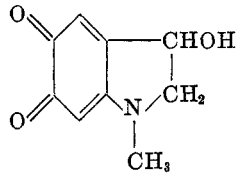
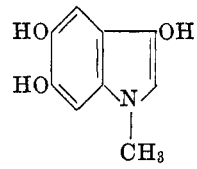
adrenolutin [3,5,6-trihydroxy-*N*-methylindole (III)]. Formulas II and III were the ones first advanced for these compounds and are still widely used; however,

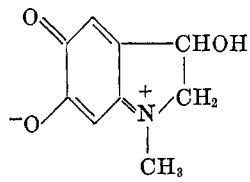


II

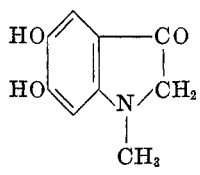


III

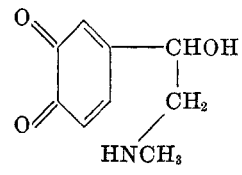
the physical and chemical properties of adrenochrome are better explained by the zwitterionic form (IV) (112, 113). Indoxyls have recently been shown to exist mainly in the keto form, both in the solid state and in solution in nonpolar solvents (130). The same considerations probably apply to adrenolutin (120), so that its formula is better written as 2,3-dihydro-5,6-dihydroxy-3-keto-*N*-methylindole, i.e., 5,6-dihydroxy-*N*-methylindoxyl (V). Throughout this review formulas IV and V will be used. The red oxidation product of adrenaline



IV

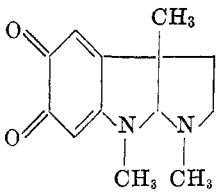


V



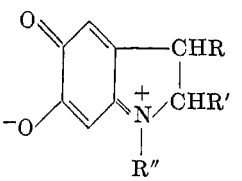
VI

was finally isolated in crystalline form from the products of an enzymatic oxidation and correctly identified by Green and Richter in 1937, who proposed the name "adrenochrome" (109). Two years previously, Weinstein and Manning had obtained a red crystalline product from the oxidation of adrenaline with silver oxide, but had erroneously believed it to be the hypothetical "adrenaline-quinone" (VI) (264). The highly fluorescent material was not isolated and characterized for a further twelve years (158, 159). The concept of the "2,3-dihydroindole-5,6-quinone" structure was introduced by Raper in 1927 for the red pigments observed during his classical studies on the oxidation of tyrosine and "dopa" (3,4-dihydroxyphenylalanine) with tyrosinase (209). In 1930 Dulière and Raper first employed silver oxide for the oxidation of dopa and epinine (3,4-dihydroxyphenyl-*N*-methylethylamine) to red quinones (73). Shortly after, a crystalline monophenylhydrazone was obtained from a similar product derived from epinine (51). A closely related, although not strictly comparable, compound containing a 2,3-dihydroindole-5,6-quinone nucleus is rubreserine (VII). The formation of red colors during the use of alkali in the isolation of the alkaloid of the calabar bean (125, 147) was noted as early as 1864. This red substance was later isolated and named rubreserine (74, 75, 201, 221), although the correct structure (VII) was not proposed for the substance until 1942 (80). Recently Sobotka and Austin have suggested the term "aminochrome" as a generic name

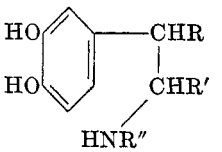


VII

Rubreserine



VIII



IX

for the highly colored 2,3-dihydroindole-5,6-quinones (VIII) obtained by the oxidative cyclization of suitable "catechol amines" (i.e., substituted 3,4-dihydroxyphenylethylamines (IX)) (232). A limited survey of the chemistry and physiology of the aminochromes has recently appeared (233). In the present review the chemistry of these compounds has been comprehensively covered and much information is presented that was not given in the previous paper. A short section on the physiological activity of the aminochromes has been included, mainly to serve as a guide to further reading on the subject.

