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Chemical and Enzymatic Routes to Methoxydopamines

BY JOHN DALY, LEOPOLD HORNER AND BERNHARD WITKOP

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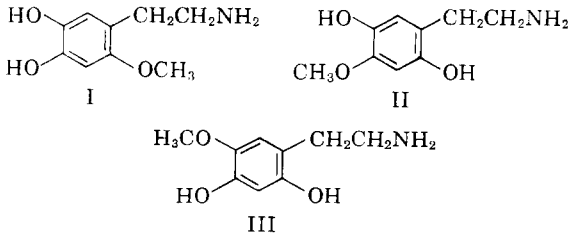
Synthetic routes to 2-methoxy-4,5-dihydroxy- (I) and 2,5-dihydroxy-4-methoxyphenethylamines have been developed. The methoxyhydroquinone amine II was identical with the product resulting from acid-catalyzed 1,2-addition of methanol to N-acyldopamine-*o*-quinones *via* allylic rearrangement. Enzymatic studies with purified catechol-O-methyltransferase confirmed these assignments. The methoxyhydroquinone II was not a substrate for this enzyme, the methoxycatecholamine I gave a new O-methylation product and the new dopamine metabolite, 2,4,5-trihydroxyphenethylamine (XXXII) yielded the missing third O-methyl isomer in this series, namely, III, which has metabolic significance.

In previous papers the formation of 2,4,5-trihydroxyphenethylamine from dopamine *in vivo* and under (aut)oxidation conditions and the 1,4 or 1,6-addition of water or methanol to the quinones of N - carbobenzyloxy- or N - benzoyldopamine (XIX) have been reported.[1,2] Renewed interest has been generated in the addition of methanol to N-carbobenzyloxy- and N-benzoyldopamine[2] by the recent publication of Horner and Göwecke[3] in which the addition of methanol to a number of catechols or *o*-quinones in the presence of acid and tetrachloro-*o*-quinone was shown to proceed by the initial *1,2*-addition of methanol, followed by an allylic rearrangement to a 2-methoxyhydroquinone which is oxidized by the tetrachloro-*o*-quinone present to a 2-methoxy-*p*-quinone.

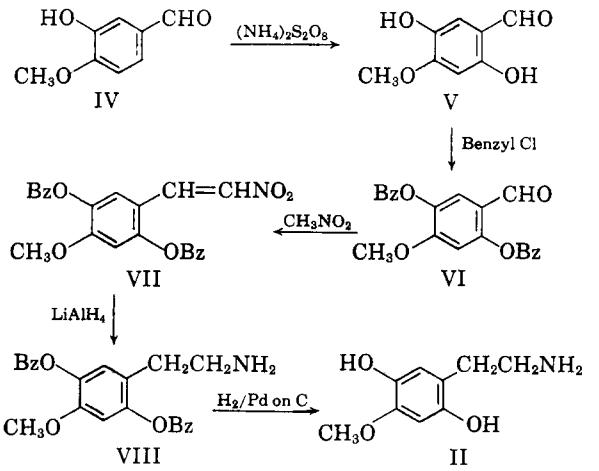
The formation of the methoxydopamine previously[2] was thought to have proceeded *via* 1,4-addition of methanol to yield the 2-methoxy-4,5-dihydroxy derivative.

The 2,4,5-positions of the three oxygen functions had been previously established by complete O-methylation of the free amine and oxidation to asarylic acid. For the methoxydopamine the structure of a 4,5-dihydroxy-2-methoxyphenethylamine (I) resulting from the 1,4-addition of methanol at the time seemed reasonable in analogy to the 1,4-addition of water and was adopted. The alternative 2,5-dihydroxy-4-methoxyphenethylamine (II) would have required the assumption of a 1,2-addition of methanol followed by an allylic rearrangement. No precedent existed for this type of reaction. The remaining third monomethyl ether, 2,4 - dihydroxy - 5 - methoxyphenethylamine (III) which need not be considered in this connection was however, obtained by the action of



catechol-O-methyltransferase on 2,4,5-trihydroxyphenethylamine. The synthesis of the position-isomeric monomethyl ethers I and II established the validity of the principle of 1,2-addition[3] in the dopamine *o*-quinone series.

The synthesis of 2,5-dihydroxy-4-methoxybenzaldehyde (V) has been reported.[4] However, an alternate and simpler route was found which utilized the Elb's oxidation[5] of isovanillin (IV) with ammonium persulfate. Attempts to condense V with nitromethane under a variety of conditions[6-8] were unsuccessful. However, after benzylation of the phenolic groups with benzyl chloride and potassium carbonate suspended in acetone, the resulting 2,5-dibenzyloxy-4-methoxybenzaldehyde (VI) smoothly condensed with nitromethane in the presence of ammonium acetate as catalyst.[8] The resulting 2,5-dibenzyloxy-4-methoxy-ω-nitrostyrene (VII) was reduced to the amine (II) in two steps. The nitrostyrene was first reduced to the free amine (VIII) with lithium aluminum



hydride in ether.[7] The amine without further purification was debenzylated catalytically in acidic (HCl) ethanol with hydrogen and 5% palladium-on-charcoal to yield 2,5-dihydroxy-4-methoxyphenethylamine hydrochloride (II).

