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

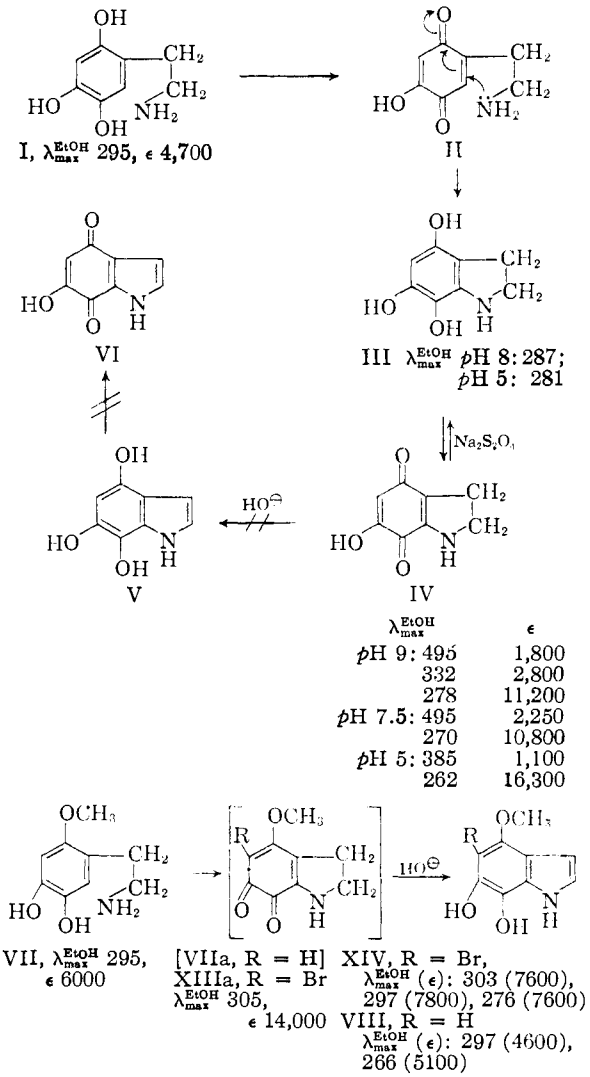
RECEIVED MAY 15, 1959

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).

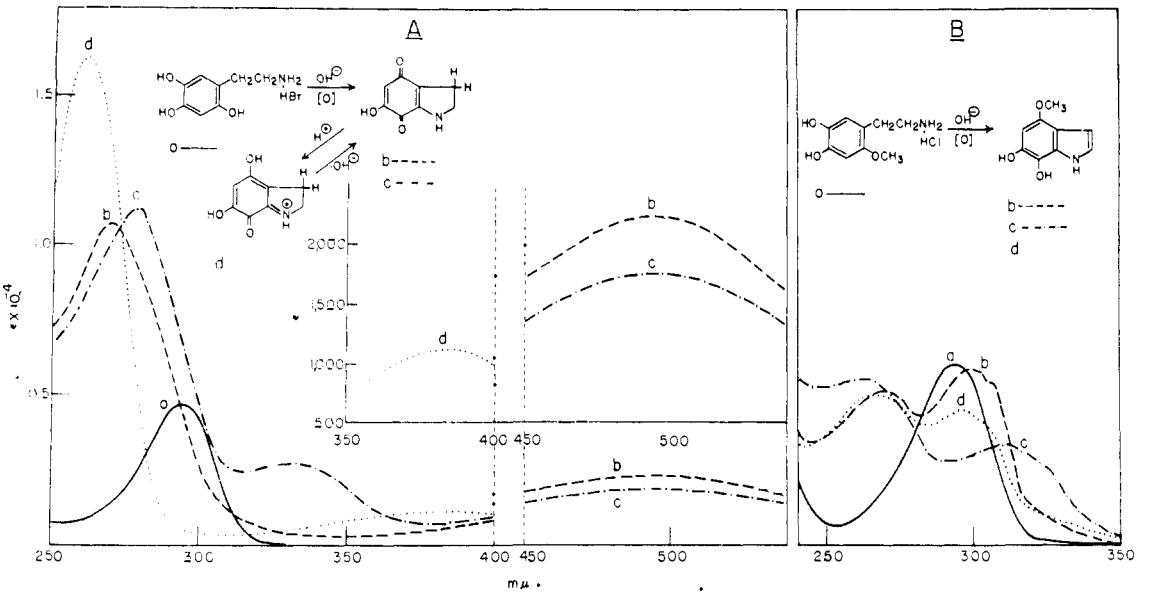
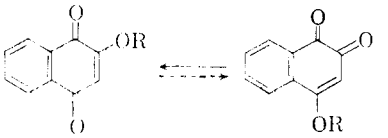


