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Preparation of Urinary Metabolites of 3,4-Dihydroxyphenylalanine and Norepinephrine, and Related Compounds[1]

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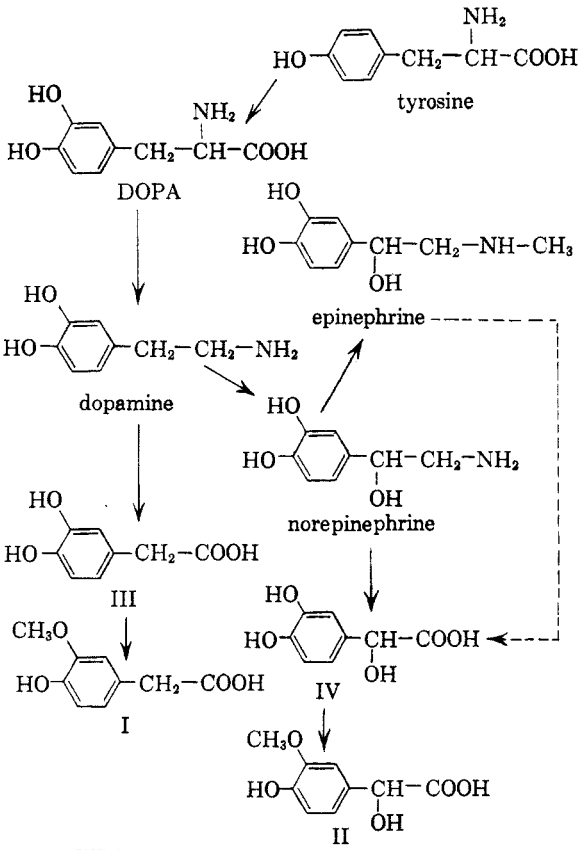
3-Methoxy-4-hydroxymandelic and 3,4-dihydroxymandelic acids were prepared *via* the corresponding nitriles and ethyl esters from vanillin and protocatechualdehyde, respectively. The stability of the mandelic derivatives under various conditions was determined by use of paper chromatography. 3-Methoxytyrosine and homovanillic acid were synthesized from vanillin and acetylglycine *via* 2-methyl-4-[3′-methoxy-4′-acetoxybenzal]-5-oxazolone. Homoprotocatechuic acid was prepared from homoveratric acid. The earlier literature on these compounds is reviewed.

Several of the phenolic acids in normal human urine[2] contain a 3-methoxy-4-hydroxyphenyl nucleus. One has been identified as 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, I),[3] and another as 3-methoxy-4-hydroxy-D-mandelic acid (II).[4,5] I and its biological precursors, 3,4-dihydroxyphenylacetic acid (homoprotocatechuic acid, III) and 3,4-dihydroxyphenylethylamine (dopamine) are major urinary metabolites of 3,4-dihydroxyphenylalanine (DOPA), and are excreted in increased amounts after administration of DOPA to man[3,6–8] and other animals.[3,6,9–11] The excretion of I and III after administration of rutin, or its aglycone, quercetin, has also been reported.[12,13] DOPA and dopamine are important intermediates in biosynthesis of the hormones, norepinephrine and epinephrine, from tyrosine.[14] Norepinephrine is catabolized, possibly *via* 3,4-dihydroxymandelic acid (IV), to II which is eliminated in the urine; the excretion of II is markedly increased in cases of pheochromocytoma which are characterized by an excessive production of norepinephrine, and also after administration of IV.[4,5] It is likely that II and IV are also metabolites of epinephrine, since both amines are substrates for amine oxidase,[15] and since the distribution of radioactive urinary metabolites is similar after administration of C^{14}-norepinephrine or epinephrine to rats.[16]



This paper reports the preparation of IV, and improved procedures for the synthesis of II and III. A convenient preparation *via* 2-methyl-4-(3′-methoxy-4′-acetoxybenzal)-5-oxazolone (VI)

(1) Supported by research grants from the National Institutes of Health, U. S. Public Health Service.

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(14) Pertinent references are cited in (3).

(15) U. S. Von Euler, *Noradrenaline*, C. C Thomas, Springfield, 1956, pp. 41–53.

(16) R. W. Schayer, R. L. Smiley, K. J. Davis, and Y. Kobayashi, *Am. J. Physiol.*, **182**, 285 (1955).

is given for 3-methoxy-4-hydroxyphenylalanine (3-methoxytyrosine, V), which was examined as a possible biological precursor of I.[3] The synthesis of I from VI is also described.