The synthesis of the methoxycatecholamine I initially started from the modified Elb's persulfate oxidation[9] of 4-benzyloxy-2-hydroxybenzaldehyde

(1) S. Senoh, B. Witkop, C. R. Creveling and S. Udenfriend, *J. Am. Chem. Soc.*, **81**, 6768 (1959).
(2) S. Senoh and B. Witkop, *ibid.*, **81**, 6222 (1959); **81**, 6231 (1959).
(3) L. Horner and S. Göwecke, *Ber.*, **94**, 1291 (1961).
(4) F. S. H. Head and A. Robertson, *J. Chem. Soc.*, 2434 (1930).
(5) K. Elbs, *J. prakt. Chem.*, **48**, 179 (1893).
(6) F. Benington, R. D. Morin and L. C. Clark, Jr., *J. Org. Chem.*, **20**, 1292 (1955).
(7) F. Ramirez and A. Burger, *J. Am. Chem. Soc.*, **72**, 2781 (1950).
(8) T. I. Crowell and F. Ramirez, *ibid.*, **73**, 2268 (1951).
(9) W. Baker and N. C. Brown, *J. Chem. Soc.*, 2303 (1948).

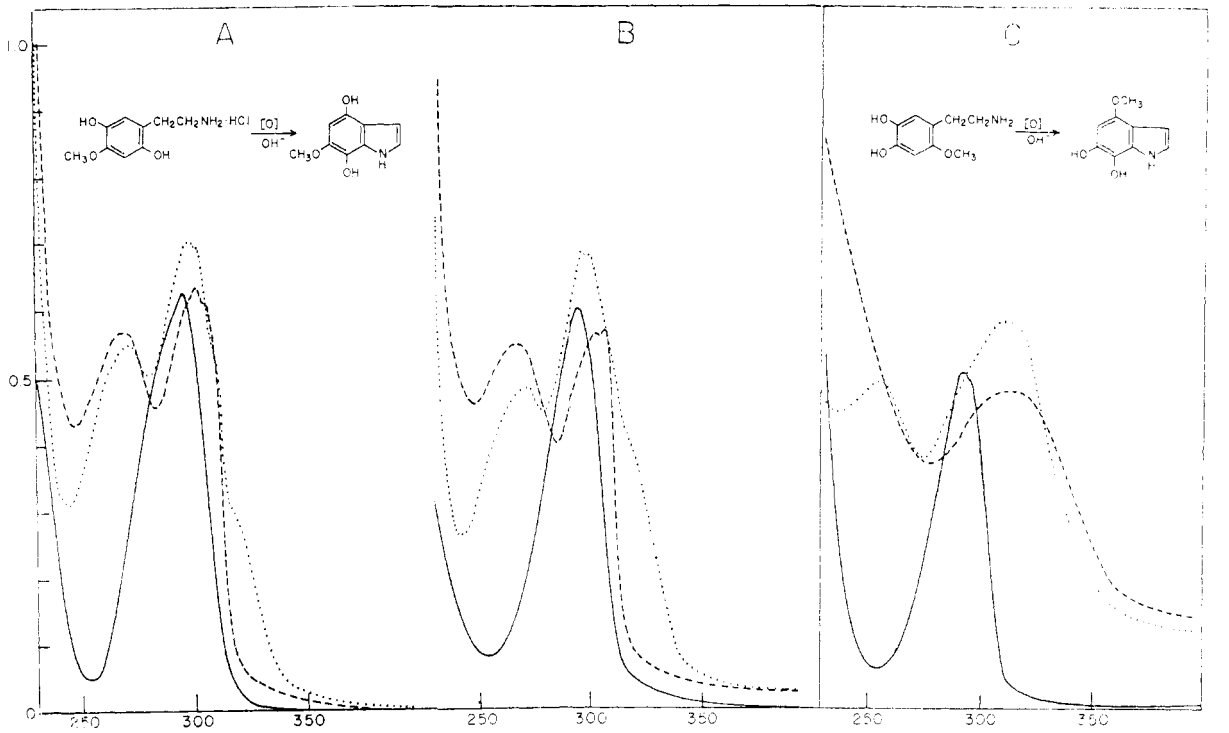
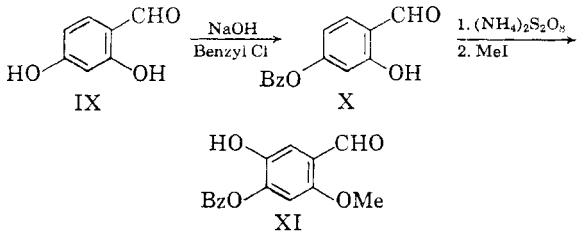


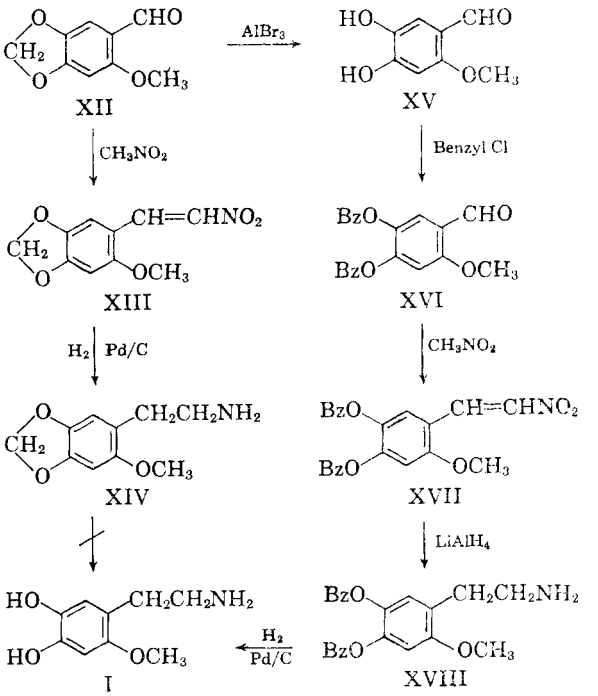
Fig. 1.—Spectral shifts observed with (A) 2,5-dihydroxy-4- methoxyphenethylamine, (B) amine described by Senoh[2] and (C) 4,5-dihydroxy-2-methoxyphenethylamine in (a) neutral alcohol (———), (b) after addition of one drop of 1 *N* NaOH (– – – –) and (c) after reacidification with HCl (. . . .).

