## Synthesis of 2,3-Methano-glutamic and -pyroglutamic Acid

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2,3-Methano-analogues of glutamic and pyroglutamic acid have been synthesized and the  $\beta$ -naphthylamide of the latter is shown to be stable to pyroglutamic amino peptidase; the crystal structure of the methyl pyroglutamate analogue has been determined.

For some years, we have been interested in the synthesis of cyclopropane amino acids, their properties, and their effects on the conformation and bioactivity of various peptides. In particular, we have observed that the incorporation of a cyclopropane amino acid into a peptide chain endows it with an enhanced stability to enzymolysis,<sup>1</sup> in vitro, leading to the possibility of enhanced peptide biolifetimes in living systems. For this reason, we felt it of considerable interest to prepare the 2,3-methano-analogues (8) and (9) of glutamic and pyroglutamic acid in order to broaden these investigations.

During this research, the synthesis of a derivative analogous to (8) was reported<sup>2b</sup> as an intermediate in the synthesis of carnosadine, a natural cyclopropane amino acid. The first step in our synthesis (Scheme 1) of (8) and (9) required the preparation of a dehydroglutamic acid derivative which had been previously prepared by two different methods.<sup>2</sup> When the dimethyl ester (1a) was condensed with benzyl carbamate in refluxing benzene, a 47% yield of (2a) was obtained along with ca. 2% of the lactone (3a). An earlier approach<sup>3</sup> led to good yields of the lactone free acid (3b) only when the condensation was carried out with the acid (1b). The lactone could then be converted into (2a) by esterification, treatment with diazabicyclo[2.2.2]undecane (DBU), and then a second esterification step. Treatment of (2b) with diazomethane gave a very stable dihydropyrazole (4) (Scheme 2) which on photolysis gave compound (5) in excellent yield (98%). The dihydropyrazole (4) was stable to boiling benzene, but was converted into a 1:1 mixture of (5) and the crystalline  $\beta$ -methyl dehydroamino acid derivative (6) on photolysis.<sup>+</sup>





(3a,b)

$$a; R = Me$$
  
$$b; R = H$$

Scheme 1. Reagents: (a)  $ZNH_2$ ,  $C_6H_6$ ,  $POCI_3$ , heat (Z = benzyloxy-carbonyl).

 $\dagger$  The structure and configuration of (6) was proved by X-ray crystallography, details of which will be reported later.

The structure of (5), obtained as an oil, was confirmed<sup> $\ddagger$ </sup> by both <sup>1</sup>H and <sup>13</sup>C n.m.r. spectroscopy.

The configuration of (5) was proved by its cyclization (Scheme 3) to the pyroglutamic acid derivative (7) after hydrogenolysis of the benzyloxycarbonyl group and refluxing



Scheme 2. Reagents: (a)  $CH_2N_2$ ,  $Et_2O$ ; (b)  $h\nu$ , 400 W Hanovia lamp, 1 h.



Scheme 3. Reagents: (a) i,  $H_2$ , 5% Pd/C; ii, Bu<sup>i</sup>OH, heat, 16 h; (b) NaOH, MeOH; (c) 6 M HCl, heat.

 $<sup>\</sup>ddagger$  (5): <sup>1</sup>H n.m.r. (CDCl<sub>3</sub>)  $\delta$  7.31 (s, 5H, ArH), 5.58 (br. s, 1H, NH), 5.08 (s, 2H, ArCH<sub>2</sub>), 3.67 (s, 3H, OMe), 2.4 (m, 2H, CH<sub>2</sub>), 1.85 (m, 1H, CH), and 1.1 (m, 2H, CH<sub>2</sub>); <sup>13</sup>C n.m.r. (CDCl<sub>3</sub>)  $\delta$  172.6, 172.4, 156.9, 136.1, 128.2, 127.8, 66.7, 52.3, 51.7, 37.7, 23.8, and 22.6; <code>v<sub>max</sub>. (NaCl) 3320, 2930, 1730, and 1510 cm^{-1}.</code>

<sup>(7),</sup> m.p. 83—84 °C; <sup>1</sup>H n.m.r. (CDCl<sub>3</sub>)  $\delta$  7.32 (s, 5H, ArH), 4.75 (s, 3H, OMe), 3.80 (dd, 1H, CH), 3.45 (dd, 1H, CH), 2.14 (m, 1H, CH), 1.8 (dd, 1H, HCH), and 0.95 (dd, 1H, HCH); <sup>13</sup>C n.m.r. (CDCl<sub>3</sub>)  $\delta$  176.4, 170.9, 52.5, 40.5, 34.2, 25.8, and 20.8;  $\nu_{max}$  (KBr) 3410, 3215, 1730, and 1695 cm<sup>-1</sup>.



Figure 1. ORTEP plot of the ester (7).

of the resulting amino ester in isobutyl alcohol solution.§ Any possibility that diketopiperazine rather than lactam formation had occurred under these vigorous conditions was eliminated by an X-ray crystallographic study (Figure 1)¶ of the crystalline‡ methyl ester (7). Of interest in the crystal structure are the large  $\phi$  dihedral angle [C(6)–N(1)–C(2)– C(1), 139°] required by the ring system, the small  $\psi$  dihedral angle [N(1)–C(2)–C(1)–O(1), 6.64°] resulting from the ester carbonyl group being tucked back under the three-membered ring, as well as the somewhat expanded angle [N(1)–C(2)– C(1), 120°] at the  $\alpha$ -carbon atom. The very small ring dihedral

\$ Refluxing in solvents boiling below 100 °C gave only partial conversion into the lactam.

¶ Crystal data: C<sub>7</sub>H<sub>9</sub>NO<sub>3</sub>,  $M_r$  = 155.15, monoclinic, space group  $P2_1/c$ , a = 11.004(6), b = 10.394(6), c = 6.708(4) Å,  $\beta = 100.24(4)^\circ$ , U = 755.0(8) Å<sup>3</sup>, F(000) = 328,  $\lambda$  (Mo- $K_\alpha$ ) = 0.71069 Å,  $\mu$  (Mo- $K_\alpha$ ) = 1.007 cm<sup>-1</sup>; Z = 4, 723 observed reflections, R = 0.055,  $R_w = 0.076$ . Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

angle N(1)-C(2)-C(3)-C(5) (2.48°) and other small dihedral angles indicate that the five-membered ring is essentially flat.

Hydrolysis of the ester (7) occurred without complications and the crystalline acid was obtained in excellent yield. Strong acid hydrolysis of (5) gave the glutamic acid analogue (9) as its hydrochloride also in good yield.

To test the effect of the cyclopropane ring on the hydrolytic stability of an amide derivative of (8), the  $\beta$ -naphthylamide (10) was prepared by a mixed anhydride coupling with the amine. Using the  $\beta$ -naphthylamide of pyroglutamic acid as a control, (10) was treated with pyroglutamic acid aminopeptidase<sup>4</sup> at pH 6.7 and at an amide to enzyme ratio of 10:1 (µmol: enzyme units). The control was hydrolysed completely in four hours, whereas (10) showed no formation of free naphthylamine in 48 h.\*\* We plan to incorporate (8) into several peptides in the future to examine both the bioactivity and stability to enzymolysis of these novel compounds.

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\*\* The reaction was monitored by t.l.c., using chloroform/ethanol/ acetic acid (90:10:1) as solvent system and u.v. light, chlorine/iodide/ toluidine for visualization.

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