[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE]

Non-enzymatic Conversions of Dopamine to Norepinephrine and Trihydroxyphenethylamines[1]

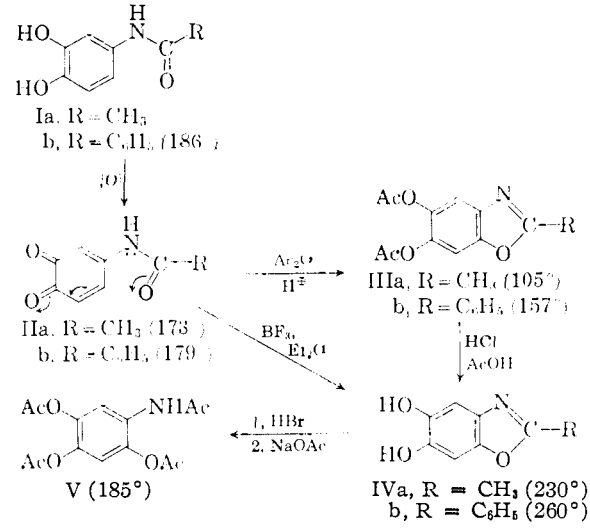
BY SIRO SENOH[2] AND BERNHARD WITKOP

RECEIVED MAY 15, 1959

*Intra*molecular acylation of the quinones of (acyl)-aminocatechols under the conditions of the Thiele reaction, or with boron trifluoride etherate, lead, *via* benzoxazole derivatives, to substituted trihydroxyanilines. This procedure is not practicable for the introduction of oxygen functions into N-acyldopamine quinones. However, *inter*molecular *1,4-* and *1,6-addition* of water, methanol and hydrogen bromide to quinones (XII) of N-acyldopamines XIa, XIb leads to ring-substituted 2,4,5-trihydroxyphenethylamines such as XIVa, XIVb, XIVc, XIXa, XIXb, or the mescaline isomer XVIId, whose structure was proved by oxidative degradation to asarylic acid (XVIII). The half-wave potentials of catecholamines are more than twice as high as those of their ring-substituted derivatives which accounts for the formation of methoxyquinones in the process of addition. The concomitant formation of norepinephrine (XVIb) and its β-O-methyl ether is interpreted as sulfuric acid- or boron trifluoride-catalyzed *1,6-addition* of water or methanol to the tautomeric *p*-quinonemethine (XIII) of N-acyldopamine *o*-quinone. Precedents for the introduction of hydroxyl groups into side chains of catechols by this pathway are cited. The yields in this conversion are ~1–3% of 2,4,5-trihydroxyphenethylamine, ~30–35% of the methoxy compound XIVc and 0.1% for 1,6-addition of water or methanol to yield norepinephrine (XVIb) and its β-O-methyl ether. Yields were determined by preparative chromatography and spectrophotometric evaluation.

Labile dihydrobenzene, *o-* and *p*-quinoid, or quinolid intermediates in the *enzymatic* hydroxylation of aromatic compounds to phenols,[3a,3b] of phenols to catechols[4] and of catechols to melanins[5] have been considered or observed spectrophotometrically, but never isolated. Such intermediates, however, exist in a number of comparable chemical reactions. For example, the *trans*-glycol of dihydrobenzene[6] easily dehydrates with acid to phenol or dehydrogenates enzymatically to catechol.[7] *o-* and *p*-quinols and their acetates undergo rearrangements,[8] external[9] and internal addition[10] reactions that lead from an initial phenol *via o-* and *p*-quinoid intermediates into the catechol, hydroquinone, resorcinol and pyrogallol series. As an extension of these reactions this investigation concerns itself with related hydroxylation mechanisms of 3,4-dihydroxyphenethylamine ("dopamine"), a biogenic amine of key importance in metabolism.

Intramolecular Hydroxylation of Aminocatechol Quinones by Neighboring Group Effects.—The normal Thiele reaction consists in the *external* addition of acetoxyl ion to an *o-* or *p*-quinone.[11] Quinol acetates, under the conditions of the Thiele reaction, rearrange by *internal* migration of the acetoxyl group through quasicyclic intermediates.[10] The analogous reaction occurs when 4-acetamino-*o*-quinone (IIa)[12] is treated with acetic anhydride under the conditions of the Thiele reaction. Deacetylation of the diacetoxybenzoxazole IIIa to the known dihydroxybenzoxazole IVa[13] is easier than hydrolytic opening of the oxazol ring which requires 48% HBr in glacial acetic acid. Trihydroxyaniline is too unstable for isolation and was obtained as the tetraacetyl derivative V. A similar sequence of transformations was carried out with the analogous benzoyl derivatives (Ib → IVb). The 2-phenyl-



benzoxazole IVb was stable to hydrolysis. The intramolecular nature of the cyclization of the acylamino group into the quinone ring was demonstrated by the use of boron trifluoride in ether on the quinones IIa and IIb which gave directly the catecholoxazoles IVa and IVb.

The analogous reaction in the naphthalene series led from 2-(acet)-amino-1,4-naphthoquinone (VI) to the naphthoxazole VIIa, although the isomeric angular tricyclic structure VIIb has not been rigidly excluded.

Examples of the intramolecular addition of an acetyl group to the *exo*-cyclic double bond of a quin-

(1) Oxidation Mechanisms. XXII. Preceding paper in this series: S. Senoh, B. Witkop, C. R. Creveling and S. Udenfriend, Colloquium on Oxygenizing Enzymes, 4th International Congress of Biochemistry, Vienna, 1958, Congress Monograph, in press.

(2) Visiting Scientist of the USPHS on leave of absence from the Institute of Food Chemistry and Osaka City University, Japan.

(3) (a) *Cf.* C. Mitoma, H. S. Posner, H. C. Reitz and S. Udenfriend, *Arch. Biochem. Biophys.*, **61**, 431 (1956). (b) For a survey of the recent literature as well as new results, *cf.* H. S. Posner, Thesis, George Washington University, Washington, D. C., October, 1958.

(4) *Cf.* H. S. Mason, "Advances in Enzymology," Interscience Publishers, Inc., New York, N. Y., Vol. 19, 1957, p. 79.

(5) *Cf.* A. B. Lerner, *ibid.*, Vol. 14, 1953, p. 73.

(6) M. Nakajima, I. Tomida, A. Hashizume and S. Takei, *Ber.*, **89**, 2224 (1956).

(7) P. K. Ayengar, O. Hayaishi, M. Nakajima and I. Tomida, 133rd ACS Meeting, San Francisco, Calif., April, 1958, Abstracts, p. 29-C.

(8) F. Wessely and R. H. Thomson, *Quart. Revs.*, **10**, 27 (1956).

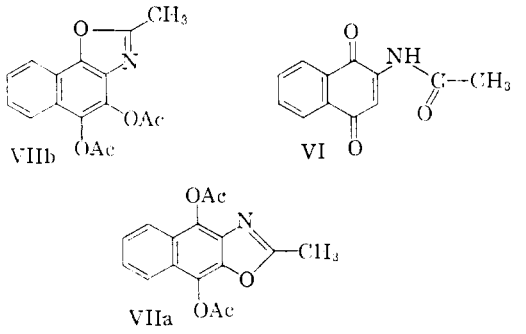
(9) E. Bamberger, *Ann.*, **390**, 164 (1912): cresorcinol dimethyl ether from *p*-toluquinol in acidic methanol.

(10) S. Goodwin and B. Witkop, THIS JOURNAL, **79**, 179 (1957). *Cf.* W. Metlesics, F. Wessely and H. Budzikiewicz, *Tetrahedron*, **6**, 345 (1959).

(11) H. Burton, *Quart. Revs.*, **6**, 316 (1952).

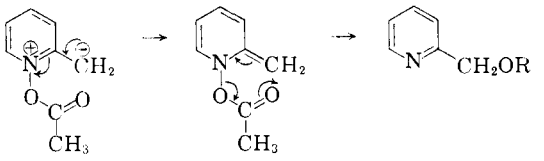
(12) F. Kehrmann and E. Hoehn, *Helv. Chim. Acta*, **8**, 218 (1925).

(13) K. Fries and F. Beyerlein, *Ann.*, **527**, 71 (1936).

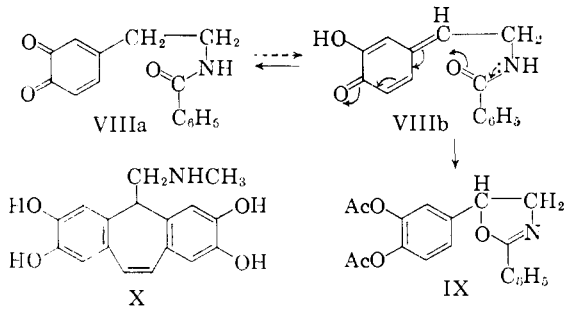


VIIb VI VIIa

oid species exist in the pyridine and quinoline series.[14] Abstraction of a hydrogen from 2-picoline



N-oxide acetate by acetate anion produces a *o*-pyridonmethine which rearranges to a hydroxymethyl pyridine derivative. A comparable *o*-quinone ⇄ *p*-quinone methine (VIIIa ⇄ VIIIb) tautomerism in the oxidized form of N-benzoyldopamine should lead to the oxazoline form of N-benzoylnorepinephrine (IX). Extensive attempts in this direction



VIIIa VIIIb X IX

were given up when it was found that external 1,4-addition of acetate supervenes in this reaction and that the oxazoline IX which, according to paper chromatographic evidence, probably is formed, cannot be hydrolyzed to norepinephrine. The action of acid on epinephrine is known to lead to deep-seated rearrangements (*e.g.*, adnamine (X)[15]).

Intramolecular Additions of Nucleophilic Agents to Quinones of N-Acylated 3,4-Dihydroxy-6-methoxyphenethylamines.—The oxidation of N-benzoyldopamine (XIa) to the quinone XII was preferably carried out with silver oxide in anhydrous methanol containing 2% of formic acid. Other solvents, such as methanol alone, dioxane with and without ether were unsatisfactory. Chromic acid or sodium dichromate was not effective in anhydrous acetic acid[16]; water had to be present for the formation of the quinone. The solution of the quinone exhibited a low extinction peak at 375 mμ which completely disappeared on standing overnight at 0°.[17]

(14) *Cf.* V. J. Traynelis and R. F. Martello, THIS JOURNAL, **80**, 6590 (1958).

