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Preparation of *m*-Hydroxyphenyl-L- and D-Lactic Acids and Other Compounds Related to *m*-Tyrosine[1]

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2-Methyl-4-(3′-acetoxybenzal)-5-oxazolone was hydrolyzed to yield *m*-hydroxyphenylpyruvic acid, which was reduced to *m*-hydroxyphenyllactic acid. *m*-Hydroxyphenyllactic acid was resolved by fractional crystallization of the quinine salts. *m*-Hydroxymandelic acid, *m*-hydroxymandelic ethyl ester, and *m*-hydroxyphenylacetic acid were prepared in good yields *via* *m*-hydroxymandelonitrile.

Recently, several factors have combined to stimulate interest in simple compounds containing a *m*-hydroxyphenyl nucleus. These include the observations that *m*-tyrosine is an excellent substrate for mammalian DOPA-decarboxylase[2-4] and participates in transamination reactions,[5] and that *m*-hydroxyphenylethylamine is a substrate for mammalian amine oxidase.[2,6] A possible metabolic importance of *m*-hydroxyphenyl compounds stems from the reported occurrence of several *m*-hydroxyphenyl compounds in animal[7-9] and human[9-13] urine, and from a reported abnormality in the excretion of *m*-hydroxyphenylacetic acid in the disease, phenylketonuria.[10,14,15] *m*-Hydroxyphenylacetic acid has also been described as a metabolite of 3,4-dihydroxyphenyl compounds in the rabbit.[16,17] It is noteworthy that many active drugs contain the *m*-hydroxyphenyl nucleus; *e.g.* edrophonium, neosynephrine, *m*-sympatol, *m*-hydroxybenzedrine, *m*-hydroxypropadrine, etc. The resolution of *m*-tyrosine was described recently.[18]

This paper reports the preparation and resolution of *m*-hydroxyphenyllactic acid, and the synthesis of *m*-hydroxymandelic acid. Improved procedures for the synthesis of *m*-hydroxymandelonitrile, *m*-hydroxyphenylpyruvic acid, and *m*-hydroxyphenylacetic acid are also presented.

m-Hydroxyphenylpyruvic acid was obtained (60% yield) by dilute acid hydrolysis of 2-methyl-4-(3′-acetoxybenzal)-5-oxazolone;[18] alkaline hydrolysis yielded an inferior product. This acid decomposes slowly when it is stored and the recovery is usually poor when it is recrystallized. It had been synthesized earlier by alkaline hydrolysis of 2-phenyl-4-(3′-acetoxybenzal)-5-oxazolone (34% yield).[19,20]

m-Hydroxyphenyllactic acid was prepared by reduction of *m*-hydroxyphenylpyruvic acid with sodium amalgam (55% yield). It was resolved by fractional crystallization of the quinine salt. The separation of the isomers was facilitated by the fact that the quinine salt of the (−)-acid formed an addition compound with methanol. An L-configuration was assigned to *m*-hydroxyphenyl-(−)-lactic acid, on the basis of a comparison of its optical properties[21] with those of the known phenyl-L-lactic and *p*-hydroxyphenyl-L-lactic acids. The only previous reports of *m*-hydroxyphenyllactic acid have indicated its excretion by dogs after the feeding of *m*-hydroxyphenylpyruvic acid,[22] and its presence in human urine.[11]

m-Hydroxymandelonitrile was prepared in 84% yield by the condensation of *m*-hydroxybenzaldehyde with sodium cyanide in the presence of sodium bisulfite, in the manner described for synthesis of *p*-hydroxymandelonitrile.[23] In an earlier procedure,

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anhydrous hydrogen cyanide was employed with calcium oxide as a catalyst (66% yield).[24]

