TABLE I

Compound	Ref.	$[M]_D$[7]	$\Delta(17\beta-17\alpha)$
4-Androsten-17β-ol-3-one	8	+314°(E)	
4-Androsten-17α-ol-3-one	9	+221°(E)	+93°
4-Androstene-3β,17β-diol	10	+140°(E)	
4-Androstene-3β,17α-diol	11	+32°(E)	+108°
4-Estren-17β-ol-3-one	12	+122°(D)	
4-Estren-17α-ol-3-one	Exptl.	+25°(D)	+97°
Etiocholane-3α,17β-diol	13, 14	+73°(E)	
Etiocholane-3α,17α-diol	14	0°(E)	+73°
1,3,5(10)-Estratriene-3,17β-diol	15	+215°(E)	
1,3,5(10)-Estratriene-3,17α-diol	11	+146°(E)	+69°
1,3,5(10)-Estratriene-3,17β-diol 3-methyl ether	12	+215°(D)	
1,3,5(10)-Estratriene-3,17α-diol 3-methyl ether	Exptl.	+152°(D)	+63°
2,5(10)-Estradiene-3,17β-diol 3-methyl ether	3	+316°(C)	
2,5(10)-Estradiene-3,17α-diol 3-methyl ether	Exptl.	+286°(D)	+30°
5(10)-Estren-17β-ol-3-one	3	+521°(C)	
5(10)-Estren-17α-ol-3-one	Exptl.	+450°(D)	+71°

in methanol (150 ml.) and water (30 ml.) containing potassium hydroxide (18 g.) was added dimethyl sulfate (3 ml.). Three additional 3-ml. portions of dimethyl sulfate were added at 30-min. intervals to the stirred reaction mixture. Stirring was continued for 1 hr. after the last addition (total reaction time 2.5 hr.), and the reaction mixture was then concentrated *in vacuo* to about 50 ml. Water was added and the mixture was filtered. The dried residue was chromatographed on Florisil, when elution with hexane-ether (9:1) furnished the 3-methyl ether (II, 1.91 g.), m.p. 112–114° (from aqueous methanol), $[\alpha]_D$ +53°, $\lambda_{max}^{CH_3OH}$ 268 mμ (2,400) and 287 mμ (2,200), λ_{max}^{Nujol} 2.96 μ.

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.47; H, 9.25. (lit.[2] m.p. 109–110°).

2,5(10)-Estradien-3,17α-diol 3-methyl ether (III). To a stirred solution of 1,3,5(10)-estratriene-3,17α-diol 3-methyl ether (II, 1.0 g.) in ether (100 ml.) and liquid ammonia (100 ml.) was added lithium metal (1.0 g.) in small pieces. To the resulting blue solution was added ethanol (32 ml.) dropwise, with stirring, over 50 min. The decolorized solution was then allowed to evaporate, and the residue was treated with water and extracted with ether. The ethereal extract was washed with water, dried (sodium sulfate) and evaporated to a solid residue. Crystallization from acetone-hexane furnished the 1,4-dihydro compound (III, 658 mg.) m.p. 108–110°. The analytical sample was obtained by recrystallization from acetone-hexane, and exhibited m.p. 112–114°, $[\alpha]_D$ +99°. It showed no selective ultraviolet absorption between 220 and 350 mμ (ϵ_{280} 60); λ_{max}^{Nujol} 3.0, 6.18, 6.3 μ.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 79.20; H, 10.18.

5(10)-Estren-17α-ol-3-one (IV). A solution of 2,5(10)-estradiene-3,17α-diol 3-methyl ether (III, 200 mg.) in methanol (16 ml.) and water (3.44 ml.) containing oxalic acid (264 mg.) was left at room temperature for 40 min. The reaction mixture was then poured into water and filtered. The dried residue was crystallized from ether-hexane, affording the β,γ-unsaturated ketone (IV, 95 mg.), m.p. 144–150°, $[\alpha]_D$ +164°, no selective ultraviolet absorption between 220 and 350 mμ, λ_{max}^{Nujol} 2.96, 5.88 μ.

Anal. Calcd. for $C_{18}H_{26}O_2$: C, 78.79; H, 9.55. Found: C, 78.27; H, 9.82.