(X) obtained by the selective alkylation[10] of 2,4-dihydroxybenzaldehyde (IX). This approach had to be abandoned when repeated efforts gave only



yields of less than 1% of a material analyzing correctly for the desired 4-benzyloxy-5-hydroxy-2-methoxybenzaldehyde (XI).

The successful synthesis of methoxycatecholamine I started with 4,5-methylenedioxy-2-methoxybenzaldehyde (XII)[11] which was condensed with nitromethane to yield the nitrostyrene (XIII). Reduction of XIII with hydrogen and palladium-on-charcoal yielded 4,5-methylenedioxy-2-methoxyphenethylamine hydrochloride (XIV). The conversion of this amine or its crude acetyl derivative to the 4,5-dihydroxy-2-methoxyphenethylamine (I) using sulfuric acid or hydrochloric acid and phloroglucinol[12,13] or aluminum bromide[14] was not successful. The aldehyde (XII), however, smoothly converted to the catechol derivative with aluminum bromide in nitrobenzene.[14] The resulting 4,5-dihydroxy-2-methoxybenzaldehyde (XV) was benzylated to XVI, which was condensed with nitromethane to the nitrostyrene XVII, which was reduced in two steps, first with lithium aluminum hydride, then by catalytic debenzylation of the amine XVIII to yield 4,5-dihydroxy-2-methoxyphenethylamine hydrochloride (I).



(10) F. Tiemann and A. Parrisius, *Ber.*, **13**, 2366 (1880).
(11) K. Campbell, P. Hopper and B. Campbell, *J. Org. Chem.*, **16**, 1736 (1951).
(12) E. Späth, *Ber.*, **60**, 1882 (1927).
(13) E. Späth and E. Mosettig, *ibid.*, **59**, 1496 (1926).
(14) E. Mosettig and A. Burger, *J. Am. Chem. Soc.*, **52**, 2988 (1930).

The properties of the synthetic methoxycatechol and methoxyhydroquinone amines I and II were compared with the dihydroxymethoxyphenethylamine obtained *via* the addition of methanol to the N-carbobenzyloxydopamine-*o*-quinone. The R_f values of the three samples are recorded in Table I and the ultraviolet spectra in neutral ethanol; after basification and after reacidification in Fig. I. The R_f values (Whatman No. 1) and spectral properties of 2,5-dihydroxy-4-methoxyphenethylamine are identical with the properties of the methoxydopamine described by Senoh[2] and their mixed melting points gave no depression. The color reactions, purple with Gibb's reagent, grey with ninhydrin, negative with molybdate and ferric chloride, were also identical and contrasted sharply with those for the methoxycatechol I which gave a brown-purple color with Gibb's reagent, grey with ninhydrin, brown with molybdate and transient green with ferric chloride. In addition the methoxyhydroquinone II gave the same dinitrophenyl- derivative as the amine described by Senoh.[2] The melting point, m.m.p. and R_f values by thin layer chromatography on Merck silica gel G (ethyl acetate)[15,16] were identical. The melting point and R_f values of the dinitrophenyl derivative from the methoxycatecholamine I were different (see Experimental).

The structure of the amine obtained by the addition of methanol to derivatives of dopaminequinone is thus established to be the methoxyhydroquinone II. This makes necessary the reinterpretation and revision of some of the subsequent reactions of the amine.[2]

TABLE I

R_f VALUES OF CATECHOLAMINES IN VARIOUS SOLVENT SYSTEMS

Substance	Solvent systems 1[a]	2[b]	3[c]	4[d]
2,4,5-Trihydroxyphenethylamine XXXII	0.56	0.47	0.17	0.08
Enzymatic methylation product of XXXII	.63	.49	.32	.16
4,5-Dihydroxy-2-methoxyphenethylamine I	.68	.50	.31	.15
Enzymatic methylation product of I	.77	.86	.48	.24
2,5-Dihydroxy-4-methoxyphenethylamine II	.76	.54	.33	.18
Amine described by Senoh[2]	.77	.54	.34	.18

[a] Methanol - 1 - butanol – benzene – water (2:1:1:1). [b] Methyl ethyl ketone–propionic acid–water (15.5:6). [c] *sec*-Butyl alcohol–formic acid–water (75:15:10). [d] 1-Butanol–acetic acid–water (4:1:1).

Chart I presents the revised sequence of transformations of N-acyldopaminequinones.