The nitrile was converted by the action of dry hydrogen chloride and ethanol, *via* the iminoester hydrochloride and *m*-hydroxymandelic ethyl ester, to *m*-hydroxymandelic acid (46% yield). This route has been used for the preparation of *p*-hydroxymandelic acid.[23] *m*-Hydroxymandelic acid was obtained in better over-all yield (60% based on nitrile) by alkaline hydrolysis of the intermediate iminoester hydrochloride, without isolation of the ethyl ester.

m-Hydroxyphenylacetic acid was prepared in 93% yield by the reduction and hydrolysis of *m*-hydroxymandelonitrile with hydriodic acid and red phosphorus. Other methods described in the literature have included the same reaction starting with *m*-methoxymandelonitrile,[25] the deamination of *m*-aminophenylacetic acid,[26] the demethylation of *m*-methoxyphenylacetic acid,[27] the reductive demethylenation of homopiperonylic acid,[28] and application of the Willgerodt reaction.[29,30]

EXPERIMENTAL

m-Hydroxyphenylpyruvic acid. 2-Methyl-4-(3′-acetoxybenzal)-5-oxazolone (24.52 g., 0.10 mole), m.p. 117–119°, prepared from *m*-hydroxybenzaldehyde and acetylglycine,[18] was refluxed with one liter of 1 *N* hydrochloric acid under nitrogen for five hours. The solution was treated with charcoal and filtered while hot. The filtrate was extracted with six 250-ml. volumes of ethyl acetate and the combined ethyl acetate solutions were extracted with three 200-ml. volumes of 1 *N* sodium bicarbonate solution. The pooled bicarbonate extracts were acidified to *p*H 1.5 with concentrated hydrochloric acid, heated to the boiling point to expel carbon dioxide, cooled, and extracted with six 200-ml. volumes of ethyl acetate. The ethyl acetate extracts were dried over sodium sulfate, treated with charcoal, and the filtrate was concentrated under reduced pressure and nitrogen. The resulting crystalline paste was dissolved in 220 ml. of boiling ethyl acetate and treated with 110 ml. of hot cyclohexane. After the solution had cooled to 5°, 0.2 g. of an amorphous solid (m.p. >250°) was removed by filtration. Four crops of *m*-hydroxyphenylpyruvic acid totalling 10.83 g. (60% yield) were recovered from the filtrate after refrigeration, concentration, and further addition of cyclohexane. Different crops melted with decomposition in the range 164–168° (initial bath temperature 160°, heating rate 3° per minute).[31] The combined crops were recrystallized from a mixture of 60 ml. of acetic acid and 240 ml. of carbon tetrachloride with charcoal treatment; *m*-hydroxyphenylpyruvic acid was obtained as fine colorless needles, 9.15 g., 85% recovery, m.p. 164–165° (dec.) (Lit.[20] m.p. 165°). The melting point decreased slowly upon prolonged storage at 5°.

*m-Hydroxyphenyl-*DL*-lactic acid.* Crude *m*-hydroxyphenylpyruvic acid (12.61 g., 0.07 mole) was dissolved in 145 ml. of 1 *N* sodium hydroxide in a round-bottomed flask. Freshly pulverized 3% sodium amalgam[32] (215 g., 0.28 g.-atom) was added in 12 equal portions at five-minute intervals with high speed agitation. Agitation was continued for one hour more. The straw-colored aqueous supernatant was removed and the amalgam was washed with water. The combined aqueous solutions were acidified to *p*H 7.0 with concentrated hydrochloric acid and washed with three 120-ml. portions of ethyl acetate to remove neutral phenolic impurities. The remaining aqueous phase was acidified to *p*H 1.6 with concentrated hydrochloric acid and extracted with four 120-ml. portions of ethyl acetate. The ethyl acetate extracts were dried over sodium sulfate, treated with charcoal, and the filtrate was concentrated under reduced pressure and nitrogen. The resulting clear gum was again taken to dryness with two portions of 1,2-dichloroethane to expel traces of water, and then was allowed to crystallize from a mixture of 25 ml. of ether and 100 ml. of 1,2-dichloroethane. *m*-Hydroxyphenyllactic acid (7.05 g., 55% yield) was recovered as clustered colorless blades, m.p. 99–100°. Further processing of the mother liquor failed to yield more product. The crude product was recrystallized from 1,2-dichloroethane for analysis, m.p. 100°.