4-Estren-17α-ol-3-one (V). A solution of 2,5(10)-estradiene-3,17α-diol 3-methyl ether (III, 200 mg.) in methanol (72 ml.), water (8 ml.) and concd. hydrochloric acid (0.5 ml.) was heated to reflux for 20 min. The solution was then evaporated, *in vacuo*, to low volume, and was diluted with water. The aqueous mixture was extracted with ether and the extract was washed with water and dried (sodium sulfate). Evaporation of the ether gave an oily residue which crystallized on trituration with hexane. Crystallization from acetone-hexane gave the α,β-unsaturated ketone (V, 95 mg.), m.p. 146–149°, $[\alpha]_D$ +9°, λ_{max} 240 mμ (16,900), λ_{max}^{Nujol} 2.96, 6.00, 6.20 μ.

Anal. Calcd. $C_{18}H_{26}O_2$: C, 78.79; H, 9.55. Found: C, 78.38; H, 9.09.

On one occasion, V was obtained in a different crystalline form,[17] m.p. 112–115°, λ_{max} 240 mμ (16,800). A mixture of this low melting material and the material of m.p. 146–149° showed m.p. 146–149°.

4-Estrene-3,17-dione (VI) *from 4-estren-17α-ol-3-one* (V). To a cooled (5°) solution of 4-estren-17α-ol-3-one (V, 50 mg.) in acetone (5 ml.) was added dropwise, with swirling, chromium trioxide–sulfuric acid reagent[4] until a permanent orange color was observed. The reaction mixture was left at room temperature for 5 min., and was then treated with methanol (0.1 ml.) and was diluted with water. The aqueous mixture was extracted with ether, and the extract was washed with water, dried (sodium sulfate) and evaporated *in vacuo* to a solid residue. Crystallization from acetone-hexane yielded 4-estrene-3,17-dione (VI, 33 mg.) m.p. 168–171°, $[\alpha]_D$ +140° ($CHCl_3$), $\lambda_{max}^{CH_3OH}$ 239 mμ (16,900), λ_{max}^{Nujol} 5.74, 5.98 μ; [lit.[3] m.p. 170–171°, $[\alpha]_D$ +137° ($CHCl_3$), λ max. 239 mμ (16,900)].

NATURAL PRODUCTS RESEARCH DEPARTMENT
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(16) Melting points were obtained on the Kofler block. Rotations were measured in dioxan solution at about 1% concentration. We are indebted to the Physical Chemistry Department, Schering Corp., for measurement of ultraviolet and infrared spectra, and of rotations. Microanalyses were carried out by Mr. E. Conner (Microanalytical Department, Schering Corp.).

(17) Wilds and Nelson (Ref. 3) observed two polymorphic forms of 19-nortestosterone.

The Preparation of Epinephrine and Norepinephrine Metabolites, 3-Methoxy-4-hydroxymandelic Acid and 3,4-Dihydroxymandelic Acid

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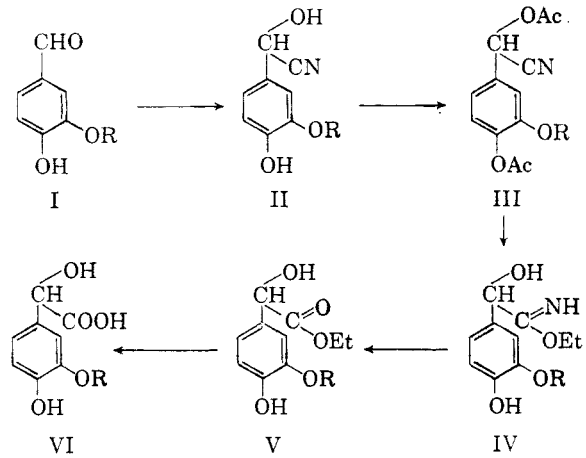
The preparation of 3,4-dihydroxy and 3-methoxy-4-hydroxymandelic acids, major metabolites of epinephrine and norepinephrine has been described recently by Shaw, *et al.*[1] Although this synthesis was repeated successfully in our laboratories, it was impractical for larger runs because of the

(1) K. N. F. Shaw, A. McMillan, and M. D. Armstrong, *J. Org. Chem.* **23**, 27 (1958).