II was prepared in 45% over-all yield from vanillin (VII), *via* the cyanohydrin (VIII) and ester (IX); this route had been used by Gardner and Hibbert,[17] who reported a 19% yield of II. A new

$$\underset{\text{VII, X}}{\text{Ar—CHO}} \longrightarrow \underset{\text{VIII, XI}}{\text{Ar—CHOH—CN}} \longrightarrow$$

$$\underset{\text{IX, XII}}{\text{Ar—CHOH—COOC}_2\text{H}_5} \longrightarrow \underset{\text{II, IV}}{\text{Ar—CHOH—COOH}}$$

II, VII, VIII, IX; Ar = 3-methoxy-4-hydroxyphenyl
IV, X, XI, XII; Ar = 3,4-dihydroxyphenyl

approach to II involving reaction of guaiacol with sodium glyoxylate has been indicated in a recent patent.[18]

VIII was obtained from VII and liquid hydrogen cyanide by Gorskii and Makarov,[19] who reported difficulty in purifying the product. Buck[20] prepared VIII in 56% yield from reaction of VII with 2 moles of sodium bisulfite and 4 moles of potassium cyanide. Hahn *et al.*[21] subsequently reported an 89% yield with approximately half as much bisulfite and cyanide; it should be noted, however, that their product was a residue of uncertain purity obtained by evaporation of extracts. Others[17,22] have used VIII from these procedures in solution without isolation, because of reputed instability.[22] In the present study, VIII was obtained in 80% yield by use of 4 moles of bisulfite and 4 moles of cyanide; the yield decreased to 25–35% and the product was less pure when only 1.1–1.2 moles of bisulfite and cyanide were employed. Crystalline VIII is stable for many months at 5°, and showed no sign of deterioration during shorter periods at room temperature.

Without isolation, VIII has been converted by the action of dry hydrogen chloride and ethanol, *via* the iminoester hydrochloride to IX (25% yield based on VII);[17,22] crystalline VIII was reported to yield only 3% of IX.[22] IX was then saponified to II in 80% yield.[17] It is possible that the solid VIII used in earlier work[22] may have contained vanillin or other impurities, since pure VIII afforded IX in 40% yield in the present work whereas impure VIII gave lower yields of impure IX. A more complete recovery of IX was impeded by the presence of by-products, however, and a better yield of II (56% based on VIII) was obtained by saponifying the intermediate iminoester hydrochloride without isolating IX. II has been reported to resinify when exposed to air;[17] in this laboratory, however, II and IX proved to be stable indefinitely.

IV was synthesized in 27% over-all yield from protocatechualdehyde (X) *via* the cyanohydrin (XI) and ester (XII). The only previous preparation of IV, an involved procedure starting with piperonal,[23] yielded a highly unstable and very impure product.

The preparation of impure XI by condensation of X with sodium bisulfite and potassium cyanide, or with liquid hydrogen cyanide was claimed in an early German patent.[24] The latter method was used subsequently by Buck[20] to obtain XI in 63% yield. A similar yield was obtained with hydrogen cyanide in this laboratory, but the product was impure and the procedure hazardous. XI of good purity was prepared in 64% yield by use of 4 moles of bisulfite and 4 moles of cyanide. The stability of crystalline XI is similar to that of VIII.

XI was converted to XII in 50% yield by the action of dry hydrogen chloride and ethanol; the yield was lowered when insufficient time was allowed for formation of the intermediate iminoester hydrochloride. IV was readily obtained in 85% yield by saponification of XII under nitrogen. The sensitivity of IV in alkaline solution to atmospheric oxidation made it expedient to isolate and purify XII, rather than to proceed directly from XI to IV. The stability of IV and XII is comparable to that of II and IX respectively.

The chromatographic behavior of the various mandelic derivatives is summarized in Table I. Paper chromatography was helpful not only as a means of assessing purity of the various compounds but also in providing further information on the stability of the nitriles, VIII and XI. Chromatography in ammoniacal isopropyl alcohol or in 20% aqueous potassium chloride solution was found to result in complete decomposition of both nitriles to the corresponding aldehydes, VII and X. Only a 20% degradation to the aldehydes occurred when 1% solutions of the nitriles in 20% potassium chloride were allowed to stand at room temperature for four hours,[25] but similar solutions of the nitriles in water alone showed the formation of 40% of the aldehydes. These findings may explain why others[19,22] operating in a more humid climate have reported difficulties in handling VIII. Slight decomposition of the nitriles also occurred upon chromatography under mildly acid conditions;

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(22) H. Schwartz and J. L. McCarthy, *Can. J. Research*, **19B**, 150 (1941).
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(24) Höchst Farbwerke, German Patent **193,634** [*Chem. Zentr.*, **79**, I, 430 (1908)].
(25) HCN liberated upon breakdown of the nitriles to the aldehydes can readily escape from the surface of a paper chromatogram; the equilibrium would thus shift in the direction of aldehyde to a greater extent than with nitrile solutions under normal storage conditions.