Anal.[33] Calc'd for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 59.58; H, 5.83.

*Resolution of m-hydroxyphenyl-*DL*-lactic acid. m*-Hydroxyphenyl-DL-lactic acid (6.38 g., 0.035 mole) and 11.35 g. (0.035 mole) of anhydrous quinine were dissolved in 200 ml. of boiling methanol. The solution was treated with 2.0 g. of charcoal, the filtrate was concentrated to dryness under reduced pressure and nitrogen, and the residue was dissolved in 75 ml. of boiling methanol. The crystals that had separated after the solution had stood overnight at room temperature were collected, washed with three 5-ml. portions of cold methanol, and desiccated over potassium hydroxide and phosphorus pentoxide at 10 mm.; crop A, 8.35 g., m.p. 194–202° (slight softening at 113°). Crop A was dissolved in 135 ml. of boiling methanol and the solution was stored overnight at 5°; 3.48 g. of crop B were recovered as colorless cuboids, m.p. 204–206°. The filtrate from this material was concentrated to dryness under reduced pressure and nitrogen; the residue was dissolved in 35 ml. of boiling methanol and the solution was allowed to stand at room temperature to yield 1.33 g. more of crop B, colorless cuboids, m.p. 204–206°; total crop B, 4.81 g. Crop B was recrystallized from 90 ml. of boiling methanol with charcoal treatment to give 3.32 g. of crop C as large colorless cuboids, m.p. 205–206° (initial bath temperature 200°, heating rate 2° per minute), $[\alpha]_D^{24}$ −161.6° (*c*, 1.0, acetic acid). A sample was recrystallized again from methanol, m.p. 205–206°, $[\alpha]_D^{24}$ −160.5° (*c*, 1.4, acetic acid).

Crop C was suspended in 75 ml. of water, treated with 13.2 ml. of 1 *N* sodium hydroxide, and extracted with four 75-ml. portions of dichloromethane to remove quinine. The aqueous phase was acidified to *p*H 1.6 with concentrated hydrochloric acid and extracted with six 75-ml. portions of ethyl acetate. The ethyl acetate extracts were dried over sodium sulfate and concentrated under nitrogen and reduced pressure to clear amber oil. This oil was dissolved in 25 ml. of ether, 50 ml. of cyclohexane was added in several portions, and the mixture was seeded with a rubbed aliquot when clouding occurred; rapid precipitation ensued. The mixture was allowed to digest for two days at room temperature, and then was stored overnight at 5°; 0.88 g. (74% yield from pure quinine salt) of *m*-hydroxyphenyl-D-lactic acid was obtained, m.p. 84–86°. After recrystallization from 1,2-dichloroethane with charcoal treatment, the D-acid was obtained as shimmering colorless flakes, 88% recovery,

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(33) Analyses were performed by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

m.p. 85–86°, $[\alpha]_D^{2.2}$ +19.5° (*c*, 1.0, water), $[\alpha]_D^{22}$ +48.2° (*c*, 1.0, 1 *N* sodium hydroxide).

Anal. Calc'd for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 59.11; H, 5.66.

