Amino Acids. 1. α-Hydroxymethylphenylalanines†

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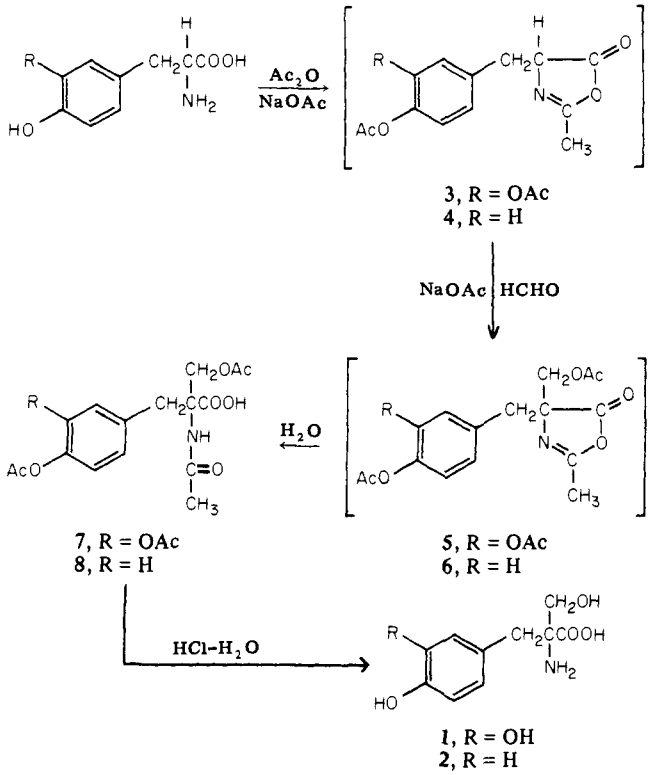
α-Hydroxymethyltyrosine and α-hydroxymethyl-Dopa were prepared from tyrosine and Dopa, respectively. Neither compound displayed a hypotensive effect with spontaneously hypertensive rats.

As part of a program to investigate the antihypertensive properties of various amino acid analogs our attention was directed to derivatives of 3,4-dihydroxyphenylalanines (Dopa) and, in particular, to the pharmacologically active α-methyl-3,4-dihydroxyphenylalanine.[1] In order to examine the effect of hydroxylating the α-methyl group, α-hydroxymethyl-Dopa (1) and α-hydroxymethyltyrosine (2) were prepared.

The base-catalyzed reaction of azlactones with acid anhydrides or acyl halides to give α-acylamino ketones[2] (Dakin-West reaction) has been well documented.[3,4] However, the reaction of azlactones with paraformaldehyde to give, after suitable hydrolysis, the α-hydroxymethyl-amino acid appears to be a novel preparative method.

Treatment of Dopa with acetic anhydride in the presence of sodium acetate afforded the crude azlactone[5] 3 which, on reaction with paraformaldehyde, gave the α-acetoxymethyl azlactone 5. Without further purification azlactone 5 was subjected to neutral hydrolysis to give *O,O,O,N*-tetraacetyl-2-hydroxymethyl-Dopa (7) as a crystalline solid. Acid-catalyzed hydrolysis provided α-hydroxymethyl-Dopa (1). (At the time this work was in progress a report appeared which describes an alternate method of synthesis.[6]) A similar series of reaction with tyrosine gave α-hydroxymethyltyrosine (2).



Pharmacology. Compounds 1 and 2 did not decrease the blood pressure of conscious rats with spontaneous hypertension in doses of 100 and 300 mg/kg by mouth. By comparison, *l*-α-methyl-Dopa produced average decreases in mean blood pressure of 44 (±10 SE, $n = 4$) and 85 mm (±24 mm SE, $n = 4$) in parallel experiments after single peroral doses of 50 and 100 mg/kg, respectively. Since the hypotensive activity of α-methyltyrosine has been previously reported,[7] these results suggest that hydroxylating the α-methyl group of either α-methyl-Dopa or α-methyltyrosine reduces or destroys the antihypertensive activity.

† This note is dedicated to Professor Edward E. Smissman in recognition of his many significant contributions to medicinal chemistry.

Experimental Section

Melting points were determined on a Thomas-Hoover oil bath apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR8 spectrophotometer. NMR spectra were determined with a Varian EM 360 spectrophotometer. Spectral data on all compounds were consistent with the proposed structures. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

***O,O,N*-Triacetyl-2-hydroxymethyltyrosine (8).** To 510 g (5 mol) of acetic anhydride were added 46 g (0.56 mol) of sodium acetate and 100 g (0.55 mol) of tyrosine. The mixture was stirred on the steam bath for 2 hr at which time 30 g (1.0 mol) of paraformaldehyde was added and the reaction mixture was stirred for an addition 6 hr on the steam bath, cooled, and diluted with 700 ml of water. The organic material was extracted into chloroform and washed with 2 l. of water, and without drying the chloroform was evaporated. The residue was dissolved in 200 ml of water and 200 ml of acetone and refluxed for 30 min, and the acetone was distilled. The residue was extracted into chloroform, washed with water, and dried ($MgSO_4$). After removing the drying agent, the product crystallized from chloroform to give 17.3 g (9.3%) of white solid which was recrystallized from methanol: mp 224–226°. Anal. ($C_{16}H_{19}NO_7$) C, H, N.