rapid breakdown of the cyanohydrins II (R = H or CH_3) during their isolation. The present paper reports a modification of Shaw's procedure suitable for the large scale preparation of the above epinephrine metabolites. The crucial step, which is of general applicability, involves the stabilization of the cyanohydrins II (R = H or CH_3) by acetylation, which permits facile isolation and purification of these intermediates in excellent yields (85–90%). The imino esters IV (R = H or CH_3) were formed by treatment of the acetylated cyanohydrins III (R = Ac or CH_3) with alcoholic hydrogen chloride which simultaneously effected alcoholysis of the acetyl groups. Acid hydrolysis afforded the esters V (R = H or CH_3) and subsequent treatment with alkali the acids VI (R = H or CH_3). 3-Methoxy-4-hydroxymandelic acid was prepared directly from III (R = CH_3) without isolation of IV (R = CH_3) and V (R = CH_3) in 46% yield. In the preparation of 3,4-dihydroxymandelic acid VI (R = H) it was advantageous to isolate and purify the ester V (R = H) prior to alkaline hydrolysis. The yield of ester was 52%. 3-Methoxy-4-hydroxymandelic acid VI (R = CH_3) forms a beautiful crystalline cyclohexylammonium salt and VI (R = H) a dicyclohexylammonium salt. Both are far less susceptible to oxidation than the corresponding free acids and may thus be used for the purification of the latter. The chromatography of the two epinephrine metabolites is described in the Experimental part.



WHERE R=H, CH_3 or Ac

EXPERIMENTAL

α-Acetoxy(3-methoxy-4-acetoxyphenyl)acetonitrile, III (R = CH_3). A solution of 910 g. (14 moles) of potassium cyanide in 1400 ml. of water was added from a dropping funnel with vigorous stirring during 2 hr., to a cold (−10°) solution of 532 g. (3.5 moles) of vanillin in 5 l. of water containing 1330 g. of sodium metabisulfite. The temperature of the reaction mixture was kept below 10° during the addition and stirring continued for 30 min. thereafter. The reaction mixture was then diluted with 1.75 l. of water and extracted nine times with 1.0 l. portions of ether. The combined ether extracts were chilled, dried over anhydrous sodium sulfate, and filtered at 5°. A mixture of 350 ml. of dry pyridine and 875 ml. of acetic anhydride was now added slowly to the ether solution with external cooling. The rate of addition was regulated so as to keep the temperature of the reaction mixture between 5° and 10°. After standing at room temperature overnight, the solvents and excess reagents were distilled at reduced pressure. The residual white, crystalline product was then triturated with ice water, filtered, washed with cold water until free of pyridine and acetic anhydride, and recrystallized from 2 l. of isopropanol; yield, 750 g. (82%), m.p. 92–94°.

Anal. Calcd. for $C_{13}H_{13}NO_5$: N, 5.32. Found: N, 5.30.

3-Methoxy-4-hydroxymandelic acid, VI (*R* = CH_3). α-Acetoxy(3-methoxy-4-acetoxyphenyl)acetonitrile, III (R = CH_3), 270 g., was suspended in 1.0 l. of absolute ethanol containing approximately 20% by weight of hydrogen chloride and allowed to stand at room temperature under nitrogen for 2 hr. with frequent shaking. The solvent and excess hydrogen chloride were then removed by distillation under reduced pressure. The oily residue was dissolved in 2.0 l. of water and stirred for 1.5 hr. under nitrogen. The solution was then cooled in an ice bath and treated with 160 g. of solid sodium hydroxide with agitation by a current of nitrogen. After standing at room temperature under nitrogen for 2 hr., the reaction mixture was acidified to *p*H 1 with 6*N* hydrochloric acid, saturated with sodium chloride, and extracted six times with 1.5 l. portions of ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated to 300 ml. under reduced pressure. Cyclohexane, 300 ml., was then added and the mixture refrigerated overnight. The crystalline product was filtered. The filtrate, when diluted with 300 ml. of cyclohexane and refrigerated, furnished a second crop of product which was washed with ethyl acetate to remove some oily impurity; total yield, 93 g. (45%). Recrystallization from approximately 250 ml. of acetonitrile gave 75 g. of 3-methoxy-4-hydroxymandelic acid, m.p. 131–133°, (reported by Shaw, *et al. loc. cit.*, m.p. 133° and by Gardner and Hibert,[2] m.p. 133°).