Fig. 1.—Spectral shifts observed with (A) 2,4,5-trihydroxyphenethylamine (I) and (B) 2-methoxy-4,5-dihydroxyphenethylamine (VII) in: (a) neutral ethanol (—), (b) after addition of one drop of 0.1 *N* NaOH (----), (c) after addition of one drop of 1 *N* NaOH (·-·-·) and (d) after reacidification with HCl (·····).

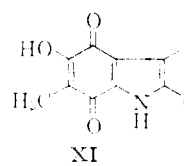
the methyl ethers (R = CH_3) have been of diagnostic value in the determination of the amounts of α- and β-quinone forms (R = H) present at equilibrium.



IX, α, E_h 0.353 X, β, E_h 0.433

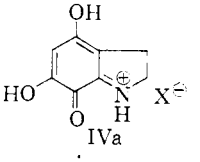
Spectrophotometric observations point to the formation of the aminochrome by the rapid sequence I → IV. Whether such a sequence is possibly involved in the test developed by Carlsson[11] for the clinical assay of dopamine, is being investigated by Dr. J. R. Crout in the National Heart Institute.

The two amines I and VII behave quite differently when base is added to their ethanolic solution in contact with air. With 0.1 *N* sodium hydroxide λ_{max} 295 mμ of I (Fig. 1, A-a) splits into two peaks, 270 and 495 mμ (Fig. 1, A-b). By comparison, hydroxy-*p*-quinone absorbs at 260 (ε 5,000) and 480–485 mμ (ε 2,100). For the quinone XI a band at 460 mμ (ε 540) has been noted.[12] A clear distinction between the aminochrome IV and the indole quinone VI was possible after treatment with a reducing agent, $Na_2S_2O_4$, which after acidification showed 281 mμ (EtOH) in agreement with a trihydroxybenzene (hydroxyhydroquinone: λ_{max} 288 (ε 2,800)),[13] but not with a hydroxyindole (*cf.* VIII). The acid shift of the aminochrome IV to 385 and 262 mμ (Fig. 1, A-d) is reminiscent of the absorption of *o*-quinone (λ_{max} 390, ε 4,200) and may possibly indicate an *o*-quinoid structure IVa present in



$\lambda_{max}^{CH_3OH}$	ε
460	5,400
331	7,200
288	14,500
240	15,100

XI



IVa

acid solution. The methoxy amine undergoes oxidative cyclization and rearrangement to the hydroxy*indole* VIII (Fig. 1, B-d) to judge from the spectral similarity of VIII with XIV (Fig. 2, D). It appears, therefore, that there is a noteworthy difference between *o*- and *p*-quinoid aminochromes. The former of type VIIa, XIIIa, adrenochrome or dopachrome undergo easy base-catalyzed isomerization to dihydroxyindoles (VIII, XIV), whereas a *p*-quinoid aminochrome of type IV is stable to base; its oxidation–reduction potential is too low for an internal hydrogen shift.

Conclusive proof for the course and mechanism of these rearrangements came from studies with the tritium labeled amines. Table I summarizes the results. As expected there is no loss of tritium in the formation of the *p*-quinoid aminochrome corresponding to IV, whereas approximately one atom of tritium is found in the solvent containing the dihydroxymethoxyindole corresponding to VIII.