In the past two decades a wide range of physiological activity has been ascribed to some of these substances and many claims and counter-claims have been advanced. However, this has served to stimulate interest in the physical and chemical properties of this interesting group of organic compounds.

II. Oxidation of Catechol Amine in Dilute Aqueous Solution

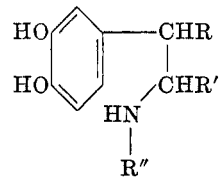
A. Nonenzymatic oxidation

The facility with which adrenaline gives color reactions with alkalis, oxidizing agents, and certain metallic salts has been known for a long time; e.g., with ferric chloride it gives a green color turning violet on addition of alkali (*cf.* 110, 172, 211, 258, 259). Some of these reactions are merely due to complex formation, but in general they are due to oxidation.

1. At alkaline pH

On the addition of alkali, dilute solutions of adrenaline exhibit a transient yellow-green fluorescence and become "inactivated" from the physiological point of view (13, 21, 105, 157, 194, 224, 250, 265). The fluorescent material does not form if oxygen (or air) is rigorously excluded from the system (105, 148, 158), and its formation has been shown to correspond to the uptake of one molecule of oxygen per molecule of adrenaline (158), i.e., the compound has the same oxidation level as adrenochrome. The substance responsible for this phenomenon was isolated and correctly identified by Lund as 5,6-dihydroxy-*N*-methylindoxyl (V) (158, 159) one year after the correct prediction of its structure on theoretical grounds (76). If no attempt is made to control the oxidation, absorption of oxygen continues after the uptake of two atoms per molecule and the initial fluorescence rapidly fades; the final products in the solution, which may be clear yellow or turbid brown in color (depending on the adrenaline concentration), correspond to an oxygen uptake of about three molecules per molecule of adrenaline (123, 158, 250).

Some other catechol amines closely related to adrenaline exhibit a similar transient fluorescence on alkaline oxidation. *N*-Ethylnoradrenaline (IX: R = OH; R′ = H; R″ = C_2H_5) and *N*-isopropylnoradrenaline (IX: R = OH; R′ = H; R″ = *i*-C_3H_7) show a strong fluorescence (11, 16, 85, 160), but the fluorescence derived from noradrenaline (IX: R = OH; R′ = R″ = H) under similar conditions is very much weaker (57, 85, 105, 160); α-methylnoradrenaline (IX:



IX

R = OH; R′ = CH_3; R″ = H) does not apparently exhibit this phenomenon at all on alkaline oxidation (85, 160). No fluorescence is observed at all during the alkaline oxidation of 3-hydroxytyramine (IX: R = R′ = R″ = H), epinine (IX: R = R′ = H; R″ = CH_3), or dopa (IX: R = R″ = H; R′ = COOH) (11, 16, 85, 105, 160).

The structural requirements for the formation of a strongly fluorescent derivative on alkaline oxidation of a catechol amine appear to be a β-hydroxy and an *N*-alkyl group in the ethylamine side chain of the molecule (11, 16, 47, 85, 181).