***O,O,O,N*-Tetraacetyl-α-hydroxymethyl-3,4-dihydroxyphenylalanine (7).** To 162 g (1.6 mol) of acetic anhydride were added 8.2 g (0.1 mol) of sodium acetate and 25.0 g (0.127 mol) of 3,4-dihydroxyphenylalanine. The mixture was heated and stirred on a steam bath for 2 hr at which time 10.0 g (0.33 mol) of paraformaldehyde was added and the mixture was stirred and heated for an additional 5 hr, cooled, and stirred with 500 ml of water. The water was decanted from oily residue and the residue was taken up in 50% aqueous acetone (500 ml) and refluxed for 5 hr. The acetone was evaporated and the resulting aqueous suspension was extracted with ethyl acetate, washed with water, and dried ($MgSO_4$). Removal of solvent afforded an oily residue which crystallized from ethyl acetate to give 4.1 g (8.2%) of a white solid which was recrystallized from ethanol: mp 224–225°. Anal. ($C_{18}H_{21}NO_9$) C, H, N.

α-Hydroxymethyltyrosine (2). To 20 ml of 6 *N* hydrochloride acid solution was added 9.0 g (0.0267 mol) of *O,O,N*-triacetyl-α-hydroxymethyltyrosine. The solution was heated on a steam bath under nitrogen for 1 hr and the water was removed by azeotropic distillation with toluene. A glassy residue was obtained which was dissolved in 50 ml of water and was stirred with 20 g of Dowex 1-X8. A precipitate formed which was decanted from the resin and collected on the filter. The white solid was recrystallized from water to give 2.0 g (35%) of white crystals, mp 313–314°. Anal. ($C_{10}H_{13}NO_4$) C, H, N.

α-Hydroxymethyl-3,4-dihydroxyphenylalanine (1). In 20 ml of 6 *N* hydrochloride acid was placed 3.1 g (0.00785 mol) of *O,O,O,N*-tetraacetyl-α-hydroxymethyl-3,4-dihydroxyphenylalanine. The mixture was heated on a steam bath under nitrogen for 2.5 hr after which a straw-colored solution was obtained. The

water was distilled with toluene under nitrogen, and the glassy residue was washed twice with a small amount of ether and dissolved in 10 ml of water. The slightly pink solution was stirred with Dowex 1-X8 for 1 hr at which time a slight precipitate formed and was filtered. The water was removed with toluene and 1.15 g (64%) of tan powder was obtained: mp 295°. Anal. ($C_{10}H_{13}NO_5$) C, H, N.

References and Notes

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Central Nervous System Depressants. 13. *s*-Triazolo-1,5-benzodiazepin-5-ones†,[1]

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Several 8-chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,5]benzodiazepin-5(6*H*)-ones, substituted in the 1 position, were prepared. These were tested for CNS activity. The most active, as a depressant, was the 1-methyl compound.

In continuation[2] of our search for better drugs acting on the central nervous system a series of *s*-triazolo-1,5-benzodiazepin-5-ones was prepared. These were made as outlined in Scheme I. P_2S_5 on 1[3] was found to give a mixture of mono- and dithiones, **2** and **3**. Since completion of this work Weber and Bauer[4] reported a similar mixture. We were able to separate the mixture by chromatography; however, this was not necessary since the mixture could be used and the products **4** or **5** purified.

The triazolo compounds could be obtained from the thione mixture by treatment with a hydrazide or via the hydrazine compound **5** and subsequent acylation and cyclization. The chloromethyl group in the 1 position provided access to other triazolo compounds by nucleophilic displacement.

Pharmacology. Compounds **4**, **7**, and **8** were subjected to a battery of tests designed to detect CNS activity. All three compounds were nontoxic as shown by no loss of righting reflex or loss of traction in standard tests[5] at 100 mg/kg. Compound **4** was active in the nicotine antagonism test[5] (ED_{50} 5 mg/kg), the strychnine antagonism test[6] (ED_{50} 28 mg/kg), the pentylenetetrazole antagonism test[7] (ED_{50} 7 mg/kg), and the hypoxic stress test (ED_{50} 60 mg/kg). A standard benzodiazepine, chlordiazepoxide, had the following ED_{50}'s in these antagonism tests: nicotine 1.0, strychnine 13, pentylenetetrazole 2.6, and hypoxic stress 10.5 mg/kg. Compound **8** was weakly active (nicotine ED_{50} 89 and pentylenetetrazole ED_{50} 25 mg/kg). Compound **7** was inactive in these tests at 100 mg/kg. These tests indicate that the 1-methyl compound **4** possesses moderate CNS depressant or tranquilizing activity.