It is possible to visualize trihydroxyphenethylamine as a key intermediate to which metabolic routes may lead from dopamine, from *m*-tyramine and 2,5-dihydroxyphenethylamine.[14]

(11) Å. Bertler, A. Carlsson, E. Rosengren and B. Waldeck, *Kungl. Fysiografiska Sällskapets i Lund Förhandlingar*, **28**, 121 (1958).

(12) *Cf.* H.-J. Teuber and G. Thaler, *Ber.*, **91**, 2262 (1958).

(13) H. S. Mason, *J. Biol. Chem.*, **181**, 803 (1949); *cf.* W. Flaig and J.-C. Salfeld, *Ann.*, **618**, 117 (1958).

(14) G. Leaf and A. Neuberger, *Biochem. J.*, **43**, 606 (1948).

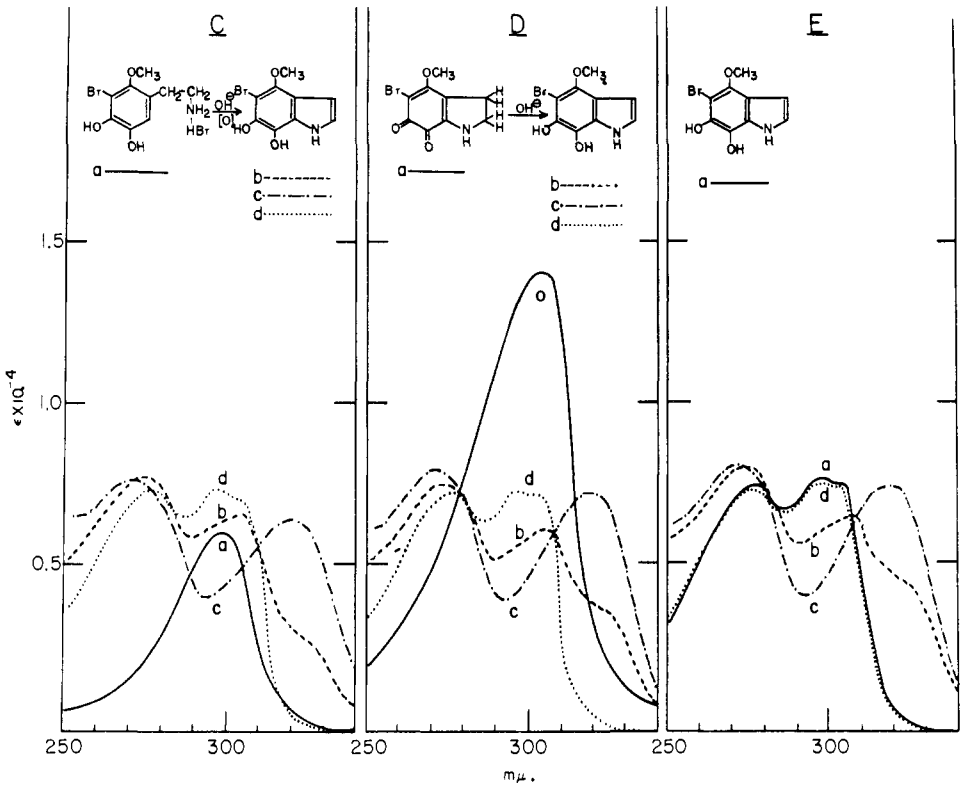


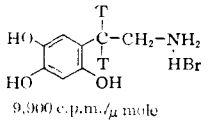
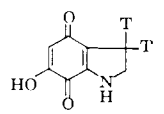
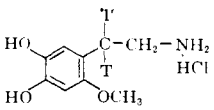
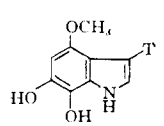
Fig. 2.—Spectral shifts observed with (C) 2-methoxy-3-bromo-4,5-dihydroxyphenethylamine (IXa), (D) the aminochromo Xa and (E) the indole derivative XII in: (a) neutral ethanol (—), (b) after addition of one drop of 0.1 *N* NaOH (----), (c) after addition of one drop of 1 *N* NaOH (·····) and (d) after reacidification with HCl (·····).