(15) M. Kawazu, *J. Pharm. Soc. Japan*, **78**, 399, 402, 978 (1958).

(16) *Cf.* L. F. Fieser, THIS JOURNAL, **70**, 3237 (1948).

The boron trifluoride-catalyzed addition of methanol to freshly prepared quinone XII led to a yellow crystalline compound in 34% yield. Spectrophotometric evidence and analytical data point to the structure of the quinone XVa of 3,4-dihydroxy-6-methoxyphenethylamine (XIVa). The analogous reaction is the $ZnCl_2$-catalyzed addition of methanol to *p*-quinone ($E_{1/2}$ 0.699 v.) leading to 2,5-dimethoxy-1,4-benzoquinone ($E_{1/2}$ 0.476 v.).[18] Indeed, the half-wave potentials of the methoxy derivatives XIV and XV were less than half the values for dopamine and its derivatives (Table I).[19] The reason for the much greater stability of XVa compared with an unsubstituted *o*-quinone XII is the presence of the structural element of a vinylogous ester group. The catechol XIVa is easily obtained on reduction with sodium hydrosulfite of the quinone XVa and is reoxidized easily with 1,2,3,4-tetrachloro-1,2-benzoquinone. Methylation or acetylation of XIVa yielded XVIIa and XVIIb, respectively.

TABLE I

Polarographic half-wave potentials and calculated oxidation–reduction potentials of a number of substituted catecholamines and their *o*-quinoid oxidation products. All measurements were done in aqueous phosphate buffer at *p*H 6.86.

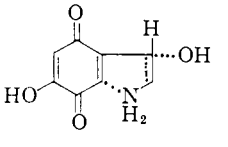
Compound	Measured $E_{1/2}$ *vs.* S.C.E., volt	Calcd. $E_{1/2}$ *vs.* H_2 electrode, volt
3,4-Dihydroxyphenethylamine (dopamine, XIc) hydrochloride	+0.139	+0.380[a]
N-Benzoyl- (XIa)	+ .101	+ .342
N-Carbobenzyloxy- (XIb)	+ .098	+ .339
3,4-Dihydroxy-6-methoxyphenethylamine (XIVc) hydrochloride	− .089	+ .152
o-Quinone of N-benzoyl- (XVa)	− .080[b]	+ .161
N-Benzoyl- (XIVa)	− .079[b]	+ .162
o-Quinone of N-carbobenzyloxy- (XVb)	− .101[c]	+ .140
N-Carbobenzyloxy- (XIVb)	− .101[c]	+ .141

[a] The related 3,4-dihydroxyphenylalanine has been found to have a E_0' of +0.370 v. at *p*H 7; E. Ball and T.-T. Chen, *J. Biol. Chem.*, **102**, 691 (1933). [b] The equimolar mixture of XIVa and XVa showed −0.105 v. (+0.136 v.). [c] The equimolar mixture of XIVb and XVb showed −0.085 v. (+0.156 v.).

Further nuclear 1,6-additions of HBr or methanol to the quinone XVa yielded XIXa and a less well characterized methoxy homolog of XIVa. The proof of the position of the nuclear bromine required the preparation of the free amine XIXb which was not obtainable by acid hydrolysis of the benzoyl derivative.

N-Carbobenzyloxydopamine (XIb) was, therefore, prepared. With the experience gained in the

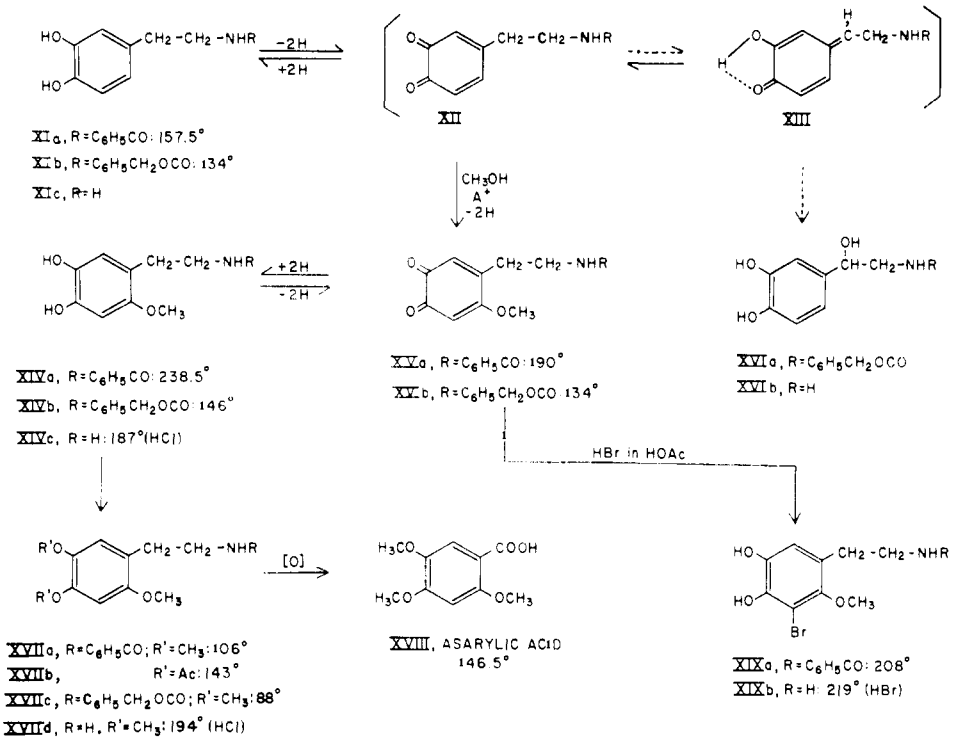
(17) This shows [*cf.* F. Ramirez and P. v. Ostwalden, *J. Org. Chem.*, **20**, 1676 (1955)] that the unknown dopamine quinone chromophor comes close to that of *o*-quinone, λ_{max} (log ε) 390 mμ (3.26) and that the values for "adrenaline quinone" ("adrenoerythrin"), λ_{max} 294 and 490 mμ [Ruiz-Gijon, *Farmacognosia*, **12**, 71 (1952); *C. A.*, **47**, 10498 (1953)], could possibly indicate a *p*-quinoid oxidation product resulting from 1,4-addition of water in the strong acid medium; *cf.* ref. 22.



(18) J. B. Conant and L. F. Fieser, THIS JOURNAL, **46**, 1858 (1924).

(19) We are greatly obligated to Prof. Charles Wiesner, University of New Brunswick, Fredericton, N. B., for arranging for the polarographic determination of the half-wave potentials.

TRANSFORMATIONS OF N-ACYLDOPAMINEQUINONES



transformations of the more easily crystallizable benzoyl derivatives, all the analogous carbobenzyloxy compounds were prepared (XIVb, XVb). Because of the easy removability of the carbobenzyloxy group, a number of new dopamine derivatives with nuclear substituents was prepared.[20] Catalytic decarbobenzyloxylation of XIVb gave 2-methoxy-4,5-dihydroxyphenethylamine (XIVc) which was demethylated to 2,4,5-trihydroxyphenethylamine, subsequently shown to arise from dopamine under conditions of oxidation, autoxidation or *in vivo* after administration to animals.[21]

Both decarbobenzyloxylation and 1,6-addition of HBr were observed when the quinone XVb was treated with HBr–HOAc. That the resulting compound was 2-methoxy-3-bromo-4,5-dihydroxyphenethylamine (XIXb) and not the equally possible isomer, *i.e.*, 2-bromo-3,4-dihydroxy-6-methoxyphenethylamine was proved by intramolecular oxidative cyclization to the (dihydro)-indole which still retained one atom of bromine.[22]

Further methylation of XIVb eventually led to 2,4,5-trimethoxyphenethylamine (XVIId), an isomer of mescaline, reported to be hallucinogenic but somewhat more toxic.[23] The conclusive proof for the structure of this amine was its oxidation to asarylic (2,4,5-trimethoxybenzoic) acid (XVIII).

The Formation of Norepinephrine.—The additions described so far are 1,4- and 1,6-additions in the *o*-quinone nucleus. If in the solution of N-acyldopamine quinone (XII) were present only a trace of the tautomeric quinone methine (XIII), besides the usual 1,4-addition, there could also be 1,6-addition which would introduce a nucleophilic group (OH, OCH_3) in the β-position of the side chain leading to norepinephrine (XVIa → XVIb) or its β-O-methyl ether.

For 1,6-addition of nucleophilic agents across the conjugated system of quinone methines there are many precedents.[24] Hindered quinone methines on refluxing in acidic methanol yield *p*-hydroxybenzyl O-methyl ether derivatives.[25] By analogy, the enzymatic conversion of homogentisic to gentisic acid has been formulated *via* an intermediate *o*-quinone methine which is hydrated to 2,5-dihydroxyphenylglycolic acid.[26]

A welcome analogy for our case may be found among the naturally occurring quinone methines.[27] Fuscin, a natural antibiotic, though it could exist as the *o*-quinone XX, is actually the quinone methine XXI.[28] It shows 1,6-addition of nucleophilic agents to yield substituted catechols of type XXII. Even in cases where stabilizing environmental fac-

(20) *Cf.* A. Burger and R. D. Foggio, THIS JOURNAL, **78**, 4419 (1956).

(21) S. Senoh, B. Witkop, C. R. Creveling and S. Udenfriend, *ibid.*, **81**, 6236 (1959).

(22) S. Senoh and B. Witkop, *ibid.*, **81**, 6231 (1959).

(23) M. P. J. M. Jansen, *Rec. trav. chim. pays-bas*, **50**, 291 (1931). No striking central effects in cats and monkeys were observed with this compound in doses which are effective for mescaline. We are indebted to Dr. Sydney Archer, Sterling-Winthrop Research Institute, for arranging for these pharmacological tests.

(24) K. Hultzsch, "Chemie der Phenolharze," Springer Verlag, Berlin-Göttingen-Heidelberg, 1950, pp. 63–87; *cf.* J. W. Ralls, *Chem. Revs.*, **59**, 329 (1959).

(25) C. D. Cook and B. E. Norcross, THIS JOURNAL, **78**, 3797 (1956).

(26) Y. Sakamoto, T. Mitsuhashi and U. Ichihara, *J. Biochem. (Japan)*, **45**, 1 (1958).