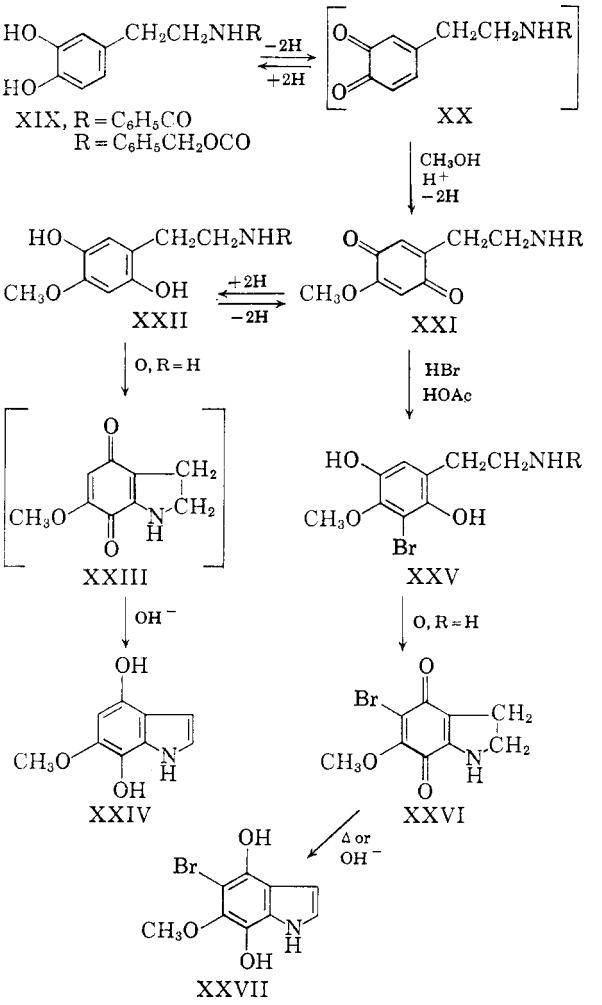
The addition of methanol to N-carbobenzoxy or N-benzoyldopaminequinone (XX) yields, *via* XXII, the yellow 4-methoxy-*p*-quinone XXI.

The benzylic methylene group in the side chain of the *p*-quinone XXI is labilized through the vinylogous quinone carbonyl. This has been proven by the base-catalyzed exchange of tritium atoms in this position.[17]

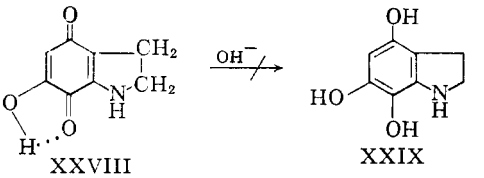
(15) J. G. Kirchner, J. M. Miller and G. J. Keller, *Anal. Chem.*, **23**, 185 (1949).

(16) E. Demole, *Chromat. Review*, **1**, 1 (1959).

CHART I: REVISION OF THE TRANSFORMATIONS OF N-ACYLDOPAMINEQUINONES AND REASSIGNMENTS OF STRUCTURES



The free amine XXII rapidly autoxidizes and cyclizes in base to form the indole XXIV. The intermediate aminochrome XXIII must have a high oxidation-reduction potential providing the driving force for the internal hydrogen shift. This is in contrast to the stability of the hydroxy-*p*-quinoid aminochrome (XXVIII) formed from 2,4,5-trihydroxyphenethylamine (XXXII) in base with oxygen.

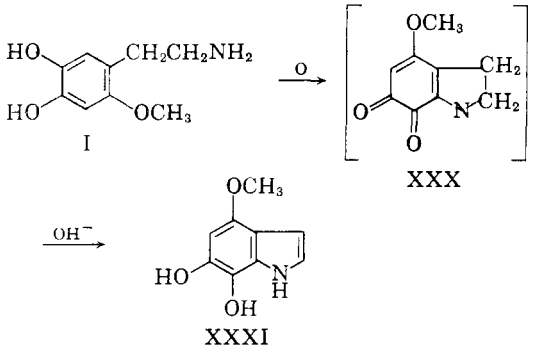


This stability probably results from the hydrogen bonding possible in the vinylogous acid structure XXVIII but not in the vinylogous ester XXIII, which enhances the stability of the hydroxy-*p*-quinoid structure both in the aminochrome XXVIII and in the phenethylamine. This difference in stability is also reflected in the lower half-wave

(17) S. Senoh, C. R. Creveling, S. Udenfriend and B. Witkop, *J. Am. Chem. Soc.*, **81**, 6236 (1959).

potential of 2,4,5-trihydroxyphenethylamine ($E_{1/2}$ *vs.* H_2 electrode + 0.114) as contrasted with 2,5-dihydroxy-4-methoxyphenethylamine ($E_{1/2}$ *vs.* H_2 electrode + 0.194). The half-wave potential for the isomeric 4,5-dihydroxy-2-methoxyphenethylamine (I) was found to be + 0.279. A higher potential and decreased stability are expected of a catechol comparable to dopamine ($E_{1/2}$ + 0.380)[2] and 3,4,5-trihydroxyphenethylamine ($E_{1/2}$ *vs.* H_2 electrode + 0.300).

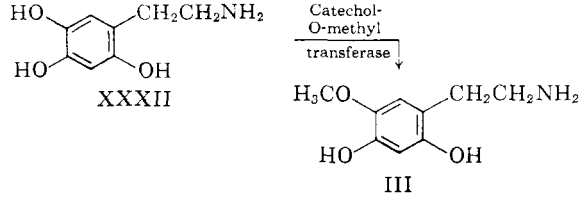
The spectra of I and II are quite similar (Fig. 1) and contrast with the spectrum of 2,4,5-trihydroxyphenethylamine.[2] Autoxidation of 4,5-dihydroxy-2-methoxyphenethylamine (I) in base occurs easily and leads probably to the indole XXXI isomeric with the indole XXIV from the methoxydihydroquinone amine II.