The preparation of crystalline aminochromes and hydroxyindoles was possible starting from the hydrogen bromide addition product to N-carbobenzyloxy-2-methoxy-4,5-dihydroxyphenethylamine-quinone, which could have either structure XIIa or XIIb. This was decided by liberation of the amine. Rapid autoxidation, cyclization and reoxidation led to the bright red aminochrome XIIIa which still contained one bromine atom. On the other hand, cyclization of XIIb might have led to a bromine-free compound XIIIb by an internal S_N2 reaction.

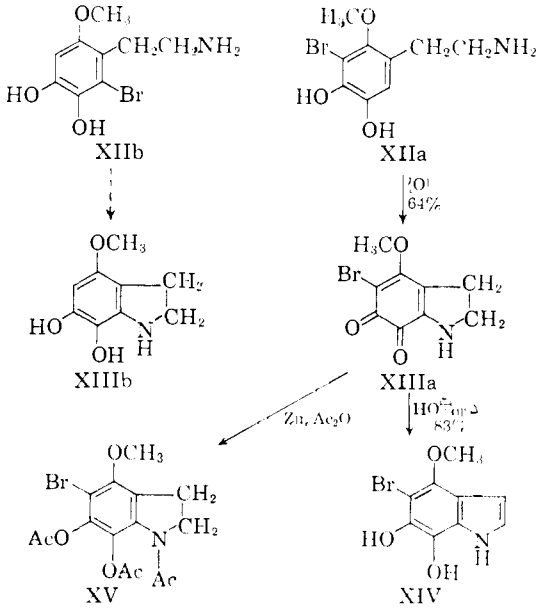
It is surprising to note that the analogous oxidative cyclization of 6-methyladrenaline to 4-methyladrenochrome should not occur.[15]

TABLE I

DISTRIBUTION OF TRITIUM ACTIVITIES IN THE FORMATION AND REARRANGEMENT OF *o*- AND *p*-QUINOID AMINOCHROMES

Specific activities of tritiated amines	Specific activities of reaction products[a]	
	Residual fraction	Volatile fraction
 9,900 c.p.m./μ mole	 10,200	Tritium content of the solvent <40
 11,100 c.p.m./μ mole	 5,900	Tritium content of the solvent 4200

[a] To the solution of 1 μmole of I-H³ (or VII-H³) in 1 ml. of ethanol was added 1 drop of 0.1 *N* NaOH. After 30 seconds 2 drops of 0.1 *N* HCl was added. Residue and solvent were separated in a closed evacuated system by lyophilization and the activities determined in the usual way (see Experimental part).



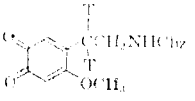
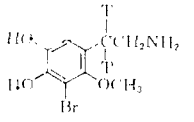
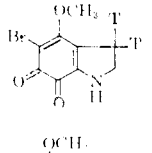
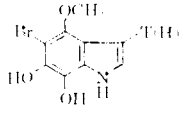
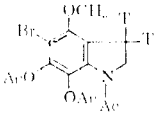
(15) *Cf.* R. S. Grewal, *Brit. J. Pharmacol.*, **7**, 338 (1952).

The well-known internal dismutation of an aminochrome to a hydroxyindole[16,17] was effected (i) by refluxing in *t*-butyl alcohol, (ii) by sublimation and (iii) by the action of base. Figure 2 summarizes the spectrophotometric observations. In basic solution the spectra of the three compounds XIIa, XIIIa and XIV become identical and retain their identity on acidification.

Mild reductive acetylation leads to the N,O,O-triacetylindole XV without any internal rearrangement as indicated by the full retention of tritium activity (see below). Stronger conditions not only effect rearrangement to the indole but also remove the nuclear bromine.

The rearrangement and the preceding steps were also followed with regard to loss of tritium starting with N-carbobenzyloxy-3,4-dihydroxyphenethylamine-β,β-H^3 (*cf.* following paper). Table II

TABLE II

Structure	Type of the reacn., c.p.m./μmole			
	750			
	720	820		
		810	690	690
			70	
				780
Ratio of residual tritium	1:1	1:1	1:0.1	1:1

shows that randomization of tritium does not occur until the aminochrome XIIIa undergoes dismutation to the dihydroxyindole XIV-H^3.