(27) For a review see: R. G. Cooke and R. H. Thomson, *Rev. Pure and Appl. Chem.*, **8**, 85 (1958).

(28) D. H. R. Barton, Symposium on Antibiotics and Mold Metabolites, Special Publication No. 5, London, The Chemical Society, 1956, p. 16; D. H. R. Barton and J. B. Hendrickson, *J. Chem. Soc.* 1028 (1956).

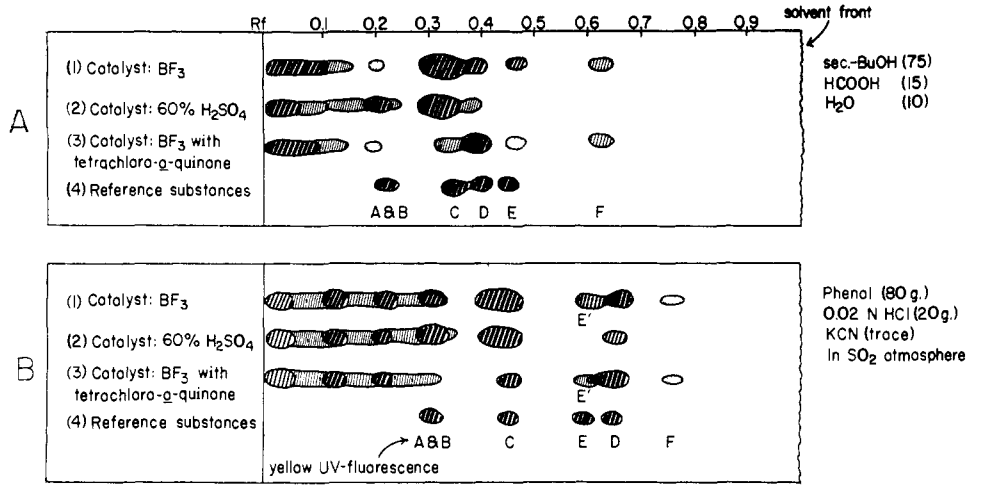
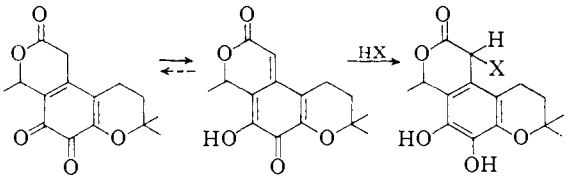
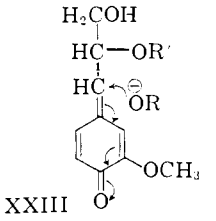


Fig. 1.—Paper chromatographic resolution of the reaction products derived from N-carbobenzyloxydopamine quinone (XII): A, norepinephrine; B, 2,4,5-trihydroxyphenethylamine; C, dopamine; D, 6-methoxydopamine; E, β-O-methylnorepinephrine; F, unknown compound.

tors, such as conjugation and additional rings, as in fuscin, are lacking, the presence of quinone methines sometimes is indicated by characteristic ab-



sorption bands or the typical products of 1,6-addition of water or methanol. The dimeric quinone methine XXIII from the enzymatic dehydrogenation of coniferyl alcohol is a recent example.[29]



XXIII

Two different sets of conditions were used for addition reactions to the dopamine quinone XII: boron trifluoride in methanol and 60% sulfuric acid. After removal of the methoxyquinone XVb by crystallization, the mother liquors were decarbobenzyloxylated catalytically and put on paper. Figure 1 shows the chromatographic resolution of the products in a non-phenolic and phenolic solvent system.

Boron trifluoride catalysis leads to β-O-methylnorepinephrine which is absent in the sulfuric acid experiment. A much greater problem is the demonstration of 1,6-addition of water and the formation of norepinephrine. Its R_f value in 5 different solvent systems (Table II) is indistinguishable from that of the isomeric 2,4,5-trihydroxyphenethylamine. This phenomenon may be called "*isography*"

(29) K. Freudenberg, G. Grion and J. M. Harkin, *Angew. Chem.*, **70**, 743 (1958).

TABLE II

R_f VALUES OF CATECHOLAMINES IN VARIOUS SOLVENT SYSTEMS

Substance	Solvent systems 1[a]	2[b]	3[c]	4[d]	5[e]
3,4-Dihydroxyphenethylamine (dopamine) (XIc)	0.44	0.35	0.64	0.25	0.57
Norepinephrine (XVIb)	.30	.22	.53	.13	.51
2,4,5-Trihydroxyphenethylamine	.29	.22	.53	.10	.49
β-O-Methylnorepinephrine (XVIc)	.58	.44	.71	.36	.66
2-Methoxy-4,5-dihydroxyphenethylamine (methoxydopamine) (XIVc)	.63	.38	.63	.15	.78
2-Methoxy-3-bromo-4,5-dihydroxyphenethylamine (XIXb)	..	.45	.72	.21	.81
2,4,5-Trimethoxyphenethylamine (XVIId)	..	.60	..	..	..
3,4,5-Trimethoxyphenethylamine (mescaline)	..	.58	..	..	..

[a] Phenol–0.02 *N* HCl–KCN (80 g.:20 ml.:trace) in saturation of SO_2. [b] *sec*-Butyl alcohol–formic acid–water (75:15:10) in N_2 atmosphere. [c] Methyl ethyl ketone–propionic acid–water (15:5:6). [d] 1-Butanol–acetic acid–water (70:15:15). [e] Methanol–benzene–1-butanol–water (2:1:1:1).

and such compounds "*isographs*." Resolution is, however, possible by treating the mixture of the two isomeric amines with methanol and hydrogen chloride. This converts the norepinephrine quantitatively to its β-O-methyl ether[30] and leaves the isomeric amine unchanged. The isomeric 6-methoxydopamine and β-O-methylnorepinephrine separate well on paper (Table III). Additional proof for the presence of norepinephrine was its spectrofluorometric determination as the noradrenolutine derivative and the pressor effects of a preparation, purified by preparative paper chromatography, on the arterial blood pressure of an anesthetized dog. The yields in the 1,6-addition of methanol or water to dopamine quinone yielding norepinephrine or β-O-

(30) B. F. Tullar, THIS JOURNAL, **70**, 2068 (1948).

TABLE III

CHROMATOGRAPHIC RESOLUTION OF MIXTURES OF TRIHYDROXYPHENETHYLAMINE AND NOREPINEPHRINE BY SELECTIVE O-METHYLATION[a]

	PhOH–0.02 *N* HCl–KCN[b] 80 g.:20 ml.:trace (satn. of SO_2 gas) Anhyd. HCl–CH_3OH[c]		*sec*-BuOH–HCOOH–H_2O 75:15:10 (N_2 atmosphere) Anhyd. HCl–CH_3OH[c]	
	Before	After	Before	After
2,4,5-Trihydroxyphenethylamine	0.29	0.30	0.25	0.25
Norepinephrine	.295	.58	.25	.51
Mixt. of trihydroxyphenethylamine and norepinephrine	.30	.30 .58	.25	.25 .51
β-O-Methylnorepinephrine	0.58		0.52	

[a] Whatman No. 1 filter paper; the two spraying reagents used were (i) 0.44% $K_3[Fe(CN)_6]$ in 0.1 *N* phosphate buffer (*p*H 7.2) or (ii) 0.1% 2,6-dichloroquinone chlorimide in ethanol, followed by 0.5% aqueous Na_2CO_3. [b] The spraying reagent (ii) could not be used in phenolic solvent systems. [c] Methylation was carried out in solution in anhydrous methanol which was saturated with dry HCl, then evaporated to dryness.

methylnorepinephrine are 0.05%, *i.e.*, <1/1000 of the 1,4-addition of methanol or water, which was 30–35% and 1–3%, respectively. It would not be correct to conclude that this disparity in the latter case is the result of the greater nucleophilicity of alkoxide as compared with hydroxide ion.[31] The two sets of conditions are too different to allow such a comparison.

The biochemical aspects of this conversion for the biogenesis of norepinephrine are discussed in the subsequent paper.[32]

Experimental[33]

4-Acetamino-*o*-benzoquinone (IIa).—The quinone IIa, prepared by the method of Kehrmann and Hoehn,[12] after recrystallization from acetone, melted and decomposed at 173° (reported 170–180°); λ_{max}^{Nujol} (μ): 3.10m (NH), 5.82s (CONH), 5.92m, 5.95m (CO); 6.15s; 6.32m; 6.65s; 7.10m.

Anal. Calcd. for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.27; H, 4.50; N, 8.05.

2-Methyl-5,6-diacetoxybenzoxazole (IIIa).—To the suspension of 400 mg. of the quinone IIa in 4 ml. of acetic anhydride there was added with agitation a few drops of concentrated sulfuric acid. The exothermic reaction produced a clear red solution, which was warmed on the steam-bath for several minutes and poured into excess cold water. After decomposition of the acetic anhydride the solution was neutralized with sodium bicarbonate, extracted with ethyl acetate three times, washed with water and evaporated to dryness *in vacuo*. The residue (516 mg.) was sublimed in high vacuum (<10^{-3} mm., bath temperature 85–95°). Recrystallization from a mixture of dichloromethane and petroleum ether gave IIIa (200 mg.), m.p. 103–105° (reported[13] 103°); $\lambda_{max}^{CHCl_3}$ (μ): 5.64s (CO); 5.89m; 6.17m; 6.32m; 6.67m; 6.83m; 7.09m; 7.31s. λ_{max}^{EtOH} (mμ): 236 (ε 9,400); 280 (ε 5,300); 285 (ε 4,900). $\lambda_{max}^{0.1\ N\ NaOH\ in\ EtOH}$ (mμ): 342 (ε 9,500).

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.57; H, 4.76; N, 5.42.

2-Methyl-5,6-dihydroxybenzoxazole (IVa).—The solution of 100 mg. of the diacetyl compound IIIa in a mixture of equal volumes of glacial acetic acid and 6 *N* hydrochloric acid was warmed on the steam-bath for 2 hours under nitrogen and then evaporated to a small volume under reduced pressure. During concentration crystals deposited which, after recrystallization from dilute acetic acid, melted at 228–230° (reported[32] 231°).

Anal. Calcd. for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.35; H, 4.38; N, 8.17.