I XXX XXXI

The bromo derivative XXV, formed by the addition of HBr to the *p*-quinone XXI,[2] as the free amine undergoes oxidation and cyclization to the red bromoaminochrome XXVI, which with base or on heating undergoes easy dismutation to the indole XXVII.

Enzymatic studies with partially purified catechol-O-methyltransferase and S-adenosylmethionine[18,19] were carried out with 2,4,5-trihydroxyphenethylamine (XXXII), 2,5-dihydroxy-4-methoxyphenethylamine (II) and 4,5-dihydroxy-2-methoxyphenethylamine (I). The methoxyhydroquinone, lacking the features of a catechol structure, was not a substrate for catechol - O - methyltransferase and was recovered unchanged. A new amine was formed from the trihydroxyamine XXXII, which differed with regard to R_f values (Table I) and color reactions (bright purple with Gibb's reagent, negative with molybdate) from both isomer I and II. In analogy to the enzymatic



XXXII III

in vitro O-methylation of dopamine[20] and (nor)-epinephrine,[21] the *meta*-O-methylation (with regard to the side chain) product from XXXII would be expected to predominate. Thus enzymatic O-methylation made accessible the third isomer, 2,4-dihydroxy-5-methoxyphenethylamine (III).

Enzymatic O-methylation of the methoxycatecholamine I produced a new compound (R_f values Table I, blue color with Gibb's reagent) which apparently is 2,5-dimethoxy-4-hydroxyphenethylamine.

In view of the facile enzymatic O-methylation of 2,4,5-trihydroxyphenethylamine (XXXII) and the importance of this metabolic pathway for catecholamines,[22,23] O-methylation probably represents a major pathway in the metabolism of 2,4,5-trihydroxyphenethylamine (XXXII) which has been shown to be a product formed from dopamine *in vivo*.[1] This expectation has now been verified by *in vivo* studies of dopamine-8-C^{14} in MAO-inhibited (JB-516) rats. The methoxy resorcinol III, in addition to *m*-O-methyldopamine,[18] is a significant metabolite probably identical (same R_F-values) with an unidentified dopamine metabolite.[24]

Experimental[25]

2,5-Dihydroxy-4-methoxybenzaldehyde (V).—Isovanillin (45 g.) was oxidized in 500 ml. of water containing 60 g. of sodium hydroxide by the slow addition of a solution of 70 g. of ammonium persulfate in 500 ml. of water while the reaction mixture was stirred over a period of 3–4 hr. at a temperature of 10–20°. Stirring was continued overnight at room temperature. The reaction mixture was then acidified to *p*H 3–4, filtered and extracted three times with ethyl acetate. Purification of the residue from ethyl acetate extract yielded approximately 10 g. of isovanillin. The aqueous solution was acidified with an excess of hydrochloric acid and heated for 30 minutes on the steam bath. After cooling, the solution was extracted with ether. The dried ether extract on evaporation *in vacuo* left 1.5 g. of 2,5-dihydroxy-4-methoxybenzaldehyde which after recrystallization from ethanol had m.p. 204–206° (lit.[4] m.p. 209°).

Anal. Calcd. for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.06; H, 4.71.

2,5-Dibenzyloxy-4-methoxybenzaldehyde (VI).—A mixture of 1.25 g. of 2,5-dihydroxy-4-methoxybenzaldehyde (V), 3 g. of anhydrous potassium carbonate and 2.0 g. of benzyl chloride in 50 ml. of acetone was stirred and refluxed for 48 hr. The hot solution was filtered and then concentrated *in vacuo*. The residual oil was extracted with boiling petroleum ether (b.p. 60–71°). On cooling, 0.65 g. of 2,5-dibenzyloxy-4-methoxy-benzaldehyde (VI), m.p. 120–123°, was obtained. Recrystallization from cyclohexane raised the m.p. to 123–124°.

Anal. Calcd. for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79. Found: C, 75.37; H, 5.95.

2,5-Dibenzyloxy-4-methoxy-ω-methoxy-ω-nitrostyrene (VII).—A solution of 0.3 g. of 2,5-dibenzyloxy-4-methoxybenzaldehyde (VI) in 5 ml. of nitromethane containing 25 mg. of ammonium acetate was heated for 5 hr. on the steam-bath. The solution was then kept at −5° until crystallization was complete. The crystals were collected by filtration and recrystallized from cyclohexane to yield 100 mg. of 2,5-dibenzyloxy-4-methoxy-ω-nitrostyrene, m.p. 132–134°.

Anal. Calcd. for $C_{23}H_{21}NO_5$: C, 70.57; H, 5.41; N, 3.58. Found: C, 70.88; H, 5.71; N, 3.57.

(18) J. Axelrod, S. Senoh and B. Witkop, *J. Biol. Chem.*, **233**, 697 (1958).

(19) J. Axelrod and R. Tomchick, *ibid.*, **233**, 702 (1958).

(20) S. Senoh, J. Daly, J. Axelrod and B. Witkop, *J. Am. Chem. Soc.*, **81**, 6240 (1959).

(21) J. Daly, J. Axelrod and B. Witkop, *J. Biol. Chem.*, **235**, 1155 (1960).

(22) J. Axelrod, J. K. Inscoe, S. Senoh and B. Witkop, *Biochim. et Biophys. Acta*, **27**, 210 (1958).

(23) M. Goldstein, A. Friedhoff and C. Simmons, *ibid.*, **33**, 572 (1959).

(24) C. M. Williams, A. A. Babuscio and R. Watson, *Am. J. Physiol.*, **199**, 722 (1960).