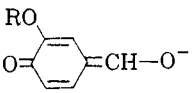
TABLE I

CHROMATOGRAPHIC BEHAVIOR OF MANDELIC DERIVATIVES[a]

Compound	Solvent and R_f				Qualitative Color Reactions		
	IA	BP	KCl	BuAc	DSA	DNPH	ASN
Vanillin	0.53	0.80	0.58	0.90	Lt. O	Lt. O	Lt. G (slow)[b]
3-Methoxy-4-hydroxy-mandelonitrile	[c]	0.42	[c]	0.89[d]	O → R	None	None
mandelic Et ester	0.85[e]	0.79	0.81	0.92	O → R	None	Bl-G (slow)
mandelic acid	0.26	0.12	0.86	0.67	O → R	Lt. Y[b]	G (slow)
3,4-Dihydroxy-benzaldehyde	0.50[f]	0.23	0.54[f]	0.84	Lt. G-Br[g]	Y-O	Dk. G → Lt. Br
mandelonitrile	[c]	0.05	[c]	0.77[d]	Lt. Br[g]	None	Y → G-Br
mandelic Et ester	0.64[h]	0.21	0.83[f]	0.83	Lt. O-Br[g]	None	Y-Br → G-Br
mandelic acid	0.10[i]	0.01	0.85[f]	0.49	Lt. O-Br[g]	Lt. O (slow)	Bk → Br

[a] *Solvent systems:* IA, isopropyl alcohol–aqueous ammonia–water, 8:1:1, 15 hr.; BP, benzene–propionic acid–water, 100:70:5, 3 hr. after 30 min. equilibration (this solvent gives same R_f values as upper layer of two phase system described earlier[2]); KCl, 20% aqueous potassium chloride (w/w), 2 hr.; BuAc, *n*-butanol–acetic acid–water, 4:1:1, 15 hr. *Spray reagents:* DSA, diazotized sulfanilic acid;[51] DNPH, 2,4-dinitrophenylhydrazine, 0.28% in 1*N* hydrochloric acid; ASN, ammoniacal silver nitrate.[51] *Colors:* Lt., light; Dk., dark; Y, yellow; R, red; Bl, blue; O, orange; Br, brown; G, grey; Bk, black. All spots were dull blue under ultraviolet light. [b] Requires 100 γ; 10 γ not detected. [c] Converted completely to aldehyde; no other spots observed. [d] Approximately 6% converted to aldehyde in 12 hr. [e] Slight trailing indicates minor dec. (amide formation ?). [f] Spots faintly visible before spray, some trailing (partial atmospheric oxidation). [g] Surrounded by white fringe. [h] Extensive trailing and abnormal color reactions indicate major dec.; R_f taken from 100-γ spot where some ester still intact. [i] Some trailing and abnormal color reactions indicate partial dec. (<50%); second minor spot (R_f 0.24), white with DSA, Lt. O with DNPH, G with ASN.

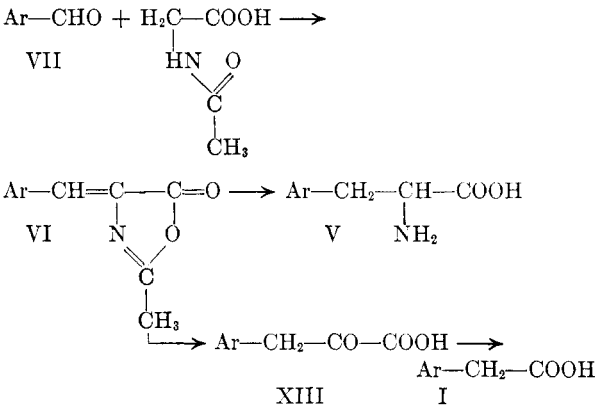
about 6% of the aldehydes were formed in 12 hours with a butanol–acetic acid–water medium. These features of instability, considered together with the failure of sodium bisulfite washing to eliminate small amounts of aldehydes from the nitriles, and with the apparent improved yield of nitriles when large excesses of bisulfite and cyanide are used, point to ready reversibility of the cyanohydrin condensation in the case of VII and X. A lowered susceptibility of the aldehyde carbon toward nucleophilic attack may be associated with a greater contribution of the quinonoid form in the mildly alkaline reaction medium, and a consequent increased resonance stabilization of the aldehydes.



R = H or CH_3

The other mandelic derivatives were relatively stable during chromatography, aside from an expected sensitivity of the 3,4-dihydroxy compounds to atmospheric oxidation, especially under alkaline conditions.