2,4,5-Triacetoxyacetanilide (V).—One hundred milligrams of IVa was refluxed in a mixture of 2 ml. of glacial acetic acid and 3 ml. of 48% hydrobromic acid under nitrogen for 10 hours and the reaction mixture concentrated to dryness under reduced pressure. To the residue was added 2 ml. of acetic anhydride and 0.5 g. of fused sodium acetate and the mixture was heated on the steam-bath for 10 hours. After the usual extraction procedure the product, recrystallized from ethanol, had m.p. 181–186° (reported[13] 188°).

4-Benzoylaminocatechol (Ib).—To a solution of 2.7 g. of 4-aminocatechol hydrobromide and 2.3 g. of fused sodium acetate in 20 ml. of water was added 2.1 g. of benzoyl chloride and the mixture shaken vigorously at room temperature for one hour. The solid reaction product was collected and recrystallized from aqueous ethanol or a mixture of acetone and chloroform, yielding 2.14 g. of crystals, m.p. 182–186° (reported[13] 169°). By concentration of the mother liquor an additional crop (0.45 g.) of crystals was obtained; λ_{max}^{Nujol} (μ): 2.34m; 3.08s; 6.13s (CONH); 6.33m; 6.47s; 6.61s; 7.80s.

Anal. Calcd. for $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11. Found: C, 67.74; H, 4.90; N, 5.84.

4-Benzoylamino-*o*-benzoquinone (IIb).—To a suspension of 1.5 g. of 4-benzoylaminocatechol (Ib) in 4 ml. of water was added gradually at 0° the solution of 1.0 g. of sodium dichromate in 5 ml. of water containing 0.5 ml. of concentrated sulfuric acid. The reaction mixture was left for 2 hours. The deposited red quinone (1.13 g.) was collected and washed several times with cold water; m.p. 170–178° dec. For analysis the substance was recrystallized from acetone to give red lustrous crystals, m.p. 175–179° dec.; λ_{max}^{Nujol} (μ): 3.08m (NH); 5.90s (CO); 6.16s (CONH); 6.25w; 6.34m; 6.61s; 7.14m; 7.80m.

Anal. Calcd. for $C_{13}H_9NO_3$: C, 68.72; H, 3.99; N, 6.17. Found: C, 68.58; H, 4.10; N, 5.99.

2-Phenyl-5,6-diacetoxybenzoxazole (IIIb).—To the suspension of 1.5 g. of the N-benzoylquinone IIb in 5 ml. of acetic anhydride was added 0.1 ml. of concentrated sulfuric acid. After the moderate exothermic reaction ceased, the mixture was warmed on the steam-bath for several minutes and left at room temperature for two hours. The reaction mixture was poured into excess cold water to decompose the acetic anhydride, neutralized with bicarbonate and left standing at 0°. The crystalline product (585 mg.) was collected and sublimed in high vacuum (<10^{-3} mm., bath temperature 120–130°). The sublimate (225 mg.) had m.p. 130–145°. After recrystallization from a mixture of dichloromethane and petroleum ether it had m.p. 153–157°. $\lambda_{max}^{CHCl_3}$ (μ): 5.64s (CO); 5.95m; 6.22m; 6.30w; 6.43m; 6.72m; 6.84s; 6.91s; 7.31s; 7.45s; 78.5m. λ_{max}^{EtOH} (mμ): 271 (ε 13,500); 304 (ε 25,000). $\lambda_{max}^{0.1\ N\ NaOH\ in\ EtOH}$ (mμ): 347 (ε 15,500).

Anal. Calcd. for $C_{17}H_{13}NO_5$: C, 65.59; H, 4.21; N, 4.50. Found: C, 65.70; H, 4.35; N, 4.53.

2-Phenyl-5,6-dihydroxybenzoxazole (IVb).—To the suspension of 100 mg. of the quinone IIb in 5 ml. of anhydrous ether 0.2 ml. of boron trifluoride etherate was added with agitation. The reaction mixture was left for 1 hour at room temperature, then refluxed gently for 4 hours. After addition of water, the ethereal layer was separated, and the aqueous layer was extracted five times with ether. The combined ether extracts were washed with water and evaporated to dryness. The residue was purified by sublimation in high vacuum (<10^{-3} mm., bath temperature 160–180°). The sublimate had m.p. 252–260° and was identical with regard to infrared spectra and mixed m.p. with the product obtained from 2-phenyl-5,6-diacetoxybenzoxazole (IIIb) by acid hydrolysis (refluxing for 5 hours in a mixture of equal parts of glacial acetic and concentrated hydrochloric acid); λ_{max}^{Nujol} (μ): 2.90m (OH); 3.00m (OH); 6.04m; 6.11m; 6.34m; 6.47s. λ_{max}^{EtOH} (mμ): 223 (ε 14,500); 268 (ε

(31) *Cf.* M. L. Bender and W. E. Glasson, A.C.S. Meeting, Chicago, Ill., Sept. 1958, Abstracts, p. 13-P.

(32) S. Senoh, C. R. Creveling, S. Udenfriend and B. Witkop, THIS JOURNAL, **81**, 6236 (1959).

(33) All melting points are corrected (Kofler block). All compounds were dried for elementary analysis at 64° over P_2O_5 for 5 hours except where indicated otherwise. The analyses were performed by Dr. W. C. Alford and associates of the Analytical Services Unit of this Laboratory.

5,600); 331 (ε 16,000). $\lambda_{max}^{0.1\ N\ NaOH\ in\ EtOH}$ (mμ): 340 (ε 10,500).

2-Methyl-4,9-diacetoxy-2′,3′-naphthoxazole (VIIa) or 2-Methyl-4,5-diacetoxy-1′,2′-naphthoxazole (VIIb).—To the suspension of 200 mg. of 2-amino-1,4-naphthoquinone in 3 ml. of acetic anhydride was added three drops of concentrated sulfuric acid and the mixture boiled for 15 minutes. After decomposition with cold water and neutralization with sodium bicarbonate the deposited product was collected and purified by sublimation. The colorless sublimate was recrystallized from dichloromethane, m.p. 178.5–180°. λ_{max}^{Nujol} (μ): 5.64s (CO); 6.24m; 6.36m; 6.52m; 7.06m.

Anal. Calcd. for $C_{16}H_{13}NO_5$: C, 64.21; H, 4.38; N, 4.68. Found: C, 64.00; H, 4.54; N, 4.69.

The same material was obtained from the Thiele reaction of 2-acetamino-1,4-naphthoquinone, whereas 2-amino-1,4-naphthoquinone under milder conditions (5 ml. of acetic anhydride and 0.1 ml. of concentrated sulfuric acid) yielded mainly 2-acetamino-1,4-naphthoquinone, m.p. 200–203° (reported[34] 204°).

N-Benzoyl-β-(3,4-dihydroxyphenyl)-ethylamine (N-Benzoyldopamine) (XIa).—To the ice-cold mixture of 3.8 g. of dopamine hydrochloride and 3.6 g. of sodium bicarbonate in 40 ml. of water and 20 ml. of ether there was added dropwise the solution of 3.0 g. of benzoyl chloride in 30 ml. of ether under vigorous agitation. After complete addition, agitation was continued at room temperature for 2 hours. The crystalline product (3.5 g.) was collected by filtration and washed with water. It had m.p. 148–154°. Another crop of 1.5 g. of crystalline material was obtained by extraction of the filtrate with ethyl acetate. After recrystallization from a mixture of ethanol–dichloromethane–petroleum ether the melting point was 156.5–157.5° (yield 3.9 g. or 70%); λ_{max}^{Nujol} (μ): 2.87m (OH); 3.01m (NH); 3.22m (OH); 6.22s (CONH); 6.37s; 6.43s; 6.55m; 6.72m; 6.95s. λ_{max}^{EtOH} (mμ): 226 (ε 17,400); 280 (ε 3,600).

Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 69.99; H, 5.89; N, 5.43.

1,4-Addition of Methanol to the *o*-Quinone XII and Reoxidation. N-Benzoyl-6-methoxydopamine Quinone (XVa). A. Boron Trifluoride Method.—To 4 g. of N-benzoyldopamine (XIa), dissolved in 50 ml. of anhydrous methanol containing 1.2 ml. of 98% formic acid, was added 6 g. of anhydrous sodium sulfate, at 0°, and 7 g. of dry freshly prepared silver oxide. The mixture was shaken vigorously in the stoppered flask for 2 minutes and filtered through a layer of anhydrous sodium sulfate and washed twice with methanol (solution A); λ_{max}^{MeOH} 220, 375 mμ of this solution was in agreement with that of *o*-benzoquinone. To this red quinone solution was added 10 ml. of boron fluoride etherate, which caused a moderate exothermic reaction and change of color to light orange. After standing for 15 minutes the mixture was boiled gently on the steam-bath for a few minutes and then poured onto a mixture of ice and water whereupon a yellow oily product separated and solidified gradually. Two recrystallizations from methanol yielded 1.5 g. (34%) of yellow crystals, m.p. 189.5–190.5°; λ_{max}^{Nujol} (μ): 3.06m (NH); 3.99s (CO); 6.05m (CO); 6.11s (CONH); 6.21m; 6.34w; 6.50m. λ_{max}^{EtOH} (mμ): 229 (ε 14,400); 260 (ε 14,800); 360 (ε 670).

Anal. Calcd. for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91; CH_3O, 10.90; mol. wt., 285.3. Found: C, 67.32; H, 5.41; N, 4.86; CH_3O, 10.10; mol. wt. (Rast method), 306.

The attempted condensation with *o*-phenylenediamine to a quinoxaline derivative yielded starting material and brown resins.

Reduction of N-Benzoyldopaminequinone with Sodium Hydrosulfite.—To the red quinone solution (solution A) mentioned above there was added an excess of sodium hydrosulfite powder with agitation until the deep reddish solution turned colorless. After the addition of water the reaction mixture was extracted three times with ethyl acetate. The combined ethyl acetate extracts were washed and evaporated to dryness. The residual oil crystallized on rubbing with a glass rod, m.p. 155–157°, no depression on admixture with authentic N-benzoyldopamine (XIa). The reduction product gave a single spot on paper in several solvent systems.

(34) L. F. Fieser and M. Fieser, THIS JOURNAL, **56**, 1776 (1934).