2. Catalytic oxidation at intermediate pH's

At an intermediate pH (6–8) adrenaline in aqueous buffer is oxidized to red substances; the speed of the reaction and the nature of the final products are dependent on the catalysts and buffers employed. Neutral solutions of adrenaline in the complete absence of traces of heavy metals probably do not undergo autoxidation at any appreciable rate (*cf.* 52, 72, 250, 253), although there is some uncertainty on this point (110, 131). In general, oxygen uptake begins only after a considerable induction period (52, 250). In such cases, traces of impurities may initiate the reaction and, later, adrenochrome catalysis (87, 250) becomes the dominant factor as the concentration of adrenochrome in the system builds up. The oxidation in aqueous buffer (acetate, bicarbonate, maleate, phosphate) is markedly catalyzed by metallic cations, especially cupric copper, manganese, and nickel (42, 43, 52, 53, 131, 250, 253). The reaction depends on the formation of an autoxidizable adrenaline–metal complex; cupric ions are more efficient catalysts than manganese or nickel ions. Magnesium or ferric ions do not form autoxidizable complexes, although the presence of ferric ions does lead to a slight increase in oxygen uptake relative to controls (52). In phosphate buffer, catalysis by cupric ions leads eventually to the uptake of three molecules of oxygen per adrenaline molecule (52, 54); catalysis by manganese or nickel ions leads to the consumption of about four molecular equivalents of oxygen (52). However, in bicarbonate buffer the copper-catalyzed oxidation stops at the two-atom (i.e., adrenochrome) stage (54, 55). Fenton's reagent oxidizes adrenaline to adrenochrome at pH 4.5 by a free-radical mechanism (178). Ferritin, the iron-contain-

ing protein, behaves similarly in the presence of hydrogen peroxide at pH 4.5 (178). Although the complex formed between iron and adrenaline at pH 7.4 appears to be stable, oxidation of the adrenaline molecule under these conditions by a process involving molecular oxygen does in fact occur (110). The oxidation effected by ferritin at pH 7.4 involves molecular oxygen and the "active" iron in ferritin, apoferritin being inactive (110). Eventually the iron-catalyzed oxidations pass the adrenochrome stage and brown melanins are produced (110). Iron-chelating agents such as EDTA greatly increase the oxidation rate; ferricytochrome-c (an iron chelate) is more active than inorganic ferric ions (110). Other catalysts that are effective in bringing about the autoxidation of adrenaline include hematin and methemoglobin (87, 110, 227) and adrenochrome itself (87, 250).

Reducing agents such as sodium bisulfite (250), BAL (2,3-dimercapto-1-propanol) (14), ascorbic acid (257), and plasma proteins (at pH 7.1) (254) inhibit the oxidation of adrenaline by molecular oxygen. Proteins and amino acids slow the oxidation rate in slightly alkaline solution but not in acid solution (257). The cupric ion-catalyzed oxidation is accelerated by certain organic compounds containing the glyoxaline nucleus; this effect can be reduced or totally abolished by the addition of certain amino acids (44).

Noradrenaline also forms an autoxidizable complex with cupric ions in bicarbonate buffer, being oxidized to noradrenochrome (56); however, this product is less stable than adrenochrome and the red solutions rapidly decompose, first to violet and finally to grey insoluble products. In phosphate buffer (pH 7.3), cupric, nickel, and manganese ions catalyze the oxidation of noradrenaline and eventually two molecules of oxygen per molecule of substrate are absorbed (56). Noradrenaline appears to undergo autoxidation in Krebs' buffer, but at a slower rate than adrenaline; in this case the difference in oxidation rate is accentuated by the presence of cupric ions (131).

Other catechol amines [e.g., epinine, 3-hydroxytyramine, dopa, and β-(3,4-dihydroxyphenyl)-*N*-methylalanine] undergo catalytic oxidation by molecular oxygen in the presence of manganese, cupric, cobalt, nickel, and to a lesser degree ferric ions, manganese ions being the most effective. During the oxidation of each substrate carbon dioxide and hydrogen peroxide are formed (107).

B. ENZYMATIC OXIDATION

It is not the object of the present review to discuss at length the oxidation of catechol amines in the presence of tyrosinase and polyphenolases. These processes have been considered in detail in earlier reviews (*cf.* 10, 83, 84, 175, 187, 238), but enzymatic processes leading to aminochrome formation will be briefly discussed.