3-Methoxytyrosine (V) was synthesized from vanillin (VII) in 53% over-all yield. VII was condensed with acetylglycine in the presence of acetic anhydride and anhydrous sodium acetate to give the oxazolone, VI, in 69% yield. VI was converted to V in 76% yield by partial hydrolysis, reduction with sodium amalgam, and hydrolysis of the resulting *N*-acetyl-V. V had been prepared in 17% yield by Bloch[26] using a similar sequence of reactions



Ar = 3-methoxy-4-hydroxyphenyl

starting with VII and hippuric acid.[27–29] V has also been made by condensation of VII with 2-thiohydantoin (65% yield),[30] homovanillin with hydrogen cyanide and ammonia (30% yield),[31] and VII with rhodanine followed by conversion to the α-thiopyruvic and α-oximino acids (46% yield).[32,33]

Homovanillic acid (I) was prepared from the oxazolone, VI, by alkaline hydrolysis, followed by oxidation of the resulting 3-methoxy-4-hydroxy-

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(27) F. Mauthner, *Ann.*, **370**, 368 (1909).

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(30) T. B. Johnson and R. Bengis, *J. Am. Chem. Soc.*, **35**, 1606 (1913).

(31) A. A. L. Challis and G. R. Clemo, *J. Chem. Soc.*, 1692 (1947).

(32) G. R. Clemo and F. K. Duxbury, *J. Chem. Soc.*, 1795 (1950).

(33) H. E. Fisher and H. Hibbert, *J. Am. Chem. Soc.*, **69**, 1208 (1947).

phenylpyruvic acid (XIII) *in situ* with hydrogen peroxide. The procedure was convenient, even though the yield of I was only 17% and the formation of several other unidentified products could be detected by paper chromatography. XIII of sufficient purity for chromatographic characterization[2] was also obtained in low yield from VI. This route to I had been applied originally to the corresponding 2-phenyl-5-oxazolone;[27] the intermediate XIII was isolated in 43% yield.[34] Other methods for the preparation of I have involved oxidation of eugenol derivatives with potassium permanganate,[35] or ozone,[31,36,37] and reaction of 3-methoxy-4-benzoxybenzyl chloride with cyanide.[38] Synthesis of I from VII and rhodanine, *via* the α-thiopyruvic and α-oximino acids, and the nitrile of I has given the highest reported yield (73%).[33]

III was obtained in 94% yield from the commercially available 3,4-dimethoxyphenylacetic acid (XIV) by the action of hydriodic acid in the presence of red phosphorus; an inferior product resulted when phosphorus was omitted, possibly because of partial oxidation by free iodine or atmospheric oxygen. The preparation of III from XIV had been indicated recently but without details.[12] III has been synthesized similarly from I,[35] from VIII,[19] from 3,4-dimethoxymandelonitrile,[39] from 3,4-methylenedioxyphenylacetic acid,[40] and from 3,4-dihydroxyphenylacetonitrile,[41] and by use of the Willgerodt reaction.[42,43]

EXPERIMENTAL

3-Methoxy-4-hydroxymandelonitrile (VIII). A solution of 260.4 g. (4.0 moles) of potassium cyanide in 400 ml. of water was added dropwise during 30 min. to a cold (−10°) solution of 152.1 g. (1.0 mole) of vanillin (U.S.P. grade) in 875 ml. of water containing 416.3 g. (4.0 moles) of sodium bisulfite. The mixture was stirred vigorously and the temperature was maintained below +10° during the addition and for 30 min. thereafter. The mixture was diluted with 500 ml. of water to dissolve the precipitated white crystals and was extracted with four 600-ml. portions of ether. The combined ether extracts were washed with two 100-ml. portions of 4*N* sodium bisulfite solution, dried over anhydrous sodium sulfate, treated with charcoal, and concentrated to dryness under nitrogen at a reduced pressure. The residue was dissolved in 200 ml. of anhydrous ether and 400 ml. of benzene was added to the solution. Following refrigeration, 124.0 g. (69% yield) of VIII was recovered as colorless crystals, m.p. 81–83°[44] (Lit.[20] m.p. 83°). The mother liquor was concentrated to dryness and the residue was crystallized from a mixture of 100 ml. of anhydrous ether and 200 ml. of benzene to yield a second crop of 19.5 g. (11% yield), m.p. 82–84°. The melting point of a mixture of the two crops was not depressed, but a mixture of either crop with vanillin (m.p. 82°) melted below 65°. VIII retained solvent tenaciously and melted over a wide temperature range before thorough drying *in vacuo*. Further crops from the mother liquor contained substantial amounts of vanillin.