B. **Sulfuric Acid Method.**—The red quinone solution (solution A), prepared from 500 mg. of N-benzoyldopamine (XIa) with silver oxide, was poured into 50 ml. of 50% aqueous sulfuric acid. The color of the reaction mixture turned immediately from deep red to yellow. The reaction mixture was warmed on the steam-bath for five minutes, diluted with water, extracted with ethyl acetate three times, washed and evaporated to dryness. The residual orange oil was recrystallized from methanol twice. The yellow crystals, m.p. 187–189°, were identical with respect to infrared spectra and mixed melting point with the compound (XVa) obtained by method A.

C. **Thiele Reaction of N-Benzoyldopamine Quinone (XII).**—The red quinone solution (solution A), prepared from 1 g. of N-benzoyldopamine (XIa) with silver oxide, was evaporated to dryness by lyophilization. To the solution of the residue in 10 ml. of acetic anhydride was added dropwise 0.5 ml. of concentrated sulfuric acid. The exothermic reaction produced a greenish solution, which was warmed on the steam-bath for 5 minutes and poured into excess cold water. After decomposition of the acetic anhydride, the solution was neutralized with sodium bicarbonate, extracted three times with ethyl acetate, washed with water and evaporated to dryness *in vacuo* (yield 1.25 g.). The residual material, sublimed in high vacuum ($<10^{-3}$ mm. bath temperature 150–170°), yielded 480 mg. of a slightly colored viscous product. This material was dissolved in a mixture of 10 ml. of 2 *N* aqueous hydrochloric acid and 10 ml. of ethanol and refluxed for 3 hours under nitrogen. The reaction mixture was concentrated to a small volume *in vacuo* and excess water added. The oil which separated was extracted three times with ethyl acetate. The combined ethyl acetate extracts were washed and evaporated to dryness under reduced pressure. The residue was examined by paper chromatography in several solvent systems. Two major spots were observed: The more mobile compound (R_f 0.82 in *sec*-BuOH–HCOOH–H_2O, 75:15:10) was identical with authentic N-benzoyldopamine. The identity was confirmed after elution of the spot from paper and comparison of the ultraviolet spectrum with that of authentic XIa. The less mobile compound (R_f 0.71 in *sec*-BuOH–HCOOH–H_2O, 75:15:10) had the same R_f as authentic N-benzoyl-DL-norepinephrine,[35] but further confirmation was rendered difficult by the failure to hydrolyze this material to norepinephrine.

N-Benzoyl-β-(2-methoxy-4,5-dihydroxyphenyl)-ethylamine (XIVa).—When the suspension of 1 g. of the finely powdered quinone XVa and 1.5 g. of sodium hydrosulfite in 20 ml. of 50% aqueous methanol was shaken at room temperature, reduction occurred immediately. The suspended quinone dissolved temporarily and the solution deposited colorless crystals. The reduction mixture was diluted with water and cooled in ice for 0.5 hour. The crystals (0.9 g., 94%) were collected by filtration and recrystallized from ethanol, m.p. 236.5–238.5°. The catechol XIVa did not show any color reaction with ferric chloride in the test-tube, but it showed the usual color reactions on paper characteristic of catechols, *e.g.*, with ferricyanide at *p*H 7.4 (0.1 *N* phosphate buffer) or with 2,6-dichloroquinone chloroimide followed by sodium carbonate. λ_{max}^{Nujol} (μ): 2.94s (OH); 3.00m (NH); 3.17m (OH); 6.12s (CONH); 6.18m; 6.23w; 6.35m; 6.45s; 6.51s; 6.72m; 7.00s. λ_{max}^{EtOH} m(μ): 226 (ε 17,500); 296 (ε 5,400).

Anal. Calcd. for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96; N, 4.88; CH_3O, 10.83; mol. wt., 287.3. Found: C, 66.51; H, 5.89; N, 4.84; CH_3O, 9.99; mol. wt., 296 (Rast method).

Reoxidation of N-Benzoyl-6-methoxydopamine (XIVa) with 3,4,5,6-Tetrachloro-1,2-benzoquinone.—When the solutions of 0.1 g. of the dopamine derivative XIVa and of 0.1 g. of chloranil, each in 2 ml. of cold methanol, were mixed, the red color of the chloroquinone solution was discharged and yellow crystals started separating from the yellow solution. After filtration and recrystallization from methanol they melted at 187–189°, not depressed on admixture with authentic N-benzoyl-6-methoxydopaminequinone (XVa).

(35) N-Benzoyl-DL-norepinephrine was prepared by the same procedure as described for N-benzoyldopamine. Recrystallization from a mixture of dioxane–benzene gave colorless crystals, m.p. 125–135°. The compound retained solvent of crystallization which could not be removed satisfactorily according to the results of several microanalyses; λ_{max}^{Nujol} (μ): 2.97m; 3.14s; 6.09s (CONH); 6.16m; 6.24m; 6.35m; 6.49s; 6.60s. λ_{max}^{EtOH} (mμ): 226 (ε 15,600); 279 (ε 3,300).

N-Benzoyl-β-(2,4,5-trimethoxyphenyl)-ethylamine (XVIIa).—To the mixture of 700 mg. of N-benzoyl-β-(2-methoxy-4,5-dihydroxyphenyl)-ethylamine (XIVa), 0.7 ml. of dimethyl sulfate and 5 ml. of water in a three-necked flask equipped with a mechanical stirrer, nitrogen inlet tube and dropping funnel there was added at room temperature with strong agitation under nitrogen 3 ml. of 10% aqueous sodium hydroxide. After complete addition the mixture was warmed to 60° for 15 minutes. When the color of the reaction mixture had faded, it was cooled and another 3 ml. of 10% aqueous sodium hydroxide and 0.4 ml. of dimethyl sulfate added. Then the mixture was warmed to 70° for 10 minutes and on the steam-bath for 15 minutes. The reaction mixture was cooled in ice, the crystalline material was filtered and washed (710 mg.). Recrystallization from aqueous methanol gave 680 mg. (88%) of the trimethyl ether XVIIa as fine colorless needles, m.p. 106–106.5°; λ_{max}^{Nujol} (μ): 3.02s (NH); 6.10s (CONH); 6.19m; 6.32m; 6.50s; 6.56s. λ_{max}^{EtOH} (mμ): 229 (ε 19,400); 290 (ε 5,300).

Anal. Calcd. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44; CH_3O, 29.5. Found: C, 68.49; H, 6.74; N, 4.50; CH_3O, 29.46.

Attempted hydrolysis of N-benzoyl-β-(2,4,5-trimethoxyphenyl)-ethylamine (XVIIa) to the free amine was unsuccessful under the following conditions: (1) refluxing in 15% potassium hydroxide in ethanol for 10 hours; (2) refluxing in 25% potassium hydroxide in 1-propanol–water (1:1) for 26 hours.

N-Benzoyl-β-(2-methoxy-4,5-diacetoxyphenyl)-ethylamine (XVIIb).—The mixture of 0.1 g. of the catechol XIVa in 3 ml. of acetic anhydride with 200 mg. of anhydrous sodium acetate was refluxed for 2 hours, poured into ice-water, neutralized with sodium bicarbonate, and extracted with ethyl acetate. The extract was evaporated to dryness and the residue recrystallized from methanol, m.p. 142.5–143°; $\lambda_{max}^{CHCl_3}$ (μ): 2.92m (NH); 5.67s (CO); 6.02s (CONH); 6.23m; 6.32m; 6.64s; 6.74s; 6.82m. λ_{max}^{EtOH} (mμ): 225 (ε 21,500); 274 (ε 3,500).

Anal. Calcd. for $C_{20}H_{21}NO_6$: C, 64.68; H, 5.70; N, 3.77. Found: C, 64.40; H, 5.58; N, 3.64.

1,6-Addition of Hydrogen Bromide to the 6-Methoxydopaminequinone (XVa): N-Benzoyl-β-(2-methoxy-3-bromo-4,5-dihydroxyphenyl)-ethylamine (XIXa).—When the solution of 0.15 g. of 6-methoxydopaminequinone (XVa) was dissolved in the ice-cold solution of 5 ml. of anhydrous 30% hydrobromic acid in acetic acid and slowly concentrated in a desiccator over sodium hydroxide under reduced pressure, the residual sirup crystallized on trituration with water. The crude product (220 mg.) melted at 190–197°. Recrystallization from methanol–ether–petroleum ether furnished colorless crystals, m.p. 205–205.5°; λ_{max}^{Nujol} (μ): 3.02m (NH); 6.10s (CONH); 6.19m; 6.22m; 6.36s; 6.43s. λ_{max}^{EtOH} (mμ): 297 (ε 5,300).

Anal. Calcd. for $C_{16}H_{16}NO_4Br$: C, 52.47; H, 4.41; N, 3.82. Found: C, 52.71; H, 4.53; N, 3.60.

Further Addition of Methanol to the Methoxy-*o*-quinone (XVa). N-Benzoyl-β-(2,3(?)-dimethoxy-4,5-dihydroxyphenyl)-ethylamine.—The suspension of 150 mg. of N-benzoyldopaminequinone (XVa) in 4 ml. of methanol and 2 ml. of boron trifluoride etherate was refluxed overnight. The resulting brown mixture was poured into water, extracted three times with ethyl acetate, and the combined extracts were washed and evaporated to dryness. The residue was extracted with chloroform and the extracts evaporated to dryness. The residue was sublimed in high vacuum ($<10^{-3}$ mg., bath temperature 130–150°). The sublimate recrystallized twice from acetone–ether to give fine colorless needles, m.p. 166.5–167.0°; $\lambda_{max}^{CHCl_3}$ (μ): 2.86m (OH); 2.97m (NH); 6.04s (CONH); 6.23m; 6.33m; 6.65s; 6.74m; 6.82s; 7.04m. λ_{max}^{EtOH} (mμ): 223 (ε 16,400); 291 (ε 5,000).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41; CH_3O, 19.6. Found: C, 65.81; H, 6.50; N, 4.97; CH_3O, 21.1.