(25) Melting points are uncorrected. The analyses were performed by Mr. H. G. McCann and associates of the Analytical Services Unit of this Laboratory.

2,5-Dihydroxy-4-methoxyphenethylamine Hydrochloride (II).—To a suspension of 300 mg. of lithium aluminum hydride in 50 ml. of ether was added 500 mg. of 2,5-dibenzyloxy-4-methoxy-ω-nitrostyrene (VII) by soxhlet ether extraction over a period of 48 hr. The excess hydride was decomposed with ice cold 5% sulfuric acid (50 ml.). The *p*H of the strongly acidic solution was adjusted to 8 with dilute sodium hydroxide. Extraction with ethyl acetate, followed by drying of the extract over sodium sulfate and evaporation to dryness *in vacuo* yielded 200 mg. of an oil, 2,5-dibenzyloxy-4-methoxyphenethylamine (VIII) which was dissolved in 50 ml. of ethanol, acidified with hydrochloric acid and reduced with hydrogen and 120 mg. of 5% palladium-on-charcoal at atmospheric pressure. The uptake of hydrogen was 29 ml. (calcd. 27 ml.). After filtration, the solution was concentrated *in vacuo* to dryness, and the residue was recrystallized from ethanol–ether to yield 30 mg. of 2,5-dihydroxy-4-methoxyphenethylamine (II) hydrochloride, m.p. 176–179°.

Anal. Calcd. for $C_9H_{14}NO_3Cl$: Cl, 16.14. Found: Cl, 16.58.

Determination of mixed melting point with a sample of the methoxydihydroxyphenethylamine hydrochloride, m.p. 183–185°, described by Senoh[2] gave a m.m.p. of 178–181°. The compounds had identical R_f values in all systems investigated (Table I), gave the same color reactions, purple with Gibb's reagent,[20] grey with ninhydrin, and *negative with sodium molybdate and ferric chloride*, and exhibited identical ultraviolet spectra in neutral, basic and reacidified ethanol (Fig. I). The amines gave the same 2,4-dinitrophenyl derivative, m.p. 131–135°, when treated with aqueous sodium bicarbonate and ethanolic 2,4-dinitrofluorobenzene under nitrogen. The DNP derivatives were purified by thin layer chromatography[15,16] on Merck silica gel G with ethyl acetate. The R_f values of the DNP derivatives from the amines were identical and differed from that of the DNP derivative of 4,5-dihydroxy-2-methoxyphenethylamine (I) hydrochloride described below. The R_f values were, respectively, 0.84, 0.84 and 0.78.

Anal. Calcd. for $C_{15}H_{15}N_3O_7$: N, 12.03. Found: N, 12.43.

4-Benzyloxy-2-hydroxybenzaldehyde (X).—To the solution of 2.0 g. of 2,4-dihydroxybenzaldehyde and one equivalent of benzyl chloride (1.85 g.) in 75 ml. of ethanol was added one equivalent of sodium hydroxide (0.58 g.) in 20 ml. of water. The stirred solution was refluxed overnight, cooled and filtered. The ethanol was removed *in vacuo* and the residue was crystallized from petroleum ether (b.p. 60–71°) to yield 2.3 g. of 4-benzyloxy-2-hydroxybenzaldehyde (X), m.p. 74–76°.

Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.29; H, 5.87.

The modified Elbs persulfate oxidation[9] was attempted on 4-benzyloxy-2-hydroxybenzaldehyde under a variety of conditions. None was successful although in an oxidation with ammonium persulfate carried out in a mixture of ethanol and water, followed by methylation of the intermediate sulfate, a very small yield (<1%) of an aldehyde was obtained, that analyzed for 4-benzyloxy-5-hydroxy-2-methoxybenzaldehyde (XI), m.p. 149–150°, the desired product.

Anal. Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found: C, 69.71; H, 5.23.

2-Methoxy-4,5-methylenedioxy-ω-nitrostyrene (XVII).—The 2-methoxy-4,5-methylenedioxy-ω-nitrostyrene was prepared from 2-methoxy-4,5-methylene-dioxybenzaldehyde (XII)[11] in the manner described above for the preparation of 2,5-dibenzyloxy-4-methoxy-ω-nitrostyrene. Two grams of aldehyde yielded 1.5 g. of nitrostyrene, m.p. 155–158°. Recrystallization from benzene gave m.p. 158–160°.

Anal. Calcd. for $C_{10}H_9NO_5$: C, 53.81; H, 4.06; N, 6.28. Found: C, 54.02; H, 4.08; N, 6.09.

2-Methoxy-4,5-methylenedioxyphenethylamine (XIV) **Hydrochloride.**—The solution of 0.45 g. of 2-methoxy-4,5-methylenedioxy-ω-nitrostyrene (XIII) in 75 ml. of glacial acetic acid was mixed with 0.2 ml. of concentrated hydrochloric acid and reduced with hydrogen in the presence of 0.25 g. of 5% palladium-on-charcoal at atmospheric pressure. The uptake of hydrogen was 220 ml. (Calcd. 240 ml.). The catalyst was removed by filtration and the solvent by distillation *in vacuo*. The residue was recrystallized from 1-propanol–ethyl acetate to yield 200 mg. of 2-methoxy-4,5-methylene dioxyphenethylamine (XIV) hydrochloride, m.p. 208–210°.

Anal. Calcd. for $C_{10}H_{14}NO_3Cl$: C, 54.18; H, 6.36; N, 6.43; Cl, 15.99. Found: C, 53.66; H, 6.42; N, 6.43; Cl, 15.50.