The filtrate from crop A was concentrated to dryness under nitrogen and reduced pressure, and the residue was crystallized from 30 ml. of boiling methanol with cooling to 5° to produce 6.39 g. of crop D, which softened to a froth at 106°, solidified again at 115°, and melted at 161–163°. Crop D was crystallized from 20 ml. of boiling methanol with cooling to 5° to give 3.81 g. (after desiccation) of crop E, m.p. 112–120°, $[\alpha]_D^{25}$ −173.0° (*c*, 1.2, acetic acid). After drying in air, crop E was in the form of colorless clusters of elongated rhombs, which decreased in weight by 0.25 g. and visibly decrepitated during desiccation. This behavior indicates the loss of one molecule of methanol of crystallization. This crop was recrystallized from 15 ml. of boiling methanol with charcoal treatment. A felted mass of long fine needles rapidly precipitated as the solution cooled. After eight hours at room temperature, numerous clusters of elongated rhombs had appeared; in 18 hours, transformation of the needles to rhombs was complete. The mixture was stored at 5° and 3.03 g. (after desiccation) of crop F was recovered, m.p. 114–120°, $[\alpha]_D^{25}$ −174.0° (*c*, 1.2, acetic acid). Crop F showed the same behavior on desiccation as crop E. It was converted to free acid in the manner described for the D-isomer; 0.77 g. (71% yield from pure quinine salt) of white powder was recovered, m.p. 84–86°. After recrystallization from 1,2-dichloroethane with charcoal treatment, *m*-hydroxyphenyl-L-lactic acid was obtained as shimmering colorless flakes, 80% recovery, m.p. 85–86°, $[\alpha]_D^{22}$ −19.1° (*c*, 1.0, water), $[\alpha]_D^{22}$ −45.0° (*c*, 1.0, 1 *N* sodium hydroxide).

Anal. Calc'd for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 59.12; H, 5.58.

The configuration of the *m*-hydroxyphenyllactic acid isomers is assigned on the basis of the observed effect of alkali on their rotations as compared to the change observed with phenyl-L-lactic acid, $[\alpha]_D^{24}$ −21.4° (*c*, 1, water),[34] $[\alpha]_D^{22}$ −31.3° (*c*, 1, 1 *N* sodium hydroxide). In addition, the magnitude and direction of rotation of the isomer assigned the L-configuration are similar to those of phenyl-L-lactic acid, and *p*-hydroxyphenyl-L-lactic acid [$[\alpha]_D$ −18.1° (*c*, 1.57, water)].[35]

m-Hydroxymandelonitrile. *m*-Hydroxybenzaldehyde (30.53 g., 0.25 mole) was dissolved in 300 ml. of 1 *N* sodium bisulfite solution at 50°. The solution was cooled to −5° with an ice-salt bath and 175 ml. of ether was added. A solution of 13.48 g. (0.275 mole) of sodium cyanide in 50 ml. of water was added dropwise during 20 minutes. The temperature was maintained below 0°, and the mixture was agitated mechanically during and for 40 minutes following the addition. The organic phase was separated and the aqueous phase was extracted with four more 175-ml. portions of ether. The ether extracts were washed with 1 *N* sodium bisulfite solution, dried over calcium chloride, treated with charcoal, filtered, and concentrated to about 200 ml. under nitrogen and reduced pressure with the bath temperature not exceeding 30°. Benzene (800 ml.) was added and the solution was concentrated to a volume of 500 ml. and stored overnight at 5°. Three crops (31.21 g., 84% yield) of cream-colored crystals were recovered, m.p. 111–112° (Lit. m.p. 110°).[24]

m-Hydroxymandelic ethyl ester. Dry hydrogen chloride was passed slowly into a solution of 29.83 g. (0.20 mole) of *m*-hydroxymandelonitrile in 12.3 ml. (0.21 mole) of absolute ethanol and 150 ml. of anhydrous ether at 5° until 7.7 g. (0.21 mole) had been absorbed (10 minutes). The mixture was stored at 5° for 24 hours, during which time the iminoester hydrochloride separated as a viscous pale yellow gum. Precautions were taken to exclude atmospheric moisture in these operations. The clear supernatant ether layer was decanted and discarded. The residual gum was desiccated over potassium hydroxide at 10 mm. for 24 hours to remove ether and excess hydrogen chloride, and was dissolved in 900 ml. of water and allowed to stand at room temperature for three hours.