Anal. Calcd. for $C_9H_{10}O_5$: C, 54.54; H, 5.08. Found: C, 54.59; H, 5.14.

Cyclohexylammonium salt. 3-Methoxy-4-hydroxymandelic acid, 33 g., was added to a hot solution of 15 ml. cyclohexylamine and 1.0 l. of isopropyl alcohol. On cooling, a colorless product was obtained. Recrystallization from isopropyl alcohol gave pure cyclohexylammonium salt of 3-methoxy-4-hydroxymandelic acid, m.p. 157–159°.

Anal. Calcd. for $C_{15}H_{23}O_5N$: C, 60.80; H, 7.43; N, 4.71. Found: C, 60.78; H, 7.39; N, 4.65. The free acid may be regenerated by passing an aqueous solution of the cyclohexylammonium salt through a column of IR-120 resin.

α-Acetoxy(3,4-diacetoxyphenyl)acetonitrile, III (*R* = *Ac*). This intermediate was prepared from 300 g. of 3,4-dihydroxybenzaldehyde as described for III (R = CH_3). The yield of crude product was 590 g. (93%). Recrystallization from 1.2 l. of ethanol gave 500 g. of pure III (R = Ac), m.p. 110–112°.

Anal. Calcd. for $C_{14}H_{13}NO_6$: N, 4.81. Found: N, 4.78.

Ethyl 3,4-dihydroxymandelate, V, (*R* = *H*). α-Acetoxy-(3,4-diacetoxyphenyl)acetonitrile III (R = Ac), 238 g., was suspended in 1 l. of ethanol containing approximately 20% hydrogen chloride and allowed to stand with occasional shaking until one phase was obtained (about 1 hr.). The solution was then evaporated under reduced pressure, the oily residue dissolved in 1 l. of water and allowed to stand at room temperature under a nitrogen atmosphere for 1 hr. The solution was saturated with sodium chloride and extracted four times with 1750 ml. portions of ethyl acetate. The pooled extracts were dried over anhydrous sodium sulfate, treated with Norit, and filtered. The filtrate was concentrated to approximately 1 l. under reduced pressure

(2) J. A. F. Gardner and H. Hibert, *J. Am. Chem. Soc.*, 66, 607 (1944).

and the solid which separated was filtered. The filtrate was evaporated to dryness to give an oily residue which on triturating with a little ethyl acetate solidified. The solid was filtered and recrystallized from acetonitrile; total yield of ethyl dihydroxymandelate, 100 g. Recrystallization from acetonitrile gave 90 g. (52%) of pure product, m.p. 147–149°, (reported by Shaw, *et. al. loc. cit.*, m.p. 153–154° and by Barger and Ewins,[3] m.p. 152–153°).

3,4-Dihydroxymandelic acid, VI (*R* = *H*). Ethyl 3,4-dihydroxymandelate V (R = H), 80 g., was hydrolyzed as described for ethyl 3-methoxy-4-hydroxymandelate. The product was recrystallized from 100 ml. of acetonitrile to give 57 g. (81%) of pure dihydroxymandelic acid, m.p. 145–146° (reported by Shaw, *et al. loc. cit.*, m.p. 137°).

Anal. Calcd. for $C_8H_8O_5$: C, 52.18; H, 4.38. Found: C, 52.15; H, 4.45. The dicyclohexylammonium salt was prepared as described above and was recrystallized from isopropyl alcohol, m.p. 209–211°.

Anal. Calcd. for $C_{20}H_{31}O_5N$: C, 65.71; H, 8.55; N, 3.83. Found: C, 65.47; H, 8.19; N. 3.84.

Chromatography. Small scale chromatograms[4] on Schleicher and Schuell (609) paper were developed with three different solvent systems: (a) 1-butanol, acetic acid, water (8:2:2), (b) isopropyl alcohol, concd. ammonia, water (8:1:1), (c) methylbutynol, 2*N* ammonia (7:3), and furnished single spots which were visualized with diazotized sulfanilic acid. The Rf values for 3-methoxy-4-hydroxymandelic acid in the above solvent systems were: (a) 0.715 (5:1:4), (b) 0.52, (c) 0.42, and for 3,4-dihydroxymandelic acid: (a) 0.51, (b) 0.14, (c) 0.15.

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(3) G. Barger and A. J. Ewins, *J. Chem. Soc.*, 95, 552 (1909).