4,5-Dihydroxy-2-methoxybenzaldehyde (XV).—An ice-cold solution of 1.5 g. of aluminum bromide in 15 ml. of dry nitrobenzene was added to a solution of 0.5 g. of 2-methoxy-4,5-methylenedioxybenzaldehyde (XII) in 100 ml. of nitrobenzene at 0–5°. The red solution was allowed to stand for 7 hr. at room temperature, followed by agitation with 200 ml. of 0.2 *N* hydrochloric acid. The mixture was extracted twice with ether. The ether extracts were dried over sodium sulfate and evaporated *in vacuo*. Addition of an excess of petroleum ether to the nitrobenzene residue caused precipitation of 250 mg. of 4,5-dihydroxy-2-methoxybenzaldehyde (XV), m.p. 178–183°. Recrystallization from ethyl acetate–benzene raised the melting point to 195–196°.

Anal. Calcd. for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 56.88; H, 4.91.

4,5-Dibenzyloxy-2-methoxybenzaldehyde (XVI).—Benzylation of 4,5-dihydroxy-2-methoxybenzaldehyde was carried out as described above for 2,5-dihydroxy-4-methoxybenzaldehyde (V). From 300 mg. of starting material was obtained, after recrystallization from cyclohexane, 210 mg. of 4,5-dibenzyloxy-2-methoxybenzaldehyde (XVI), m.p. 69–70°.

Anal. Calcd. for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79. Found: C, 75.61; H, 6.05.

4,5-Dibenzyloxy-2-methoxy-ω-nitrostyrene (XVII).—The condensation of 4,5-dibenzyloxy-2-methoxybenzaldehyde (XVI) with nitromethane was carried out as described above for 2,5-dibenzyloxy-4-methoxybenzaldehyde (VI). From 180 mg. of aldehyde, 85 mg. of 4,5-dibenzyloxy-2-methoxy-ω-nitrostyrene (XVII), m.p. 136–137°, was obtained after recrystallization from cyclohexane.

Anal. Calcd. for $C_{23}H_{21}NO_5$: C, 70.57; H, 5.41; N, 3.58. Found: C, 70.68; H, 5.51; N, 3.45.

4,5-Dihydroxy-2-methoxyphenethylamine (I) Hydrochloride.—The preparation of 4,5-dihydroxy-2-methoxyphenethylamine (I) hydrochloride was carried out as described above for the isomeric 2,5-dihydroxy-4-methoxyphenethylamine (II) hydrochloride. Reduction of 200 mg. of the nitrostyrene XVII with lithium aluminum hydride yielded 150 mg. of the oily 4,5-dibenzyloxy-2-methoxyphenethylamine XVIII which was reduced catalytically in acidic (HCl) ethanol with 5% palladium-on-charcoal. After recrystallization of the product from ethanol–ether, 26 mg. of 4,5-dihydroxy-2-methoxyphenethylamine (I) hydrochloride, m.p. 202–205° dec. was obtained.

Anal. Calcd. for $C_9H_{14}NO_3Cl$: C, 49.21; H, 6.42; N, 6.38; Cl, 16.14. Found: C, 49.80; H, 6.46; N, 5.87; Cl, 15.97.

Resolution of a mixture of the isomeric amines I and II occurred in at least one solvent system (Table I). The methoxycatecholamine I was also distinguished through its color reactions, brown-purple with Gibb's reagent, grey with ninhydrin, *brown with sodium molybdate* and *transient green with ferric chloride.*

The amine was converted to the 2,4-dinitrophenyl derivative, m.p. 117–119° as described above. Purification was carried out by thin layer chromatography on Merck Silica Gel G with ethyl acetate as a solvent.

Anal. Calcd. for $C_{15}H_{15}N_3O_7$: N, 12.03. Found: N, 12.24.

Polarographic Determination of Half-wave Potentials.[26]—The potentials of the amines were measured in *p*H 6.86 phosphate buffer with respect to a 1 *N* calomel electrode or a saturated calomel electrode at 25°. The values given are the mean of forward and backward runs taken in a self-recording polarograph. The following half-wave potentials (see ref. 2) expressed *versus* the normal hydrogen electrodes were obtained: 2,4,5-trihydroxyphenethylamine (XXXII) hydrobromide, $E_{1/2}$ = 0.114 v.; 2,5-dihydroxy-4-methoxy-

(26) We are greatly indebted to Dr. Prelog and Dr. Engel, Laboratorium für organische Chemie, Eidg. Technische Hochschule, Zürich, for arranging for the determinination of the polarographic half-wave potentials.

phenethylamine (II) hydrochloride, $E_{1/2}$ = 0.194 v.; 4,5-dihydroxy-2-methoxyphenethylamine (I) hydrochloride, $E_{1/2}$ = 0.279 v., and the related 3,4,5-trihydroxyphenethylamine hydrochloride, $E_{1/2}$ = 0.300 v.