N-Carbobenzyloxy-β-(3,4-dihydroxyphenyl)-ethylamine (N-Carbobenzyloxydopamine) (XIb).—To the mixture of 9.5 g. of dopamine hydrochloride and 9.0 g. of sodium bicarbonate in 100 ml. of water and 50 ml. of ether there was added dropwise the solution of 8.6 g. of carbobenzyloxy chloride in 40 ml. of ether under vigorous agitation in an ice-bath. Stirring was continued for 5 hours at 0°. The reaction mixture then was extracted three times with ethyl acetate, the combined extracts were washed with water and evaporated to dryness under reduced pressure. The residual oil (12.7 g.) solidified gradually and was recrystallized from methanol–ether–petroleum ether, yielding 10.6 g. (73%) of colorless crystals, m.p. 133.5–134°.

The catechol XIb gave a deep green color reaction with ferric chloride; $\lambda_{max}^{CHCl_3}$ (μ): 2.83m (OH); 2.92m (NH); 3.07m (OH); 5.85s (CONH); 6.21m; 6.64s. λ_{max}^{EtOH} (mμ): 283 (ε 3,000).

Anal. Calcd. for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96; N, 4.88. Found: C, 66.87; H, 5.86; N, 4.81.

1,4-Addition of Methanol to N-Carbobenzyloxydopaminequinone (XIIb) and Reoxidation to the Methoxyquinone XVb.—To 10 g. of N-carbobenzyloxydopamine (XIb), dissolved in a mixture of 150 ml. of anhydrous methanol containing 4 ml. of 98% formic acid and 20 g. of anhydrous sodium sulfate, was added at 0° 20 g. of dried freshly prepared silver oxide. The flask was stoppered and shaken vigorously for 2 minutes. The reaction mixture was filtered through anhydrous sodium sulfate and washed twice with methanol. To the resulting red quinone solution was added 30 ml. of boron trifluoride etherate. The red color immediately turned to light orange. After 15 minutes the mixture was boiled gently on the steam-bath for 2 minutes, poured into a mixture of ice and water and left in the ice-box overnight. After decantation the solid was washed with water and dried. Trituration with methanol produced crystals. Two recrystallizations from methanol gave 2.4 g. (22%) of yellow prisms (XVb), m.p. 133–134°; $\lambda_{max}^{CHCl_3}$ (μ): 2.92m (NH); 5.81s (CONH); 5.90s (CO); 6.05s (CO); 6.22s; 6.67s. λ_{max}^{Nujol} (μ): 3.03m (NH); , 5.89s (COHN); 5.98s (CO); 6.05s (CO); 6.14m; 6.19s; 6.47s. λ_{max}^{EtOH} (mμ): 260 (ε 13,800); 360 (ε 670).

Anal. Calcd. for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.43; N, 4.44; CH_3O, 9.84. Found: C, 64.76; H, 5.48; N, 4.54; CH_3O, 9.82.

Boron Trifluoride-catalyzed 1,6-Addition of Methanol to the Quinone-methine XIIIb: Formation of β-O-Methylnorepinephrine and (by Hydrolysis) Norepinephrine.—The aqueous supernatant from the decantation from, and the methanolic mother liquors of XVb, were combined and extracted three times with ethyl acetate. The combined extracts were washed and concentrated to dryness under reduced pressure. The residue, 8 g. of a brown resin, was debenzylated catalytically in the presence of 2 g. of 10% palladium-on-charcoal in a mixture of 300 ml. of methanol and 4 ml. of concentrated hydrochloric acid. The CO_2 formed was absorbed in a side tube filled with Ascarite. Within 5 hours 540 ml. of hydrogen was taken up. Under exclusion of air the reduction mixture was filtered from the catalyst and evaporated to dryness *in vacuo*.

The residue (5.6 g.) was dissolved in hot methanol, seeded with authentic dopamine hydrochloride and chilled. The product was filtered and recrystallized from methanol, yielding 1.1 g. of colorless crystals, m.p. 245–246°, identical with authentic dopamine hydrochloride with regard to R_f, infrared and ultraviolet spectra. The filtrate from the dopamine hydrochloride was evaporated to dryness under sulfur dioxide to yield 3.8 g. of a resin, which was examined by preparative chromatography as follows: 80 mg. of the crude product in a small volume of methanol was put on two sheets of S. & S. No. 598 filter paper and developed in a phenolic system (80 g. of phenol and 20 ml. of 0.02 *N* hydrochloric acid in the presence of a trace of potassium cyanide in an atmosphere of sulfur dioxide gas, ascending method). At the same time, 20-γ samples of the following five substances expected from the reaction were put on the margins of each sheet as standards for comparison: dopamine hydrochloride, DL-norepinephrine (XVIb) hydrochloride, 2,4,5-trihydroxyphenethylamine hydrobromide, 6-methoxydopamine (XIVe) hydrochloride and β-O-methylnorepinephrine hydrochloride. (The R_f values of the standard samples are summarized in Table III.) After complete development the paper was drained in the hood for 10–15 minutes and from the wet paper the residual phenol was washed out three times with benzene and twice with ether. The paper then was dried by a stream of air. A representative longitudinal strip was cut off and sprayed with 0.44% ferricyanide in *p*H 7.4, 0.1 *N* phosphate buffer (sensitivity approx. 5–10 γ). The brown spots produced by the ferricyanide spray showed under the ultraviolet lamp violet fluorescence for dopamine, intensive bright yellow fluorescence for norepinephrine, weak purple

fluorescence for β-methoxydopamine and violet fluorescence for β-O-methylnorepinephrine.

The five latitudinal strips, corresponding to 6-hydroxydopamine and norepinephrine (fraction A and B), dopamine (fraction C), β-O-methylnorepinephrine (fraction E'), 6-methoxydopamine (fraction D) and fraction F containing an unknown substance (see Fig. 1), were cut off and eluted with each 10 ml. of 0.1 *N* hydrochloric acid containing sulfur dioxide. Each elution was concentrated to dryness in a vacuum desiccator charged with sodium hydroxide. The residue of each fraction was rechromatographed in the same phenolic solvent system. The chromatograms were cut up again following the markers of the reference catecholamines. After elution with 0.1 *N* hydrochloric acid in the presence of sulfur dioxide and evaporation to dryness, each fraction was re-examined for homogeneity by paper chromatography in several solvent systems described in Table III and run with standard samples for comparison. For the detection of catecholamines in non-phenolic solvent systems, a freshly prepared 0.1% ethanolic solution of 2,6-dichloroquinone chlorimide followed by spraying with 0.2% aqueous sodium carbonate was used. This reagent is more sensitive than the ferricyanide reagent and detects not only phenolic compounds but also their methyl ethers such as mescaline and the isomeric 2,4,5-trimethoxyphenethylamine (XVIId). C^{14}-Labeled dopamine, norepinephrine and β-O-methylnorepinephrine were used as markers which showed that the visible spots were matched by peaks of radioactivity using a strip counter.

The semi-quantitative determination of the catecholamines in each fraction was carried out as follows: 1. Rechromatographed fraction C gave only one spot in several solvent systems identical with authentic dopamine. Direct calculation of the yield of dopamine based on the molar extinction of λ_{max} 280 mμ showed that 50–55% of the product of debenzylation (3.8 g. after removal of crystalline dopamine) consisted of dopamine hydrochloride. 2. Rechromatographed fraction D was identical with authentic 6-methoxy-dopamine with regard to R_f and ultraviolet spectra. This fraction represented 10–15% of the crude product. 3. The chromatographed fraction E' contained dopamine and 6-methoxydopamine as main products. In order to convert β-O-methylnorepinephrine to norepinephrine, fraction E' was dissolved in 3 *N* aqueous hydrochloric acid in the presence of sulfur dioxide and evaporated to dryness *in vacuo*. A solution of the residue in a small volume of 3 *N* hydrochloric acid was put on paper and developed in *sec*-butyl alcohol–formic acid–water (75:15:10) under nitrogen. The three latitudinal strips corresponding to norepinephrine, dopamine and 6-methoxydopamine were cut out, eluted with 0.1 *N* hydrochloric acid and examined spectrophotometrically. There was present <0.1% norepinephrine, 3–4% dopamine and 4–5% 6-methoxydopamine. 4. On rechromatography fraction A and B gave a single spot identical with 6-hydroxydopamine, corresponding to a yield of 2–4%. However, a small amount of accompanying norepinephrine was detected when fraction A and B was treated with cold anhydrous methanol saturated with anhydrous hydrogen chloride, evaporated to dryness *in vacuo* and chromatographed in *sec*-butyl alcohol–formic acid–water. A new spot identical with β-O-methylnorepinephrine was found corresponding to a yield of <0.05% based on the molar extinction of the solution obtained on extraction of the spot.

In another determination 100 mg. of the crude hydrogenolysis product was treated with 5 ml. of 3 *N* hydrochloric acid in the presence of sulfur dioxide and evaporated to dryness *in vacuo*. The residue was chromatographed in the phenolic solvent system described above. The "norepinephrine fraction A and B" was eluted with 0.1 *N* hydrochloric acid in the presence of sulfur dioxide, and evaporated to dryness *in vacuo*. The residue was purified again by chromatography (phenolic solvent system) and examined spectrofluorometrically using the adrenolutine method. This method differentiates sharply from any possible contaminant, because dopamine, 6-hydroxy- or 6-methoxydopamine are negative in the adrenolutine test. The yield of norepinephrine by this method was 50–60 γ/100 mg. or 0.05%. In addition this fraction was tested pharmacologically by its effect on the arterial blood pressure in the anesthetized dog. The slight but distinct rise in blood pressure confirmed the presence of norepinephrine. Comparable amounts of dopamine or its 6-hydroxy and 6-methoxy derivatives were without effect.

Reduction of N-Carbobenzyloxydopaminequinone with Sodium Hydrosulfite.—To the red quinone solution obtained from the silver oxide oxidation of 1 g. of N-carbobenzyloxydopamine there was added an excess of sodium hydrosulfite ($Na_2S_2O_4$) solution in a small volume of water. The deep red solution turned colorless. Water was added and the reaction mixture extracted three times with ethyl acetate. The combined ethyl acetate extracts were washed and evaporated under reduced pressure. The residual sirup crystallized on scratching, m.p. 131–133°, undepressed on admixture with authentic N-carbobenzyloxydopamine (XIb). After catalytic debenzylation the product showed one single spot on paper identical with authentic dopamine.