Experimental[18]

Polarographic Determination of the Half-wave Potentials.[19] 2,4,5-Trihydroxyphenethylamine (I) Hydrobromide.—The amine, 2.1 mg., was dissolved in 10 ml. of aqueous phosphate buffer, *p*H 6.87. Potentials were taken with respect to a saturated sulfate cell which had a measured potential of +0.395 v. with regard to a saturated calomel electrode at 25°. At this temperature 2 forward and backward runs of the amine were taken on a self-recording polarograph and the arithmetic mean of the half-wave was taken to eliminate any error coming from the log of the system. One observed $E_{1/2}$ = −0.554 v. *vs.* satd. sulfate reference cell (−0.159 v. *vs.* calomel reference cell, +0.083 v. *vs.* normal hydrogen electrode).

2-Methoxy-4,5-dihydroxyphenethylamine (VII) Hydrochloride.—By the same technique 2.2 mg. of the amine VII gave $E_{1/2}$ −0.465 v. *vs.* sulfate cell (−0.070 v. *vs.* calomel cell, +0.172 v. *vs.* normal hydrogen electrode (where satd. calomel electrode = +0.242 v. with regard to normal hydrogen electrode)).

Spectrophotometric Studies. Formation of 4-Methoxy-6,7-dihydroxyindole (VIII).—The oxidative cyclization and rearrangement of 6-methoxydopamine (VII) hydrochloride to the corresponding dihydroxyindole VIII by the action of a drop of 0.1 *N* and 1.0 *N* sodium hydroxide were followed spectroscopically. The results are shown in Fig. 1, B.

Formation of 4,6,7-Trihydroxydihydroindolequinone (IV).—The oxidative cyclization and oxidation of 2,4,5-trihydroxyphenethylamine (I) hydrobromide to the trihydroxydihydroindolequinone (IV) by the action of a drop of 0.1 *N* and 1.0 *N* sodium hydroxide were followed spectroscopically. The results are shown in Fig. 1, A. The solution of the quinone IV showed a reversible color phenomenon, pink in basic and yellow in acidic solution. The extinction of the absorption maximum (495 mμ) decreased gradually, probably as the result of polymerization.

Formation of 4,6,7-Trihydroxydihydroindole (III).—To the pink basic solution of the quinone IV in ethanol ($\lambda_{max}^{EtOH\ (pH\ 7.5)}$ 495 (ϵ 2,250); 270 mμ (ϵ 10,800)) was added an excess of aqueous $Na_2S_2O_4$ solution (*p*H 7.5) and mixed. The colorless solution showed $\lambda_{max}^{EtOH\ (pH\ 7.5)}$ 287 mμ and $\lambda_{max}^{EtOH\ (pH\ 5-6)}$ 281 mμ. In order to cancel the significant absorption of $Na_2S_2O_4$ in this region, the reference cell contained an equal amount of $Na_2S_2O_4$.

β-(2-Methoxy-3-bromo-4,5-dihydroxyphenylethylamine (XIIa) Hydrobromide.—Two grams of N-carbobenzyloxy-6-methoxydopaminequinone[1] was dissolved at 0° in 10 ml. of anhydrous 30% hydrobromic acid in glacial acetic acid solution and the solution concentrated to a small volume in a vacuum desiccator over sodium hydroxide in the cold room. The crystalline hydrobromide which separated from the brown reaction mixture was collected on a fritted glass filter and washed with a small volume of cold glacial acetic acid, yielding 1.83 g. and an additional crop of 0.25 g. from the mother liquors (total yield 2.08 g., 95.5%). After recrystallization from glacial acetic acid the hydrobromide had m.p. 218–219° dec.; λ_{max}^{Nujol} (μ): 2.87m (OH); 2.95w (NH); 3.14s (OH); 6.18m; 6.25m; 6.67s. λ_{max}^{EtOH} (mμ): 298 (ϵ 5,800).