The ethyl ester was extracted from the colorless aqueous solution with six 900-ml. portions of ether. The combined ether extracts were dried over sodium sulfate and concentrated to dryness under reduced pressure and nitrogen. The resulting oily ester was dissolved in 600 ml. of boiling benzene, treated with charcoal, and the filtrate was concentrated to dryness; the solid residue was crystallized first from benzene and then with charcoal treatment from water to give 19.67 g. (50% yield) of *m*-hydroxymandelic ethyl ester, m.p. 102–103°. A sample was recrystallized from isopropyl ether for analysis, m.p. 104°.

Anal. Calc'd for $C_{10}H_{12}O_2$: C, 61.21; H, 6.17. Found: C, 61.17; H, 6.29.

m-Hydroxymandelic acid. An aqueous hydrolysate of *m*-hydroxymandelic iminoester hydrochloride, prepared in the manner described in the preceding section, was treated with 66 ml. of 10 *N* sodium hydroxide and refluxed under nitrogen for two hours. The solution was cooled to room temperature, acidified to *p*H 7.5 with 6 *N* hydrochloric acid, and extracted with four 500-ml. volumes of ethyl acetate to remove most of the colored impurities. The aqueous phase was acidified to *p*H 1.5 and extracted with six 500-ml. volumes of ethyl acetate. The *p*H 1.5 extracts were dried over sodium sulfate and concentrated under reduced pressure and nitrogen. The resulting colorless oil was taken up in 200 ml. of boiling ethyl acetate, treated with charcoal, and the filtrate was again concentrated. The residue was crystallized from a mixture of 100 ml. of ethyl acetate and 150 ml. of cyclohexane to give 16.77 g. (50% yield) of *m*-hydroxymandelic acid as colorless needles, m.p. 130–131°. A second crop (3.24 g., 10% yield) was recovered from the filtrate, m.p. 129–131°. For analysis, the compound was recrystallized from 50% (v/v) ethyl acetate-cyclohexane with charcoal treatment, m.p. 131–132°.

Anal. Calc'd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.30; H, 4.98.

A solution of 19.62 g. (0.10 mole) of *m*-hydroxymandelic ethyl ester in 450 ml. of water and 22 ml. of 10 *N* sodium hydroxide was processed in the manner described above. Two crops of *m*-hydroxymandelic acid were obtained; 8.40 g. (50% yield), m.p. 129–131°, and 2.52 g. (15% yield), m.p. 128–129°.

m-Hydroxyphenylacetic acid. *m*-Hydroxymandelonitrile (14.95 g., 0.10 mole) was refluxed with 300 ml. of 7.6 *N* hydriodic acid and 18.59 g. (0.60 g.-atom) of red phosphorus for 16 hours. The reaction mixture was cooled and concentrated to dryness under reduced pressure and nitrogen.[36] Three 150-ml. portions of water were added and the mixture was concentrated to dryness each time. The residue was taken up in 150 ml. of boiling water and treated with charcoal. The filtrate was adjusted to *p*H 7.5 with 10 *N* sodium hydroxide and washed with three 150-ml. portions of ether. The aqueous phase was acidified to *p*H 1.8 with 6 *N* hydrochloric acid and extracted with five 225-ml. volumes of ether. The ether extracts were dried over sodium sulfate, treated with charcoal, and the filtrate was concentrated to dryness under reduced pressure and nitrogen. The crystalline residue was dissolved in 150 ml. of warm ether, 450 ml. of cyclohexane was added, and the solution was stored at 5° overnight; 14.10 g. (93% yield) of colorless clustered blades were recovered in two crops, m.p. 130–131°, unchanged by recrystallization from ether-cyclohexane (Lit. m.p. 129°).[26,27]

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(36) In one experiment, concentration was started while the reaction mixture was still hot. After a few minutes, an explosion shattered the still head, possibly due to a warm mixture of phosphine (and P_2H_4) with air in the assembly.