectroscopic properties, structure VII-A of acyldiketopiperazine was assigned to the new compound. VII is in fact rapidly hydrolyzed by 0.1N sodium hydroxide at room temperature (tripeptide acid V being the main product); allowed to stand at room temperature in methanol, it gives after 24 hrs 50% of Tos-L-Ala-OCH_3 and cyclo(L-phenylalanyl-D-prolyl); VII is stable when treated with AcOH-$HClO_4$ or conc.H_2SO_4 under conditions in which cyclol - compounds are dehydrated[7]. Mass spectrum showed no M^+-H_2O peak; characteristic peaks were: m/e(%) M^+ 469(18), 441(3), 314(65), 244(62), 198(100, $CH_3CH{=}NH^+$-Tos), 155(88);metastable peak for the transition $M^+ \rightarrow$ m/e441. In the nmr spectrum (100 MHz, TMS, $CDCl_3$) protons of the alanine residue showed signals at δ ppm 1.27(d, 3H, J=7.0Hz, $\underline{CH_3}$-CH-), 5.22 [multiplet (1H) of 8 lines, CH_3-$\underline{CH}$-NH-Tos], 5.75 (d,1H, exchangeable with D_2O, J=10Hz,-CH-$\underline{NH}$-Tos). The above assignement was possible by decoupling experiments as well as by exchange with D_2O. The phenylalanine residue showed two multiplets (3H) centered respectively at $\delta\sim3.0$ (8 lines) and at $\delta\sim5.1$ (4 lines) relative to the ABX system -CH-CH_2Ph, with a geminal coupling constant J_{AB}= 14Hz and $J_{AX}\cong$ 3.5Hz, $J_{BX}\cong$ 5.5Hz. The proline residue gave complex multiplets

centered at δ 1.8 (4H, $-CH_2-CH_2-$) and at δ 3.4 (3H, $-CH_2-\overset{|}{N}-$ and $-\overset{|}{N}-\underline{CH}-CH_2-$). Other signals δ 2.34 (s, 3H, $\underline{CH_3}-C_6H_4$) and δ 6.95, 7.25, 7.80(m, 9H, aromatic protons). Ir spectrum: $\nu_{C=O}$(KBr) 1725, 1695, 1660 cm^{-1}; no bands presents in the frequency range of the amide II band 1500-1590 cm^{-1}.

Isolation, by alcoholysis of VII-A, of cyclo(L-phenylalanyl-D-prolyl) shows that epimerization at the proline center takes place. By using for the cyclization the p-nitrophenyl ester of the tripeptide VIII[8] containing L-L-D sequence, we isolated in fact the same acyl-diketopiperazine VII-A as from the L-L-L isomer V. The stereochemical lability of the cis-form of cyclo(phenylalanyl-prolyl) is already known[6]; on the other hand no epimerization has been reported in cyclol systems of this type[5,6].

T A B L E

Compound		m.p.(°C)	$[\alpha]_D^{20}$	
I	Tos-L-Ala-L-Phe	198-200	+6°	(c,2 EtOH)
II	3(S)benzyl-6(S)methyl-1(N-tosyl)-piperazine-2,5-dione	159-161	-48°	(c,2 EtOH)
III	Tos-L-Ala-L-Phe-OCH_3	101-102	-15°	(c,1.5 EtOH)
IV	Tos-L-Ala-L-Phe-NH-NH_2	187-189	-48°	(c,1.5 EtOH)
V	Tos-L-Ala-L-Phe-L-Pro		-69°	(c,1 EtOH)
VI	Tos-L-Ala-L-Phe-L-Pro-OCH_3	184-185	-90°	(c,1 MeOH)
VII-A	N(Tos-L-alanyl)-L-phenylalanyl-D-proline anhydride	191-192	+85°	(c,0.6 AcOEt)
VIII	Tos-L-Ala-L-Phe-D-Pro		-9°	(c,1 EtOH)
IX	Tos-L-Ala-L-Phe-D-Pro-OCH_3	175-176	+15°	(c,1 AcOEt)
X	Tos-L-Ala-D-Phe-OCH_3	111-112	-36°	(c,1 EtOH)
XI	Tos-L-Ala-D-Phe-NH-NH_2	196-197	-37°	(c,1 EtOH)
XII	Tos-L-Ala-D-Phe-L-Pro		-30°	(c,1 MeOH)
XIII	Tos-L-Ala-D-Phe-L-Pro-OCH_3	126-127	-48°	(c,1 AcOEt)
XIV	N(Tos-L-alanyl)-D-phenylalanyl-L-proline anhydride	178-179	-164°	(c,1 AcOEt)