Anal. Calcd. for $C_9H_{12}NO_3Br\cdot HBr$: C, 31.54; H, 3.53; N, 4.08; Br, 46.6. Found: C, 31.97; H, 3.90; N, 3.96; Br, 47.03.

Free Base XIIa.—To the solution of 60 mg. of sodium bicarbonate in 3 ml. of water was added 120 mg. of the hydrobromide of XIIa under nitrogen. When the stoppered flask was shaken and placed in an ice-bath a colorless crystalline product separated from the initially clear solution. The voluminous crystalline precipitate was collected, washed with cold water and dried immediately. All operations had to be carried out under a stream of nitrogen. One obtained 75 mg. (82%) of the free amine XIIa, m.p. 136–139°, which was easily oxidized by air in the wet state or in solution.

Anal. Calcd. for $C_9H_{12}NO_3Br$: N, 5.34; Br, 30.2; CH_3O, 11.8. Found: N, 5.47; Br, 29.90; CH_3O, 11.91.

4-Methoxy-5-bromo-6,7-dihydroxydihydroindolequinone (XIIIa).—To the solution of 1.4 g. of β-(2-methoxy-3-bromo-4,5-dihydroxyphenyl)-ethylamine (XIIa) hydrobromide in 10 ml. of water was added at 0° 0.7 g. of sodium bicarbonate whereupon the free base was precipitated. Then oxygen was bubbled through the mixture. When the flask was stoppered and shaken vigorously, the suspension turned red and gradually deposited a red precipitate. After 10 minutes, the reaction vessel was recharged with oxygen and shaken for another 10 min. This procedure was repeated once more. The deep red reaction mixture was cooled in ice, the precipitate was collected, washed with cold water and dried. The crude quinone melted at 123–126°. After recrystallization from dichloroethane and petroleum ether, one obtained bright red crystals (670 mg., 64%), m.p. 127.5–128.5°; λ_{max}^{Nujol} (μ): 3.06s (NH); 6.06s (CO); 6.22s; 6.39m; 7.09m.

(16) J. D. Bu'Lock and J. Harley-Mason, *J. Chem. Soc.*, 2248 (1951).

(17) *Cf.* H. J. Richter and B. C. Weberg, THIS JOURNAL, **80**, 6446 (1958).

(18) All melting points are corrected. All boiling points are uncorrected. Analyses have been performed by Dr. W. C. Alford and associates of the Analytical Services Unit of this Laboratory. All ultraviolet spectra were measured in a self-recording Cary spectrophotometer.

(19) We are greatly indebted to Prof. Charles Wiesner, University of New Brunswick, for kindly arranging for the micro-determination of the polarographic half-waves in his department.

λ_{max}^{KB} (μ): 3.05s (NH); 6.06s (CO); 6.22s; 6.36m; 6.48m. λ_{max}^{EtOH} (mμ): 305 (ϵ 14,000).

Anal. Calcd. for $C_9H_8NO_3Br$: C, 41.88; H, 3.12; N, 5.43; Br, 30.9; CH_3O, 12.03. Found: C, 42.75; H, 3.49; N, 5.75; Br, 30.24; CH_3O, 13.42.

Rearrangement of the Aminochrome XIIIa to the Isomeric Dihydroxyindole XIV. (A) Heat-catalyzed Isomerization.—The suspension of 105 mg. of the aminochrome XIIIa in 7 ml. of redistilled *t*-butyl alcohol was refluxed for three hours under nitrogen. The reaction mixture was cooled and evaporated to dryness under reduced pressure. The residue was purified by sublimation ($<10^{-3}$ mm., bath temperature 110–115°). After recrystallization from ether, one obtained colorless prisms (87 mg., 83%), m.p. 138.5–140.5°.

The dihydroxyindole XIV gave a deep green color reaction with ferric chloride reagent, whereas the aminochrome XIIIa gave the same green color reaction *via* a transient orange color.