Boron Trifluoride-catalyzed 1,4-Addition of Methanol in the Presence of 3,4,5,6-Tetrachloro-1,2-benzoquinone.—To the red quinone solution obtained from the silver oxide oxidation of 2 g. of N-carbobenzyloxydopamine (XIb) there was added 0.9 g. of 3,4,5,6-tetrachloro-1,2-benzoquinone in methanol and 5 ml. of boron trifluoride etherate. The reaction mixture was warmed on the steam-bath for 15 minutes and poured into a mixture of ice and water. The viscous oil which separated after decantation was suspended in cold methanol. The crystalline product was recrystallized from methanol (580 mg.), m.p. 132–134°, undepressed on admixture with the methoxyquinone XVb. The combined mother liquors from XVb in a small volume of methanol were reduced with an excess of sodium borohydride. The reduction mixture was poured into water, extracted with ethyl acetate and evaporated. The residual oil was dissolved in a small volume of ethanol. By addition of ether, tetrachlorocatechol was precipitated. The filtrate was evaporated to dryness and decarbobenzyloxylated catalytically in methanol (H_2 uptake 134 ml.). The reduction product (1.15 g.) was examined by paper chromatography. Figure 1 shows that by this procedure the yield of 6-methoxydopamine was increased and that of dopamine decreased.

Sulfuric Acid-catalyzed 1,6-Addition of Water to Yield Norepinephrine.—The quinone solution obtained from 1 g. of XIb was poured into 50 ml. of 60% aqueous sulfuric acid and kept at room temperature for one hour. The reaction mixture was diluted with water and extracted three times with ethyl acetate, washed and evaporated to dryness. The residue after catalytic debenzylation in ethanol was examined paper chromatographically using the same procedure described before (see Fig. 1). It contained dopamine, 2,4,5-trihydroxyphenethylamine, 6-methoxydopamine and a small quantity of norepinephrine which did not separate from 6-hydroxydopamine in the solvent systems used. The norepinephrine fraction was rechromatographed in two different solvent systems. The eluted spots were assayed spectrofluorometrically by the noradrenolutin method, as well as by their effect on the blood pressure in the anesthetized dog using known amounts of norepinephrine and 6-hydroxydopamine as standards of reference. Although exact yields could not be calculated, there was no doubt about the presence of norepinephrine.

N-Carbobenzyloxy-2-methoxy-4,5-dihydroxyphenethylamine (XIVb). (A) With Sodium Hydrosulfite.—The suspension of 1.0 g. of the quinone XVb and 1.5 g. of sodium hydrosulfite ($Na_2S_2O_4$) in 15 ml. of 60% aqueous methanol was agitated until the color was discharged. Water was added to the reaction mixture. The colorless crystals which deposited on cooling at 0° were collected, washed with water and dried (530 mg.). The filtrate was extracted three times with ethyl acetate and the combined extracts were washed and evaporated to dryness (100 mg.). The total yield was 630 mg. (63%), m.p. 142–144°. Recrystallization from methanol–ether yielded fine needles, m.p. 145.5–146.5°. This catechol did not give a color reaction with ferric chloride; λ_{max}^{Nujol} (μ): 2.94w (OH); 3.01m (NH); 3.16m (OH); 5.86s (CONH); 6.11m; 6.21w; 6.30w; 6.58s; 6.68m; 7.07s. λ_{max}^{EtOH}(mμ): 292 (ε 4,900).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.26; H, 6.07; N, 4.53.

(B) **With Sodium Borohydride.**—To the suspension of 1.60 g. of the quinone XVb in 10 ml. of methanol was added 120 mg. of sodium borohydride with agitation. The yellow color was discharged immediately. The reaction mixture was filtered, the clear filtrate was diluted gradually with water and cooled in ice. The crystals which separated after scratching were collected, washed with water and dried, yielding 1.60 g. of colorless needles (99%), m.p. 142.5–144°.

Reoxidation of N-Carbobenzyloxy-2-methoxy-4,5-dihydroxyphenethylamine (XIVb) with 3,4,5,6-Tetrachloro-1,2-benzoquinone.—When the solutions of 100 mg. of XIVb and of tetrachloro-*o*-benzoquinone, each in 2 ml. of methanol, were combined, a yellow solution was obtained which deposited crystals on cooling in ice. They were identical with the quinone XVb, m.p. 131–132°.

2-Methoxy-4,5-dihydroxyphenethylamine (XIVc) Hydrochloride (6-Methoxydopamine Hydrochloride). (A) By Catalytic Decarbobenzyloxylation.—The catalytic decarbobenzyloxylation of 1.1 g. of the catechol XIVb was carried out in 30 ml. of methanol containing 1 ml. of concentrated hydrochloric acid with 400 mg. of 10% palladium-on-charcoal at room temperature and ordinary pressure in a flask equipped with a side tube filled with Ascarite. The uptake was 83 ml. (98%) of hydrogen (65 ml. within 1 hour and 17 ml. within further 5 hours). The reaction mixture was filtered, the catalyst washed with methanol containing sulfur dioxide, the combined filtrate evaporated to a small volume under reduced pressure and kept in the refrigerator. The colorless prisms were filtered and washed with a small volume of cold concentrated hydrochloric acid. There was obtained 320 mg., m.p. 187–188°. An additional crop of 290 mg., m.p. 186–188°, was obtained by concentration of the mother liquor. The total yield was 610 mg. (97%). The sample on examination by chromatography in several solvent systems showed only one spot (Table III); λ_{max}^{EtOH} (mμ): 222 (ε 5,500); 295 (ε 6,000).

Anal. Calcd. for $C_9H_{13}NO_3 \cdot HCl$: C, 49.21; H, 6.42; N, 6.38. Found: C, 49.29; H, 6.42; N, 6.24.

(B) **One-step Conversion of 6-Methoxy-N-carbobenzyloxydopaminequinone (XVb) to the Amine XIVb.**—The suspension of 630 mg. of the quinone XVb in 40 ml. of methanol containing 0.2 ml. of concentrated hydrochloric acid was reduced catalytically with 200 mg. of 10% palladium-on-charcoal at room temperature and ordinary pressure (Ascarite side tube). Within 6 hours 95 ml. of hydrogen (theor. 97.2 ml.) was taken up. After filtration the catalyst was washed with methanol and the combined filtrate was evaporated. The residual sirup gave two spots; one gave a blue color with 0.44% ferricyanide in 0.1 *N* phosphate buffer *p*H 7.4. The sirup was triturated with a small volume of methanol, filtered and the filtrate was evaporated to dryness under reduced pressure. The residual sirup crystallized gradually on addition of a few drops of ethanol. The solid was dried on a porous porcelain plate, dissolved in isopropyl alcohol. To this solution was added ethyl acetate. Some fractionation was required until crystallization started. After cooling in ice the crystals were collected and dried. The substance showed a single spot on paper, identical with that of the amine XIVc obtained by procedure A.

(C) **Decarbobenzyloxylation of the Catechol XIVb with Anhydrous 30% Hydrobromic Acid in Glacial Acetic Acid.**—When 0.15 g. of the catechol XIVb was dissolved in 2 ml. of anhydrous 30% hydrobromic acid in glacial acetic acid, the odor of benzyl bromide and the evolution of carbon dioxide were observed. The mixture was evaporated to dryness in a vacuum desiccator in the cold room. When the residual sirup was triturated with ethyl acetate, it crystallized. The R_f values of the compound in several solvent systems were identical with the catecholamine XIVc obtained by the procedures (A) and (B).

Anal. Calcd. for $C_9H_{13}NO_3 \cdot HBr$: C, 40.91; H, 5.34; N, 5.29. Found: C, 40.35; H, 5.65; N, 4.94.

N-Carbobenzyloxy-2,4,5-trimethoxyphenethylamine (XVIIc).—Using a three-neck flask equipped with a mechanical stirrer, nitrogen inlet tube and dropping funnel, 6 ml. of 10% aqueous sodium hydroxide was added dropwise to the mixture of 1.6 g. of the catechol XIVb, 1.9 g. of dimethyl sulfate and 10 ml. of water at room temperature with strong agitation under nitrogen. After complete addition the mixture was warmed to 60° for 15 minutes, cooled again and another 6 ml. of 10% aqueous sodium hydroxide and 1.9 g. of dimethyl sulfate was added. Then the mixture was warmed to 70° for 10 minutes, and for a further 10 minutes on the steam bath. On cooling in ice the methylation product (1.59 g.) crystallized. The filtrate was extracted with ethyl acetate. The extract gave an additional crop of 0.12 g. The total yield was 1.71 g. (98%) of colorless crystals, m.p. 86–88°. After recrystallization from methanol the melting point was 87.5–88°; λ_{max}^{Nujol} (μ): 300s (NH); 5.79s (CONH); 6.19m; 6.27w; 6.50s; 6.60s. λ_{max}^{EtOH} (mμ): 230 (ε 9,400); 290 (ε 4,900).

Anal. Calcd. for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.31; H, 6.85; N, 3.92.

2,4,5-Trimethoxyphenethylamine (XVIId) Hydrochloride.—The catalytic decarbobenzyloxylation of 1.7 g. of N-carbobenzyloxy-2,4,5-trimethoxyphenethylamine (XVIIc) was carried out in 100 ml. of ethanol containing 1 ml. of concentrated hydrochloric acid with 500 mg. of 10% palladium-on-charcoal at room temperature and ordinary pressure (side tube with Ascarite). The uptake was 150 ml. of hydrogen within 6 hours. After filtration the catalyst was washed with ethanol and the combined filtrate was evaporated to dryness under reduced pressure. The crystalline residue (1.22 g., 98%) had m.p. 185–190°, and, after recrystallization from ethanol–ether, 193.5–194.5° (reported[23] 190°). λ_{max}^{EtOH} (mμ): 232 (ε 8,300); 291 (ε 4,600).

Anal. Calcd. for $C_{11}H_{17}NO_3 \cdot HCl$: C, 53.30; H, 7.32; N, 5.66; CH_3O, 37.58. Found: C, 53.12; H, 7.39; N, 5.59; CH_3O, 37.75.

DNP Derivatives of XVIId.—The aqueous solution of the trimethoxyamine XVIId hydrochloride was treated with aqueous sodium bicarbonate and 2,4-dinitrofluorobenzene. The orange-red product was recrystallized from ethanol, m.p. 164.5–165.5° (reported[23] 158°).

Anal. Calcd. for $C_{17}H_{19}N_3O_7$: N, 11.14. Found: N, 10.79.