Enzymatic O-methylation Studies.—Enzymatic O-methylation of 2,5-dihydroxy-4-methoxyphenethylamine (II), 4,5-dihydroxy-2-methoxyphenethylamine (I) and 2,4,5-trihydroxyphenethylamine (XXXII) was attempted using 2 ml. of an enzyme preparation[20] containing the enzyme catechol-O-methyltransferase, 1 ml. of 0.5 *M* phosphate buffer, *p*H 7.9, 0.05 ml. of 0.5 *M* magnesium chloride, 5 μmole. Before addition of the substrate, the tubes were flushed with nitrogen and the incubation for 2 hrs. at 37° was carried out under nitrogen. Controls were also carried out in which no S-adenosylmethionine was present. After incubation the solutions were acidified with 0.5 ml. of 2 *N* hydrochloric acid and centrifuged at 10,000 rpm in a Servall centrifuge for 10 minutes. The decanted solutions were lyophilized, the residue extracted with 5 ml. of ethanol, the extract concentrated to dryness *in vacuo* and taken up in 0.4 ml. of ethanol. Paper chromatography (Table I) of these extracts showed methylation products for both 4,5-dihydroxy-2-methoxyphenethylamine (I) and 2,4,5-trihydroxyphenethylamine (XXXII) while 2,5-dihydroxy-4-methoxyphenethylamine (II) gave no reaction. The product from 4,5-dihydroxy-2-methoxyphenethylamine (I) gave a blue color with Gibb's reagent. The product from 2,4,5-trihydroxyphenethylamine (XXXII) gave a bright purple color with Gibb's reagent and no color with sodium molybdate.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

The Reaction of α-Chloro-α,α-diphenylacetanilide with Sodium Hydride

BY JOHN C. SHEEHAN AND JOHN W. FRANKENFELD

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The reaction of α-chloro-α,α-diphenylacetanilide with sodium hydride in an inert medium produced three compounds of the oxindole and indoxyl type; no α-lactam was isolated. Two oxindoles (3,3-diphenyloxindole and 1,3-diphenyloxindole) were identified by independent syntheses and the structure of the third product (2,2-diphenylindoxyl) was established by spectral data and by conversion to 3,3-diphenyloxindole by acidic reagents. The formation of oxindoles and indoxyls from α-haloanilides under basic conditions has not been reported previously.

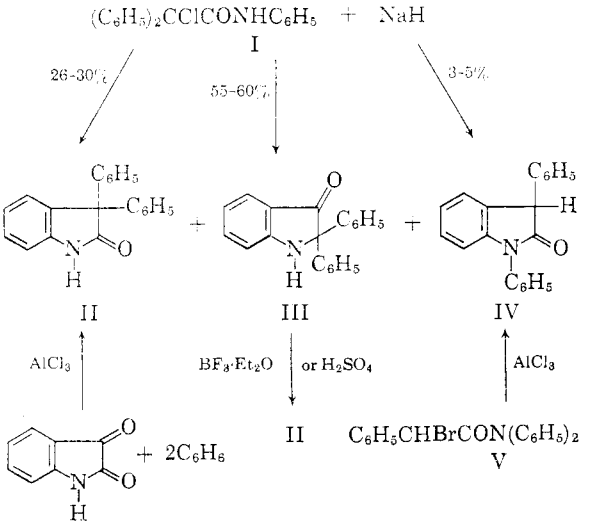
In a recent communication[1] the reaction of two α-haloanilides with sodium hydride was described and the reaction products were formulated as aziridinones (α-lactams). In view of our interest in small ring lactams we undertook to reinvestigate this work using α-chloro-α,α-diphenylacetanilide (I). In our hands the reaction took a different course leading to the formation of products which, although they resembled in physical and spectral properties the compounds reported previously,[1] have been identified as oxindole- and indoxyl-type compounds. No α-lactam was isolated from the reaction mixture. However, the formation of the products obtained may be rationalized by means of an α-lactam intermediate.

When α-chloro-α,α-diphenylacetanilide[2] (I, m.p. 87–88°) was treated with sodium hydride at 40–60° (under an inert atmosphere and with careful exclusion of oxygen) three compounds were obtained. These have been identified as 3,3-diphenyloxindole (II), 2,2-diphenylindoxyl (III) and 1,3-diphenyloxindole (IV).

This is the first reported example of the formation of oxindoles and indoxyls by the action of basic reagents upon α-haloanilides.

It was found that gradual addition of a twofold excess of sodium hydride to the α-chloroanilide I at 40–60°, followed by a brief period at 80°, provided the cleanest reaction. However, varying the reaction conditions considerably (including inverse mode of addition) did not affect the ratio of products materially. No reaction was observed below 40°.

The oxindole II crystallized readily from the reaction mixture leaving behind the more soluble III and IV. The infrared (bands at 3190, 1718, 1680, 1615 and 1589 cm.$^{-1}$) and ultraviolet (λ_{max}^{EtOH} 255 mμ, log ε 3.81) spectra suggested a 5-membered lactam fused to an aromatic system. One such compound, the formation of which can be rationalized easily, is the known[3a,b] 3,3-diphenyloxindole. This compound, prepared from isatin and benzene as described,[3a] proved to be identical to the reaction product II.



Fractional crystallization of the residue from ether and ether–petroleum ether mixtures yielded the indoxyl III and the second oxindole IV in the percentages shown. Compound IV (m.p. 114°) has infrared and ultraviolet spectra similar to those of 3,3-diphenyloxindole (II) except that the infrared ($CHCl_3$) clearly shows the absence of N–H absorption. These observations together with mechanistic considerations suggested the hitherto unknown 1,3-diphenyloxindole structure for IV. This oxin-

(1) S. Sarel and H. Leader, *J. Am. Chem. Soc.*, **82**, 4752 (1960).
(2) H. Klinger, *Ann.*, **389**, 253 (1912).
(3) (a) J. Wegmann and H. Dahm, *Helv. Chim. Acta*, **29**, 415 (1946); (b) C. H. Hassal and A. E. Lippman, *J. Chem. Soc.*, 1059 (1953).