Anal. Calcd. for $C_9H_8NO_3Br$: N, 5.43; Br, 30.9; CH_3O, 12.03. Found: N, 5.82; Br, 31.75; CH_3O, 12.4; $\lambda_{max}^{CHCl_3}$ (μ): 2.84s (OH); 2.87s (indole NH); 5.90w; 6.11s; 6.22m; 6.34s. λ_{max}^{EtOH} (mμ): 215 (ϵ 30,000); 276 (ϵ 7,600); 297 (ϵ 7,800); 303 (ϵ 7,600).

(B) **By Sublimation.**—When 25 mg. of the aminochrome XIIIa was sublimed in high vacuum ($<10^{-3}$ mm.) keeping the bath temperature first close to its melting point (115–120°), an almost colorless sublimate was formed which had m.p. 136.5–139°, identical with XIV with regard to m.p., ferric chloride test and ultraviolet and infrared spectra.

(C) **Base-catalyzed Isomerization.**—The rearrangement of 2,3-dihydro-4-methoxy-5-bromo-6,7-dihydroxyindolequinone (XIIIa) to the corresponding dihydroxyindole XIV by the action of a drop of 0.1 *N* and 1.0 *N* sodium hydroxide was followed spectroscopically by comparison with the three reference compounds, *i.e.*, β-(2-methoxy-3-bromo-4,5-dihydroxyphenyl)-ethylamine (XIIa), the aminochrome XIIIa and 4-methoxy-5-bromo-6,7-dihydroxyindole (XIV) obtained from the heat-catalyzed isomerization. The results are shown in Fig. 2.

Reductive Acetylation of the Aminochrome XIIIa.—The suspension of 70 mg. of the aminochrome XIIIa and 150 mg. of zinc dust in 2 ml. of acetic anhydride was boiled for 5 minutes under nitrogen. The mixture was poured into water, neutralized with sodium bicarbonate, and extracted three times with ethyl acetate. The combined extracts were washed and evaporated to dryness. The residual oil on trituration with ether became a crystalline solid, m.p. 148–150°. The triacetyl compound XV was purified by sublimation ($<10^{-3}$ mm., bath temperature 120–130°), and recrystallized from ether, m.p. 149–151°. The yield was 15 mg.

Anal. Calcd. for $C_{15}H_{16}NO_6Br$: C, 46.65; H, 4.18; N, 3.64; Br, 20.63. Found: C, 47.75; H, 4.52; N, 4.17; Br, 20.15; λ_{max}^{Nujol} (μ): 5.67s (CO); 6.00s (>NCO); 6.23m; 6.31m; 7.17m. λ_{max}^{EtOH}: 218; 257; 302 mμ.

Acetylation of the Aminochrome XIIIa Proceeding with Removal of Nuclear Bromine.—A suspension of 150 mg. of the aminochrome XIIIa and 300 mg. of zinc dust in 3 ml. of acetic anhydride was refluxed for 6 hours. The mixture was poured into water, neutralized with bicarbonate, and extracted with ethyl acetate. The extracts were evaporated to dryness. The residual material was sublimed in high vacuum and recrystallized from ether, yielding 50 mg. of colorless needles, m.p. 131–134°. The substance gave a negative Beilstein test; $\lambda_{max}^{CHCl_3}$ (μ): 5.66s (CO); 6.03s (>NCO); 6.19m; 6.69s; 6.90s; 7.10s. λ_{max}^{EtOH}: 215; 254; 300 mμ.

Anal. Found: C, 62.79; H, 5.90; N, 5.33.

Tritium Studies.—Tritium activities of samples were measured by a Packard liquid scintillation spectrophotometer. The sample solution was prepared by the following procedure: To the solution of a certain amount (approximately 100 γ) of the material in 2 ml. of methanol was added 10 ml. of the "toluene solution" which consisted of 3 g. of DPO (2,5-diphenyl-oxazole) and 10 mg. of PoPoP (1,4-bis-[2-(5-phenyloxazolyl)]-benzene) in one l. of toluene (sample solution A). To another batch of the same solution was added a certain amount of standard tritium solution (sample solution A^S). As a background blank, 2 ml. of methanol was added to 10 ml. of the "toluene solution" (background blank solution B). To another batch of the same solution was also added a certain amount of standard tritium solution (background blank solution B^S). These four solutions for each separate fraction were measured using Channel 9 (1310 volt) and the counting region 10 to 100. The results were calculated as follows:

