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THREO- AND ERYTHRO-β-HYDROXY-L-ASPARAGINES

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The recent findings of Campbell et al. (1) regarding the action of L-asparaginase on various asparagine-requiring tumours have prompted the syntheses and investigation of analogues of this amino acid (2).

We wish to report the preparation of the β-amides of threo- and erythro-β-hydroxy-L-aspartic acids by ammonolysis of β-benzyl threo-β-hydroxy-L-aspartate (3) and β-methyl erythro-β-hydroxy-L-aspartate respectively. The latter ester was prepared by direct esterification of erythro-β-hydroxy-L-aspartic acid obtained by resolution of its racemic N-benzyl derivative by means of L-histidine and subsequent hydrogenolysis (4).

The amides differ in their stability in acidic medium. The one belonging to the threo- series when left for several hours in N HCl is partly hydrolysed to threo-β-hydroxy-L-aspartic acid, detected on chromatograms, which caused a sharp drop in optical rotation. Erythro-β-hydroxy-L-asparagine, however, was stable under the same conditions.

The "β-hydroxy asparagine" isolated from normal human urine (5) could not be identified with either of the isomers synthesised by us, having different properties (R_f values and IR spectrum).

Threo-β-hydroxy-L-asparagine - β-Benzyl threo-β-hydroxy-L-aspartate (3) (1 g) was dissolved in 25% aqueous ammonia (10 ml). After 7 days at room temperature the solution was evaporated to dryness in vacuo. The solid residue was dissolved in hot water and the pH adjusted to 5 by addition of 6N HCl. The substance precipitated and was recrystallised from water (0.6 g, yield quantitative); m.p. 278° (decomp.); $[\alpha]_D^{22}$ -30.6° (c 2.45 in N HCl) (Found: C, 32.2; H, 5.9; N, 18.9; N (Van Slyke), 9.6. $C_4H_8N_2O_4$ requires: C, 32.4; H, 5.4; N, 18.9; N (Van Slyke), 9.4%).

β-Methyl erythro-β-hydroxy-L-aspartate - To a suspension of erythro-β-hydroxy-L-aspartic acid (4) (5 g) in anhydrous methanol (50 ml) was added concentrated HCl (6 ml). The mixture was heated under reflux for 3 hrs. The

solvents were removed in vacuo, the residue was dissolved in ethanol and the pH adjusted to 8 by addition of pyridine. The resulting precipitate was recrystallised from 50% aqueous ethanol. (3.5 g, 65%); m.p. 229° (decomp.); $[\alpha]_D^{22}$+65.5° (c, 1.8 in N HCl) (Found: C, 36.8; H, 5.8; N, 8.5; N (Van Slyke), 8.7. $C_5H_9NO_5$ requires: C, 36.8; H, 5.5; N, 8.6; N (Van Slyke), 8.6%).

Erythro-β-hydroxy-L-asparagine was prepared in an analogous manner to the threo isomer by ammonolysis of the above ester; m.p. 260° (decomp.); $[\alpha]_D^{22}$+63.4° (c 1.9 in N HCl) (Found: C, 32.4; H, 5.9; N, 18.9; N (Van Slyke), 9.2. $C_4H_8N_2O_4$ requires: C, 32.4; H, 5.4; N, 18.9; N (Van Slyke), 9.4%).

Paper chromatography in 80% phenol-water (light-brown colour with ninhydrin) gave R_f values 0.18 and 0.13 for threo- and erythro-β-hydroxy-L-asparagine respectively. On TLC (silica gel) in n-butanol-acetic acid-pyridine-water (2:1:1:4) they were 0.39 and 0.23 respectively.

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