(4) J. C. Underwood and L. B. Rockland, *Anal. Chem.*, 26, 1553 (1954).

The Presence of Mescaline in *Opuntia cylindrica*

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In 1948 Guillermo Cruz-Sanchez[1] described the pharmacology of *Opuntia cylindrica* and an alkaloid derived therefrom. In two other papers with Dr. Carlos Gutierrez-Noriega[2,3] were reported the effects of oral administration of this alkaloid in doses varying from 5 to 11.5 g. per kilo to a total of thirty-four subjects, of whom two developed a brief psychotic state. The method of preparation of the alkaloid, some of its physical and chemical properties, the psychological changes, as well as the dosage of the alkaloid employed, suggested the possibility of the presence of mescaline. However, the material was never adequately purified and the laboratory facilities for satisfactory identification were unavailable. The only other reference to *Opuntia cylindrica* we have been able to find is in a review article by Buscaino.[4]

Through the courtesy of Dr. Vincente Zapata Ortiz of the University of San Marcos, Peru, an alcohol extract was sent us for examination. Further, through the courtesy of Dr. Leoncio Zapata, a physician on the staff of this hospital, we received several kilograms of the whole dried plant of *Opuntia cylindrica*. We have been able to identify the alkaloid present as mescaline, present in concentration of 0.9% of the whole dried plant. There is no more than a slight trace of additional alkaloids.

EXPERIMENTAL

Isolation and characterization of Mescaline. Three hundred grams of the powdered dried *Opuntia* was moistened with a mixture of methanol-aqueous ammonia (20:1). The mass was transferred to a glass chromatography column which was set up for continuous Soxhlet extraction with chloroform. After 24 hr. of extraction the residue left after evaporation of 10 ml. of final chloroform extract did not give a filter paper spot with ninhydrin. The extract was partly evaporated in a stream of air at room temperature, treated with an excess of 5% acetic acid, and extracted with water. The aqueous solution was extracted with benzene, which took up most of the lipids. This was brought to *p*H 7–7.6. Benzene extraction yielded a trace of material giving a ninhydrin test, but this could not be isolated. The *p*H of aqueous solution was raised to 10 with sodium hydroxide. Benzene extraction now resulted in quantitative transferral of the ninhydrin-positive material to the benzene. Benzene was washed twice with distilled water, evaporated to dryness in a stream of warm air, and finally over phosphorus pentoxide. Exactly 2 g. of one extraction at this point dissolved in 10 ml. of 95% ethanol and was titrated to *p*H 3.0 with 6.19 ml. of 1*N* sulfuric acid, brought to dryness, and the residue extracted with benzene. The white, semicrystalline residue weighed 1.31 g. which, on the basis of the titration in sulfuric acid, corresponds to a molecular weight of 211 for the raw base. The sulfate was recrystallized three times from water-ethanol and the melting point compared with authentic mescaline sulfate in a Fisher-Johns melting point apparatus. The crystals of the *Opuntia* sulfate melted sharply at 184° simultaneously with mescaline sulfate. The crystals of *Opuntia* and of mescaline sulfates were mixed and recrystallized in alcohol; they melted sharply at 184°. The chromatogram on Whatman No. 1 filter paper of authentic mescaline sulfate, *Opuntia* sulfate, and a mixture of the two gave a single ninhydrin-positive spot, Rf 0.48, when developed by ascending chromatography with butanol, acetic acid, water (4:1:1). The picrates of *Opuntia* and mescaline were prepared. After three recrystallizations they melted sharply and simultaneously on the cover of the slip at 224°. The maximum yield of crude base was 0.9%. The filtrates recovered after the recrystallizations of the mescaline were further worked up. Finally a crystalline, very bitter material was obtained in very small yield, which was chromatographed and compared with mescaline. When

(1) G. Cruz-Sanchez, *Revista de Farmacologia y Medicina Experimental (Lima)*, 1, 253 (1948).

(2) C. Gutierrez-Noriega and G. Cruz-Sanchez, *Revista de Neuro-Psiquiatria*, 10, 422 (1947).

(3) C. Gutierrez-Noriega and G. Cruz-Sanchez, *Revista de Neuro-Psiquiatria*, 11, 155 (1948).

(4) V. Buscaino, *Gazz. Sanit.*, 20, 417 (1949).