The factor correcting the observed number of counts for the self-adsorption of the material was $(B^S-B)/A^S-A)$; the actual number of counts per minute = $(A-B) \times (B^S-B)/(A^S-A)$; the number of counts per minute per μmole (c.p.m./μmole) = $(A-B) \times (B^S-B)MW/(A^S-A)w$ where A, A^S, B, B^S are observed counts per minute for each solution, w is sample weight in γ and MW is molecular weight number of the material.

β-(2-Methoxy-3-bromo-4,5-dihydroxyphenyl)-ethylamine-β,β-H^3 Hydrobromide.[1]—Five hundred milligrams of N-carbobenzyloxy-6-methoxydopaminequinone-β-β-H^3, specific activity 750 c.p.m./μmole, was dissolved at 0° in 4 ml. of anhydrous 30% hydrobromic acid in glacial acetic acid solution and the solution was concentrated to a small volume in a vacuum desiccator over sodium hydroxide in the cold room. The purification of the reaction product was carried out as described for XIIa. One obtained 415 mg., m.p. 218–219° dec., specific activity 720 c.p.m./μmole, as measured in a liquid scintillation counter (Table II).

4-Methoxy-5-bromo-6,7-dihydroxy-dihydroindolequinone-3,3-H^3.—The aqueous solution of 700 mg. of β-(2-methoxy-3-bromo-4,5-dihydroxyphenyl)-ethylamine-β,β-H^3 hydrobromide, specific activity 820 c.p.m./μmole, was neutralized with 400 mg. of sodium bicarbonate and subjected to oxidative cyclization as described for XIIIa. One obtained 290 mg. of red crystals, m.p. 127–128.5°, specific activity 810 c.p.m./μmole (Table II).

Isomerization of the Aminochrome to the Dihydroxyindole.—The suspension of 60 mg. of 4-methoxy-5-bromo-6,7-dihydroxy-dihydroindolequinone-3,3-H^3, specific activity 690 c.p.m./μmole, in 5 ml. of *t*-butyl alcohol was refluxed under nitrogen as described for XIV. One obtained 38 mg. of colorless crystals, m.p. 139.5–141°, specific actvity 70 c.p.m./μmole (Table II).

1,6,7-Triacetyl-4-methoxy-5-bromodihydroindole-3,3-H^3.—The reductive acetylation of 30 mg. of 4-methoxy-5-bromo-6,7-dihydroxy-dihydroindolequinone-3,3-H^3, specific activity 690 c.p.m./μmole, was carried out with zinc dust in acetic anhydride as described for XV. There was obtained 10 mg. of colorless needles, m.p. 140–145°, specific activity 780 c.p.m./μmole (Table II).

Heat-catalyzed Exchange of Tritium of 4-Methoxy-5-bromo-6,7-dihydroxyindole-3-H^3.—The solution of 15 mg. of 4-methoxy-5-bromo-6,7-dihydroxyindole-3-H^3, specific activity 50 c.p.m./μmole, in *t*-butyl alcohol was refluxed for 15 hours under nitrogen. The solution was evaporated to dryness *in vacuo*. The residue had specific activity of 20 c.p.m./μmole. Most of the activity was in the solvent.

Acid-catalyzed Exchange of Tritium of Dihydroxyindole.—To the solution of 2.228 mg. of 4-methoxy-5-bromo-6,7-dihydroxyindole-3-H^3 was added 2 drops of concentrated hydrochloric acid and the solution allowed to stand for 60 hours at room temperature under nitrogen. The solution was evaporated to dryness under reduced pressure. As a reference, 2.117 mg. of the same dihydroxyindole-3-H^3 was dissolved in methanol and left for 60 hours under nitrogen and evaporated to dryness under reduced pressure. The tritium activities of both residues were measured. The former had 20 c.p.m./μmole; the latter had 50 c.p.m./μmole.

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