Acid tripeptides are glassy oils; they give crystalline derivatives upon treatment with diazomethane.

No attempts were made to crystallize p-nitrophenyl esters. They were used, chromatographycally pure, immediately after the preparation.

The observation that stable cyclols of VII-C type contain both alanine and proline in the same (L) configuration and that cyclolization is a stereospecific step[5,7] induced us to examine the reactivity of the active ester of the tosyl-tripeptide XII[8] with L-Ala-D-Phe-L-Pro sequence. In this case the preferred trans-form of the diketopiperazine residue leaves in fact proline in the L configuration.

Treatment of the p-nitrophenyl ester of XII under the same conditions used for the cyclization of the corresponding diastereomers, gave in high yield a $C_{24}H_{27}N_3O_5S$ compound, to which structure XIV was assigned. Chemical and spectral properties were in fact analogous to those found for VII-A. Cyclo(D-phenylalanyl-L-prolyl)[9] was isolated by alcoholysis. Mass spectrum m/e (%) M^+ 469(18), 441 (2), 314(77), 244(73), 198(91), 155(100, $CH_3C_6H_4SO_2^+$); no M^+-H_2O was present. The nmr spectrum showed a general similarity to that of VII-A. A difference is observed for the $-CH_2-Ph$ protons; they are magnetically non-equivalent in VII-A but equivalent in XIV, as evidenced by the doublet centered at δ ppm 3.08 (J=5.5 Hz) and by the triplet of $-\underline{CH}-CH_2-Ph$ centered at δ ppm 4.65 (J=5.5 Hz). Ir spectrum $\nu_{C=O}$(KBr) 1715, 1695, 1660 cm^{-1}.

When the tosyl-tripeptides V and XII were treated under the conditions of mixed anhydride synthesis with ethyl-chlorocarbonate, good yields of VII-A and XIV could be obtained.

In the tosyl-peptides so far examined, formation of acyl-diketopiperazines is then preferred; examination of tosyl-tripeptides not containing proline, is now in progress.

FOOTNOTES AND REFERENCES

1) M.Zaoral and J.Rudinger, Collect.Czech.Commun., 26, 2316 (1961).

2) C.Berse, T.Massiah and L.Piché, Can.J.Chem., 41, 2763 (1961).

3) All new compounds gave correct elemental analysis.

4) In this case small quantity of a trans isomer of II could be detected; isolated by tlc was characterized on the basis of elemental analysis and mass fragmentation pattern; m.p. 171-173°C.

5) G.Lucente and A.Romeo, Chem.Comm., 1605 (1971).

6) H.Ott, A.J.Frey and A.Hofmann, Tetrahedron, 19, 1675 (1963).

7) A.Hofmann, H.Ott, R.Griot, P.A.Stadler and A.J.Frey, Helv.Chim.Acta, 46, 2306 (1963).

8) Prepared following the procedure given for the L-L-L isomer.

9) m.p.147-149°C; $[\alpha]_D^{20}$ = -93° (c, 0.2 H_2O). Litt.[6] for the enantiomer: m.p.148 150°C; $[\alpha]_D^{20}$ = +92° (c, 0.2 H_2O).