Asarylic Acid (XVIII) by Permanganate Oxidation of 2,4,5-Trimethoxyphenethylamine (XVIId).—To the solution of 0.5 g. of 2,4,5-trimethoxyphenethylamine (XVIId) hydrochloride in 40 ml. of water containing 1.5 ml. of pyridine was added dropwise the solution of 1.15 g. of potassium permanganate in 35 ml. of water at room temperature. The permanganate color was discharged immediately. The reaction mixture was filtered, and the residual manganese dioxide was washed several times with hot water containing sodium hydroxide. The combined filtrate was evaporated to a small volume under reduced pressure, acidified with concentrated hydrochloric acid and extracted three times with a mixture of ether and ethyl acetate. The organic extract was washed with water, then extracted twice with aqueous sodium bicarbonate solution. The aqueous bicarbonate extracts were acidified with concentrated hydrochloric acid and re-extracted three times with a mixture of ether–ethyl acetate. The combined ether–ethyl acetate extracts were evaporated to dryness. The residual oil crystallized on trituration with ether. The crude acid was sublimed in high vacuum ($<10^{-3}$ mm., bath temperature 80–85°) and recrystallized from aqueous ethanol, m.p. 145.5–146.5°, undepressed on admixture with authentic asarylic acid. The infrared and ultraviolet spectra were also identical with authentic XVIII; λ_{max}^{KBr} (μ): 3.14m (OH); 3.42m; 3.45m; 3.57m; 5.82s (CO); 6.21s; 6.31m; 6.59s; 6.78s; 7.08s. λ_{max}^{EtOH} (mμ): 226 (ε 21,800); 258 (ε 9,900); 312 (ε 6,400).

Anal. Calcd. for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70. Found: C, 56.67; H, 5.87.

Asarylic acid was prepared by methylation of 2,4,5-trihydroxybenzoic acid[36] in acetone with a large excess of potassium carbonate and dimethyl sulfate overnight. After addition of water to the reaction mixture it was acidified with hydrochloric acid, extracted three times with ethyl acetate. The combined extracts were evaporated to dryness, whereupon the ester of asarylic acid crystallized, m.p. 95–96°. The ester was saponified by boiling with excess aqueous methanolic sodium hydroxide on the steam-bath for 10 minutes. After cooling, the hydrolysate was acidified with concentrated hydrochloric acid. Asarylic acid was obtained as fine needles from the solution on cooling in ice, m.p. 146.5–147° after recrystallization from dilute ethanol.

2,4,5-Trihydroxyphenethylamine (6-Hydroxydopamine) Hydrobromide.—The solution of 600 mg. of 6-methoxydopamine (XIVc) hydrochloride in 25 ml. of a mixture of equal volumes of 48% aqueous hydrobromic and glacial acetic acid was refluxed gently under nitrogen overnight. The solution was diluted with 25 ml. of water containing sulfur dioxide, decolorized with active charcoal and filtered. The colorless filtrate was evaporated to a small volume under reduced pressure and kept at 0°. The colorless fine needles

(36) Bargellini and Martegiani, *Gazz. chim. ital.*, **42** II, 353 (1912).

were filtered and washed with a small volume of cold 48% hydrobromic acid. There was obtained 650 mg. (95.5%), m.p. 218–219°. The substance gave a single spot on paper in several solvent systems (Table III). The ferric chloride test gave a brown color; λ_{max}^{EtOH} (mμ): 297 (ε 4,700).

Anal. Calcd. for $C_8H_{11}NO_3 \cdot HBr$: C, 38.41; H, 4.84; N, 5.60. Found: C, 38.42; H, 5.08; N, 5.60.

Acknowledgment.—We are indebted to Dr. Albert Sjoerdsma and Mr. C. R. Creveling, National Heart Institute, for assistance in the pharmacological and spectrofluorometric tests for norepinephrine.

BETHESDA 14, MD.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE]

Formation and Rearrangements of Aminochromes from a New Metabolite of Dopamine and Some of its Derivatives[1]

BY SIRO SENOH[2] AND BERNHARD WITKOP

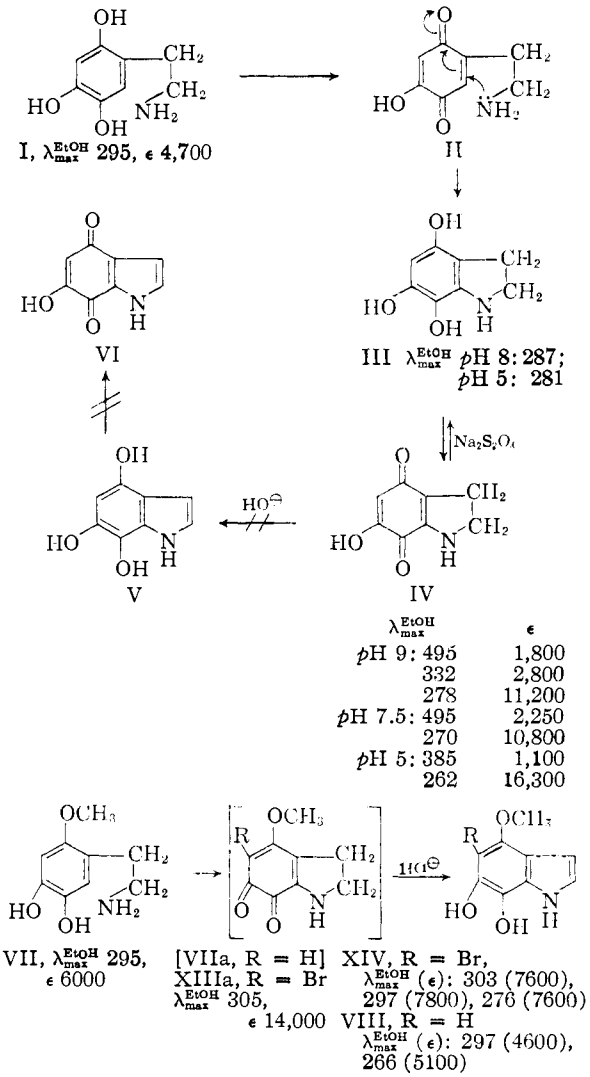
RECEIVED MAY 15, 1959

Spectrophotometric, polarographic and tritium studies demonstrate the easy oxidative cyclization of 2,4,5-trihydroxyphenethylamine (I), the new metabolite from dopamine, to the *p*-quinoid aminochrome IV, while 2-methoxy-4,5-dihydroxyphenethylamine (VII) goes to the *o*-quinoid aminochrome which is easily rearranged to the dihydroxyindole VIII. The low oxidation–reduction potential of I ($E_{1/2}$ +0.083 v.) is indicative of a *p*-quinoid oxidation product II whereas $E_{1/2}$ +0.172 v. of the methoxydopamine VII is characteristic of an *o*-quinoid oxidation product. The amine I containing two tritium atoms in the benzyl position of the side chain retained all activity on cyclization to the final product, the aminochrome IV, whereas the analogously tritium-marked VII lost as required one atom of tritium in the conversion to the indole VIII. The conversion of 2-methoxy-3-bromo-4,5-dihydroxyphenethylamine (XIIa) *via* the crystalline aminochrome XIIIa to 4-methoxy-5-bromo-6,7-dihydroxyindole (XIV) extended and confirmed these observations and established the position of the bromine in the starting amine.

The oxidation products of catecholamines, among them the so-called aminochromes,[3] are of importance in the determination of (nor)epinephrine.[4] So far they have no clear physiological role,[5] no significant pharmacological effects and only minor therapeutic applications.[6]

The easy oxidation or metabolic conversion of dopamine to 2,4,5-trihydroxyphenethylamine (I)[1] adds to the recent interest in (dihydro)-indoles with oxygen functions in position 4,[7] 6 and 7[8] which would arise by intramolecular oxidative cyclization of the ethaneamine side chain (I–VI).

The reversible polarographic half-wave (oxidation–reduction) potentials of these amines can be determined without interference of the side chain, since any possible intramolecular addition would be too slow to be significant.[9] Compound I has a much lower oxidation–reduction potential ($E_{1/2}$ + 0.083 v.) than its methoxy derivative VII ($E_{1/2}$ + 0.172 v.) which is incapable of forming a *p*-quinone, reminiscent of the similar case IX ⇄ X (R = H or CH_3)[10] where the oxidation–reduction potentials of



(1) Oxidation Mechanisms. XXIII. Preceding paper, *cf.* THIS JOURNAL, **81**, 6222 (1959).

(2) Visiting Scientist of the USPHS on leave of absence from the Institute of Food Chemistry and Osaka City University, Japan.

(3) H. Sobotka and J. Austin, THIS JOURNAL, **73**, 3077 (1951).

(4) A. Lund, *Acta Pharm. Tox.*, **5**, 75, 121 (1949); **6**, 137 (1950); U. S. v. Euler and J. Floding, *Acta Physiol. Scand.*, **33**, Suppl. 118, 45 (1955).

(5) Adrenochrome, *e.g.*, is not present in blood: S. Szara, J. Axelrod and S. Perlin, *Am. J. Psychiatry*, **115**, 162 (1958).

(6) For a recent review *cf.* H. Sobotka, N. Barsel and J. D. Chanley, "Progress in the Chemistry of Natural Products," edited by L. Zechmeister, Springer Verlag, Wien, Vol. 14, 1957, pp. 217–243; R. A. Heacock, *Chem. Revs.*, **59**, 181 (1959).

(7) *Cf.* Psylocybine, A. Hofmann, A. Frey, H. Ott, Th. Petrzilka and F. Troxler, *Experientia*, **14**, 397 (1958).

(8) The indole related to mescaline, *i.e.*, 5,6,7-trimethoxyindole, seems to be devoid of central effects in cats [R. D. Morin, F. Bennington and L. C. Clark, Jr., *J. Org. Chem.*, **22**, 331 (1957); **23**, 19 (1958)]; *cf.*, *J. Org. Chem.*, **24**, 917 (1959).

(9) E. G. Ball and T. T. Chen, *J. Biol. Chem.*, **102**, 691 (1933); K. Wiesner, *Biochem. Z.*, **313**, 48 (1942); **314**, 214 (1943).

(10) L. F. Fieser and M. A. Peters, THIS JOURNAL, **53**, 793 (1931).