Raper and his coworkers in their extensive studies on tyrosinase (from plants or invertebrates) demonstrated that this enzyme catalyzed the oxidation of several catechol amines to red pigments (73, 123, 208, 209, 210), formulated as 2,3-dihydroindole-5,6-quinones. Later similar catechol oxidases were shown to

be present in mammalian tissues (128), and the oxidation of dopa by plant and animal tyrosinases was shown to follow the same path in each case (173). In 1937 Green and Richter isolated adrenochrome from the oxidation of adrenaline in the presence of a highly purified catechol oxidase obtained from the mushroom *Agaricus campestris* (109). They further showed that the oxidation was catalyzed by a cyanide-insensitive enzyme present in heart and skeletal muscle and by the widespread cytochrome–indophenol oxidase system (109). These observations were soon confirmed and the conversion was shown to be of the order of 95 per cent (202). Adrenochrome formation occurred with consumption of one molecule of oxygen per molecule of substrate (34); the responsible enzyme was a copper protein (34). The cytochrome–cytochrome oxidase system also brings about the oxidation of adrenaline to adrenochrome (34, 87, 227), and recently the presence of an enzyme system in human blood that catalyzes this reaction has been demonstrated (127, 196). The oxidation of adrenaline in the presence of catechol oxidases has been extensively studied in recent years (*cf.* 34, 131, 144, 146, 151, 206, 246, 250, 257, 260); unless specially purified catechol oxidases are used, the oxygen uptake passes the one-molecule stage (i.e., the adrenochrome level) (250) and up to three molecules of oxygen per molecule of adrenaline are consumed (34, 146, 246, 250).

In the presence of polyphenolases adrenaline absorbs more oxygen than noradrenaline at pH 7, but the converse applies at pH 5 (131). The "inactivation" of noradrenaline by catechol and cytochrome oxidases has been briefly reviewed (83). Eseroline takes up to two atoms of oxygen per molecule in the presence of tyrosinase to form a red product, now known to be rubreserine (177).

III. Preparative Methods

A. GENERAL COMMENTS

The enzyme- or metallic ion-catalyzed air oxidation of catechol amines in dilute aqueous solution has not, in general, been utilized as a preparative method for the aminochromes. Notable exceptions are the original preparation of adrenochrome (109) and the preparation of rubreserine by the direct oxidation of eseroline by gaseous oxygen (221). In the past two decades the use of inorganic oxidizing agents (particularly silver oxide and potassium ferricyanide) has found widespread use for the preparation of these compounds either in the solid state or in solution.

B. ADRENOCHROME

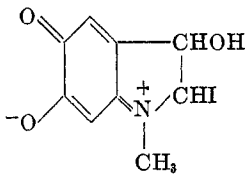
There are numerous references in the literature to the isolation of solid adrenochrome from the products obtained when adrenaline is oxidized in methanol (usually containing a little formic or acetic acid) with silver oxide (46, 77, 88, 113, 121, 135, 158, 162, 167, 205, 218, 229, 232, 256). Since the procedure was first described in 1942 (256) several modifications of the basic technique have been