Experimental Section[8]

8-Chloro-1-methyl-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,5]benzodiazepin-5(6*H*)-one (4). A solution of 3 g of a mixture of **2** and **3**[4] and 2 g of acethydrazide in 300 ml of *n*-BuOH was heated under reflux for 24 hr during which time N_2 was passed through the solution. After evaporation in vacuo the residue was washed (H_2O) and chromatographed on silica gel eluting with 3% MeOH in $CHCl_3$. The product (1.2 g) was recrystallized from EtOH yielding 0.8 g of white crystals, mp 297–298°. The principal spectral bands are ir (Nujol mull) 1695 (C=O), 1600, 1540, 1500 (C=C/C=N), 1320, 1205, 1105, 830, 755, 720, 695 cm^{-1} (C=C/arom); NMR ($CDCl_3$) δ 2.66 (s, 3, CH_3), ab centered at 3.58 and 4.23 (2, J = −14 Hz, 4-CH_2), and between 6.95 and 7.55 (m, 8, arom H's); mass spectrum $M^{\cdot+}$ 424 (1 Cl). Anal. ($C_{17}H_{13}ClN_4O$) C, H, Cl, N.

8-Chloro-1,3-dihydro-4-hydrazino-1-phenyl-2*H*-1,5-benzodiazepin-2-one Hydrate (5). To a suspension of 12.9 g of a mixture of **2** and **3** in 350 ml of MeOH was added dropwise 9.6 ml of hydrazine hydrate during which time N_2 was passed through the mixture. After stirring at room temperature overnight the resulting white solid was collected, washed (MeOH), dried, and recrystallized from MeOH yielding 4 g of crystalline solid, mp 102–103°. An additional 2.5 g of less pure material was obtained from the filtrates. Ir and NMR support the structure and NMR indicates that it is a hydrate. Anal. ($C_{15}H_{13}ClN_4O \cdot 1.5H_2O$) C, H, Cl, N.

8-Chloro-1-(chloromethyl)-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,5]benzodiazepin-5(6*H*)-one (7). A mixture of 4 g (0.0133 mol) of **5** and 30 ml of THF, under N_2, was cooled to 0° and 1.5 g (0.0133 mol) of $ClCH_2COCl$ in 5 ml of THF was added dropwise with stirring. After stirring at 0° for 35 min and at room temperature for 1 hr, the solution was poured into ice water, mixed with a little $CHCl_3$, and neutralized with $NaHCO_3$. The resulting solid was collected, washed (H_2O and Et_2O), and dried yielding 4 g of white solid, mp 210–215°. Ir and NMR indicate this is the expected 2-(7-chloro-5-phenyl-3*H*-1,5-benzodiazepin-2-yl)-chloroacetyl hydrazide (**6**).

Without further purification 1.5 g of **6** in 20 ml of AcOH was heated, under N_2, in a bath at 140° for 4 hr. After cooling the resulting solid was collected, washed (H_2O and Et_2O), and dried yielding 1.1 g of **7**, mp 282–285° dec. A sample was recrystallized from MeOH–CH_2Cl_2: mp 306–308° dec. The principal spectral bands are ir (Nujol mull) 3060 (=CH), 1695 (C=O), 1600, 1595, 1525, 1505, 1495 (C=C/C=N), 1320, 855, 760, 695 cm^{-1} (C=C/arom); uv (EtOH) 229 nm (ϵ 34,200), 283 (1950), 291 (sh, 1650); mass spectrum $M^{\cdot+}$ 258 (2 Cl). Anal. ($C_{17}H_{12}Cl_2N_4O$) C, H, N; Cl: calcd, 19.74; found, 20.30.

8-Chloro-1-[(dimethylamino)methyl]-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,5]benzodiazepin-5(6*H*)-one (8). A mixture of 1.07 g (3.0 mmol) of **7** and 30 ml of THF was cooled to 0° under N_2. A methanolic solution of 5 g of Me_2NH and 0.5 g of KI was added and the mixture was stirred at room temperature for 4 hr. The solution was evaporated in vacuo, mixed with aqueous $NaHCO_3$, and extracted with $CHCl_3$. After washing (H_2O) and drying (Na_2SO_4) the solution was evaporated. On trituration with EtOAc the oil crystallized yielding 0.98 g of white solid, mp 232–234°. The principal spectral bands are ir (Nujol mull) 3060,

† Dedicated to the memory of Professor Edward E. Smissman.