Ethyl 3-methoxy-4-hydroxymandelate (IX). Dry hydrogen chloride was passed into a solution of 89.6 g. (0.50 mole) of VIII in a mixture of 24.0 g. (0.52 mole) of absolute ethanol and 400 ml. of anhydrous ether at 5° until 19.3 g. (0.53 mole) was absorbed (15 min.). The mixture was stored at 5° for 48 hr., during which time gummy crystals of the iminoester hydrochloride separated. Precautions were taken to exclude atmospheric moisture in these operations. The supernatant ether layer was discarded; the gummy residue was dried *in vacuo* over potassium hydroxide for 24 hr. to remove ether and excess hydrogen chloride, and then was stirred with 900 ml. of water for 4 hr. The aqueous layer was separated from about 10 ml. of oil which was shaken with three more 100-ml. portions of water. The combined aqueous layers were treated with charcoal; the filtrate was used either for isolation of the ester or for preparation of the acid.

The ester was extracted from the aqueous filtrate with four 750-ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate, treated with charcoal, and concentrated to dryness under nitrogen at a reduced pressure. The residual yellow oil was dissolved in 750 ml. of anhydrous ether and 900 ml. of cyclohexane was added to the solution. The resulting crystal cake was fragmented after 24 hr. at 5°, and 225 ml. more cyclohexane was added. After another day at 5°, 45.1 g. (40% yield) of IX was recovered, m.p. 74–76°. A sample was recrystallized from water with charcoal treatment; rosettes of colorless blunt needles were recovered, m.p. 77–78° (Lit.[17] m.p. 77°), unchanged by further recrystallization from ether-cyclohexane. The ether-cyclohexane mother liquor was concentrated at a reduced pressure to dryness. The resulting 34.5 g. of yellow oil failed to yield more crystalline IX and was hydrolyzed with 1*N* sodium hydroxide, in the manner described in the next section, to give 11.55 g. (12% yield, based on VIII) of II, m.p. 131–132°.

3-Methoxy-4-hydroxymandelic acid (II). An aqueous hydrolysate of 3-methoxy-4-hydroxymandelic iminoester hydrochloride, prepared in the manner described in the preceding section, was treated with 150 ml. of 10*N* sodium hydroxide and refluxed under nitrogen for 2 hr. The solution was cooled to room temperature, acidified to *p*H 7.0 with hydrochloric acid, and extracted with six 900-ml. portions of ethyl acetate to remove neutral impurities. The aqueous phase was acidified to *p*H 1.5 and extracted with six 900-ml. portions of ethyl acetate. The combined *p*H 1.5 extracts were treated with charcoal, dried over anhydrous sodium sulfate, and concentrated at a reduced pressure to dryness. The brown residue was dissolved in 600 ml. of boiling ethyl acetate, the solution was treated with charcoal, and 900 ml. of cyclohexane was added to the filtrate. Following refrigeration, 52.2 g. (53% yield from VIII) of II was obtained, dec. 130–132° (sample in bath at 125°, heating rate 2° per minute). A further 3.0 g. (3% yield), dec. 129–131°, was recovered from the mother liquor. The compound was recrystallized from acetonitrile with charcoal treatment and recovered (80%) as a hard colorless to faintly yellow crystalline scale, dec. 131–133° (Lit.[17] m.p. 133°).

A solution of 25.3 g. (0.112 mole) of IX, m.p. 74–76°, in 225 ml. of 1*N* sodium hydroxide was processed in the manner

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(35) F. Tiemann and N. Nagai, *Ber.*, **10**, 201 (1877).

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(38) A. Ya. Berlin, S. M. Sherlin, and T. A. Serebrennikova, *Zhur. Obshcheĭ Khim.*, **19**, 759 (1949) [*Chem. Abstr.*, **44**, 1058 (1950)].

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(43) J. A. King and F. H. McMillan, U. S. **2,568,011** [*Chem. Abstr.*, **46**, 3081 (1952)].

(44) Melting points are corrected and were taken in open capillary tubes.

described above. Two crops of II were recovered (ethyl acetate–cyclohexane); 17.0 g. (77% yield), dec. 131–133°, and 1.2 g. (5% yield), dec. 130–132°.

3,4-Dihydroxymandelonitrile (XI). A solution of 65.1 g. (1.00 mole) of potassium cyanide in 100 ml. of water was added dropwise during 30 min. to a cold (−10°) mixture of 150 ml. of ethyl acetate and 34.5 g. (0.25 mole) of 3,4-dihydroxybenzaldehyde[45] dissolved in 250 ml. of 4*N* sodium bisulfite solution. The mixture was stirred vigorously under nitrogen and the temperature was maintained at −5° to 0° during the addition and for 30 min. thereafter. The crystals which precipitated were dissolved by adding 150 ml. of water, the ethyl acetate layer was separated, and the aqueous phase was extracted with five more 150-ml. portions of ethyl acetate. The combined ethyl acetate extracts were washed with two 25-ml. portions of 4*N* sodium bisulfite solution, dried over anhydrous sodium sulfate, treated with charcoal, and concentrated to dryness at a reduced pressure under nitrogen. The residual 37 g. of oil was dissolved in 200 ml. of anhydrous ether and 100 ml. of cyclohexane was added. The solution was filtered to remove a small amount of dark brown flocculent material, and more cyclohexane (100 ml.) was added to the filtrate until incipient clouding occurred. The solution was seeded with several 5-ml. aliquots which had been scratched vigorously to promote crystallization, and was stored at 5° with occasional fragmentation of the resulting crystal shell until crystallization was completed;[46] 20.3 g. (49% yield) of XI was obtained as cream colored rosettes of blades, m.p. 94–95° (slow dec., sample in bath at 90°, heating rate 2° per min.) (Lit.[20] m.p. 95°), raised to 96–98° (dec.) by recrystallization from ether-cyclohexane, then from ether–1,2-dichloroethane–petroleum ether (b.p. 30–60°) (1:1:2, v/v). The filtrate from the first crop was concentrated to dryness, and the residue was crystallized from ether-cyclohexane to yield a second crop of 8.05 g. (20% yield), m.p. 89–92°. The second crop was dissolved in 50 ml. of ether; the solution was washed with two 5-ml. portions of 4*N* sodium bisulfite solution, dried over anhydrous sodium sulfate, treated with charcoal, and concentrated to dryness at a reduced pressure. The residue was crystallized from a mixture of 50 ml. of ether and 50 ml. of 1,2-dichloroethane by addition of 100 ml. of petroleum ether (b.p. 30–60°); 6.03 g. of additional XI (total yield 64%) was recovered, m.p. 96–98° (dec.).

The aldehyde content of XI was determined by paper chromatography (*vide infra*) since the melting point is not completely reliable as a criterion of purity; crude first crop, 3%; twice recrystallized first crop, 2%; purified second crop, 2%.[47] The crude first crop and purified second crop were used for the next step.

Ethyl 3,4-dihydroxymandelate (XII). Dry hydrogen chloride was passed into a solution of 11.77 g. (0.071 mole) of XI in a mixture of 3.37 g. (0.073 mole) of absolute ethanol and 50 ml. of anhydrous ether at 5° until 2.67 g. (0.073 mole) was absorbed (15 min.). The mixture was stored at 5° for 72 hr. during which time gummy crystals of the iminoester hydrochloride separated. Precautions were taken to exclude atmospheric moisture during these operations. The ether supernatant was discarded and the gummy residue was dried *in vacuo* over potassium hydroxide for 24 hr. to remove ether and excess hydrogen chloride. The iminoester hydrochloride was dissolved in 90 ml. of water and the solution was allowed to stand at room temperature for 1 hr. After addition of 25 g. of sodium chloride, the solution was extracted with five 90-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, treated with charcoal, and concentrated to dryness at a reduced pressure under nitrogen. The solid residue (12.9 g.) was dissolved in 65 ml. of absolute ethanol and 65 ml. of cyclohexane was added to the solution; a further 65 ml. of cyclohexane was added after 24 hr. at 5°. After another day at 5°, 7.16 g. (51% yield) of XII was recovered in two crops, m.p. 151–153°. A sample of XII was recrystallized from ethanol-cyclohexane (1:3, v/v) with charcoal treatment and was recovered (84%) as colorless glittering plates, m.p. 153–154° (Lit.[23] m.p. 152–153°).

3,4-Dihydroxymandelic acid (IV). A solution of 5.00 g. (0.0236 mole) of XII in 100 ml. of 2*N* sodium hydroxide under nitrogen was stirred for 4 hr. at room temperature. The solution was acidified to *p*H 1.0 with hydrochloric acid, 25 g. of sodium chloride was added, and the solution was extracted with four 200-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, treated with charcoal and concentrated to dryness at a reduced pressure under nitrogen. The solid residue was crystallized from a mixture of 100 ml. of ethyl acetate and 100 ml. of cyclohexane; 3.67 g. (85% yield) of IV was obtained, dec. 136° (sample in bath at 134°, heating rate 2° per minute). IV was recrystallized from ethyl acetate–cyclohexane with charcoal treatment, and was recovered (91%) as colorless needles, dec. 137°.

Anal.[48] Calcd. for $C_8H_8O_5$: C, 52.18; H, 4.38. Found: C, 51.46; H, 4.35.

Paper chromatography of mandelic derivatives: stability of mandelonitriles. Ascending chromatography was carried out on Whatman No. 1 paper with 10 γ amounts of each compound applied as a standard solution in ethyl acetate. Procedures and precautions described earlier for paper chromatography of phenolic acids[2] were followed. The resulting R_f values in various solvent systems and the qualitative color reactions with several spray reagents are recorded in Table I.

The aldehyde content of a nitrile under various conditions was determined by chromatographing 1 to 6 γ of aldehyde in 1-γ increments together with a series of nitrile spots containing similar amounts of aldehyde with benzene–propionic acid–water, 100:70:5 (BP), as the solvent. The areas of the spots were compared visually after spraying with the reagents listed in Table I. The accuracy is ±15%.

The nitriles VIII and XI decomposed quantitatively to the aldehydes VII and X respectively during chromatography in isopropyl alcohol–aqueous ammonia–water, 8:1:1 (IA), or in 20% (w/w) aqueous potassium chloride solution. The aldehydes were identified on the basis of their characteristic color reactions and R_f values. For further identification, the nitriles (20-γ spots) were chromatographed in the IA solvent, spots of the respective aldehydes were applied to the dried sheets, and then chromatography was carried out in a second dimension[2] with the BP solvent; the R_f's of the spots arising from decomposition of the nitriles were identical with those of the respective aldehydes in the second dimension. Two dimensional chromatography could not be used to follow decomposition of the nitriles on potassium chloride chromatograms because of adverse effects of high salt concentration upon chromatography in the second dimension. In this case, 0.50-g. samples of each nitrile dissolved in 50 ml. of 20% aqueous potassium chloride solution were allowed to stand at room temperature for four hours under nitrogen. Similar samples were prepared in water alone. The KCl solutions were extracted thoroughly with ethyl acetate, the combined extracts were dried over anhydrous sodium sulfate, and appropriate aliquots were chromatographed with authentic aldehyde spots in the BP solvent. The aqueous solutions of the nitriles were chromatographed directly in the same manner. The R_f's of the spots from decomposition of the

(45) A solution of commercial material (Aldrich Chemical Co.) in hot ethyl acetate was treated with charcoal and concentrated to a tan colored residue, m.p. 152–154°.

(46) Crystallization may require many days unless the solution is seeded heavily and the crystal shell is fragmented frequently.

(47) These are maximum values, since part of the aldehyde may have formed during chromatography.

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

nitriles again were identical with those of the respective aldehydes; under these circumstances, however, decomposition of the nitriles to the aldehydes proceeded to an extent of only about 20% in 20% potassium chloride solution, but increased to about 40% in water.

2-Methyl-4-[3'-methoxy-4'-acetoxybenzal]-5-oxazolone (VI). To a mixture of 152.1 g. (1.0 mole) of vanillin (U.S.P. grade), 117.1 g. (1.0 mole) of acetylglycine,[49] and 82.1 g. (1.0 mole) of anhydrous sodium acetate was added 300 ml. (3.0 mole) of boiling acetic anhydride. The mixture was maintained at 100° for seven hours and then allowed to stand overnight at room temperature. The resulting crystal mass was fragmented and stirred vigorously for 30 min. with 300 g. of ice and 300 ml. of ice water. The mixture was filtered, and the filter cake was washed twice with cold 33% aqueous acetic acid to remove dark tars, and twice with cold water. After drying *in vacuo* over concentrated sulfuric acid and potassium hydroxide, 207.0 g. of crude VI was obtained as a mustard colored powder, m.p. 141–150°. VI was recrystallized from 700 ml. of boiling benzene with charcoal treatment; 189.7 g. (69% yield) of dark yellow powder was recovered in three crops, m.p. 144–148°. This material was used for subsequent syntheses. For analysis, a sample was recrystallized twice more from benzene; VI was obtained as glittering yellow crystals, m.p. 149–151°.

Anal. Calcd. for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76; N, 5.08. Found: C, 61.20; H, 4.87; N, 5.02.

3-Methoxy-4-hydroxyphenylalanine (3-methoxytyrosine) (V). A suspension of 27.53 g. (0.10 mole) of VI, m.p. 144–148°, in 120 ml. of 2.5*N* sodium hydroxide was heated to the boiling point under nitrogen. The resulting solution was cooled to room temperature; 306.4 g. (0.40 g.-atom) of freshly pulverized 3% sodium amalgam[50] was added in ten equal portions at five-minute intervals with high speed stirring under nitrogen, and stirring was continued for two hours more. The aqueous supernatant was removed and the amalgam was washed with water. The combined aqueous solutions were acidified to *p*H 1.5 with hydrochloric acid and extracted with six 300-ml. portions of ethyl acetate. The combined ethyl acetate solutions were dried over anhydrous sodium sulfate, treated with charcoal and concentrated to dryness at a reduced pressure to give 23.7 g. of yellow gummy *N*-acetyl-3-methoxytyrosine. This gum was refluxed for 18 hr. with 400 ml. of 1*N* hydrochloric acid; the solution was then concentrated to dryness at a reduced pressure. The crystalline residue was dissolved in 100 ml. of boiling water; the solution was treated with charcoal and filtered. The filtrate was adjusted to *p*H 5.5 by careful addition of ammonium hydroxide. Following refrigeration, the product was collected and dried *in vacuo* over phosphorus pentoxide and potassium hydroxide; 15.93 g. (76% yield) of anhydrous V were recovered, dec. 267° (sample in bath at 265°, heating rate 2° per minute) (Lit.[32] m.p. 257°). The air-dried compound was hydrated and lost weight slowly upon drying *in vacuo*. Crude V was recrystallized from boiling water (83% recovery); a minor amount of infusible impurity was removed by filtration and the decomposition point was raised 1°.

Anal. Calcd. for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.76; H, 6.31; N, 6.80.

3-Methoxy-4-hydroxyphenylacetic (homovanillic) acid (I). A suspension of 17.55 g. (0.064 mole) of VI, m.p. 144–148°, in 130 ml. of 5*N* sodium hydroxide was refluxed under nitrogen for 22 hr. The solution was cooled to 3° and 100 ml. of 10% hydrogen peroxide was added dropwise during 20 min. with stirring and continued cooling. The mixture was cooled for 2 hr. more and then allowed to stand for 24 hr. at room temperature. The solution was acidified to *p*H 1.2 with hydrochloric acid and extracted with six 200-ml. volumes of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, treated with charcoal, and concentrated to dryness at a reduced pressure. The oily residue was crystallized from 40 ml. of boiling water with charcoal treatment to yield 2.67 g. of brown crystals in two crops, m.p. 138–143°. The crude product was recrystallized from 1,2-dichloroethane with charcoal treatment to give 1.96 g. (17% yield) of I as buff colored plates, m.p. 142–143° (Lit.[37] m.p. 143°).

Anal. Calcd. for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 58.94; H, 5.63.

3-Methoxy-4-hydroxyphenylpyruvic acid (XIII). A suspension of 13.7 g. (0.05 mole) of VI, m.p. 144–148°, in 100 ml. of 10*N* sodium hydroxide was refluxed under nitrogen for 6 hr. The solution was cooled to 5°, diluted to 400 ml. with water, acidified to *p*H 7.0 with hydrochloric acid, filtered to remove a small amount of infusible precipitate, and extracted with four 100-ml. portions of ether to remove neutral by-products. The aqueous phase was boiled briefly to expel ether, cooled, acidified to *p*H 1.8 with hydrochloric acid and extracted with six 200-ml. portions of ether. The *p*H 1.8 extracts were dried over anhydrous sodium sulfate, treated with charcoal, and concentrated at a reduced pressure to dryness. The resulting 4 g. of brown solid was dissolved in 20 ml. of 1*N* sodium bicarbonate solution. The solution was heated to the boiling point, treated with charcoal, and acidified to *p*H 1.6 with hydrochloric acid. Following refrigeration, 1.40 g. (13% yield) of XIII was recovered in two crops as tan colored microcrystals, m.p. 159–161° (dec.) (Lit.[34] m.p. 161°). A darkening of color and tar formation when aqueous solutions of XIII were heated indicated partial decomposition.

3,4-Dihydroxyphenylacetic (homoprotocatechuic) acid (III). A suspension of 6.20 g. (0.20 g.-atom) of red phosphorus (Merck) in 100 ml. of 55–58% hydriodic acid (Merck, reagent grade, sp. gr. 1.7) was refluxed until the brown color of free iodine had vanished (about 15 min.). The suspension was cooled and 19.62 g. (0.10 mole) of homoveratric acid (Eastman Kodak) was added. The mixture was refluxed under nitrogen for three hours; methyl iodide was evolved rapidly at first, but was no longer evident in the condenser after 30 min. The mixture was cooled to room temperature, and concentrated to dryness at a reduced pressure under nitrogen; the residue was again concentrated to dryness with three successive 150-ml. portions of water to remove most of the hydriodic acid. The residue was taken up in 100 ml. of boiling water and the phosphorus was removed by filtration. The filtrate was saturated with sodium chloride and extracted with four 75-ml. portions of ethyl acetate. The combined ethyl acetate extracts were washed with 25 ml. of 4*N* sodium bisulfite. The bisulfite solution and the extracted aqueous phase were then combined and extracted with another 75 ml. of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, treated with charcoal, and concentrated to dryness at a reduced pressure. The solid residue was crystallized from ethyl acetate–cyclohexane (1:1) to yield 15.83 g. (94% yield) of III in two crops of colorless crystals, m.p. 130–131°. A sample was recrystallized from 1,2-dichloroethane containing 7% methanol (v/v) and was recovered as thin plates or dense dendrites, m.p. 131–132° (Lit.[39] m.p. 127–128°).

Anal. Calcd. for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.07; H, 4.84.

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