introduced, e.g., variations in adrenaline concentration, reaction time, reaction temperature, and crystallization temperature (0° to −80°C.), etc. Batches of up to 50 g. of adrenaline have been oxidized at a time with no apparent lowering of yield (46, 162). Many of the earlier preparations were described as being quite unstable (80, 109, 256); even in the solid state at room temperature they gradually decomposed with the formation of insoluble black powders. Adrenochrome prepared by this method often contains up to 0.1 per cent of silver (colloidal and/or ionic) (121), and this might conceivably account for the relative instability of the solid and its solutions. It has recently been shown that passage of the reaction mixture through an anion-exchange resin bed, in the chloride form, prior to crystallization greatly reduces this contamination and the product so obtained appears to be indefinitely stable at room temperature (121). A similar purification seems to have been effected by the use of solid sodium sulfate filter beds (232); centrifugation of the reaction mixture prior to crystallization also gives a stable product (88). Adrenochrome is obtained by the methods described above as dark red-violet rods (if the crystals are crushed, the resulting powder is bright red in color); yields of up to ca. 60 per cent are claimed. The melting point is not a very satisfactory criterion of purity, since the substance decomposes vigorously on melting and values from below 100°C. to 136°C. have been quoted (see references cited above). Silver oxide has been used satisfactorily in other solvents; ethanol has been employed but the product obtained was said to be unstable (80). When methanol containing 12 per cent of a 1:1 mixture of water and ethyl formate was used as the reaction medium, a 74 per cent yield of crude adrenochrome was claimed (236). Owing to the high solubility of adrenochrome in water and the instability of strong aqueous solutions, water is not a satisfactory solvent if isolation of the product is desired, but the adrenochrome solutions obtained can be converted directly to the monosemicarbazone or oxime in high yield (235) or rearranged with alkali to adrenolutin (120). Although the majority of the preparations have been carried out with L-adrenaline, the D- and DL-isomers have been oxidized satisfactorily (26, 167, 232). Potassium ferricyanide in aqueous bicarbonate buffer has been employed extensively to effect the oxidation when adrenochrome solutions are required (47, 55, 76, 109, 260). Other oxidizing agents that have been employed (excluding the catalytic and enzymatic air oxidations) include: lead dioxide in dilute acid (pH 2–6) (47, 207); manganese dioxide (57, 69, 160, 161); mercuric salts in aqueous buffer (pH ca. 6) (40, 41, 77); sodium or potassium persulfate (a trace of ferrous sulfate markedly accelerates the reaction) (113, 240); ceric sulfate (77); Fenton's reagent (ferrous sulfate and hydrogen peroxide) at pH 4.5 (178); potassium permanganate (191, 243); oxygen in the presence of palladized charcoal (33); sodium nitrite (81); iodine under conditions of low acidity (e.g., in the presence of calcium carbonate) (39, 63, 77, 109, 188, 190); and iodic acid and potassium iodate (0.5 mole) (i.e., without excess oxidizing agent and at a neutral pH) (68, 163).

dl-Adrenochrome-β-C^{14} has been prepared from the corresponding labelled adrenaline by oxidation with silver oxide in methanol (222).

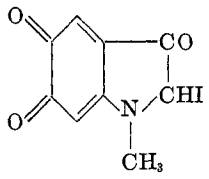
C. 2-BROMO- AND 2-IODOADRENOCHROME

The oxidation of adrenaline with an excess of potassium iodate (or iodic acid)



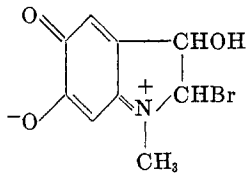
X

2-Iodoadrenochrome



XI

2-Iodoöxoadreno-chrome



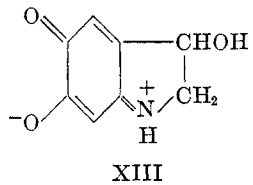
XII

2-Bromoadreno-chrome

in aqueous solution where no attempt is made to control the acidity levels leads to the formation of 2-iodoadrenochrome (X) (39, 212, 232). Six equivalents of iodine are required for the production of the iodochrome (39). Oxidation of adrenaline with iodine usually stops at the adrenochrome stage, although with increasing acidity there is some tendency to produce the 2-iodo derivative (39, 63, 77). Iodic acid (1 mole) in 85 per cent methanol at 40°C. is said to give 2-iodoöxoadrenochrome (XI), but if only half the quantity of oxidant is used (at room temperature) adrenochrome is obtained (163, 164). Bromine in acetate buffer not only oxidizes adrenaline to adrenochrome but effects simultaneous substitution in the indole nucleus, giving rise to 2-bromoadrenochrome (XII) (39, 109, 113, 232). The mechanisms of these reactions are considered in Section VI,E.

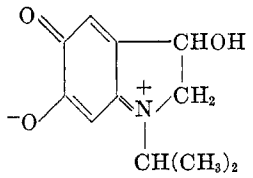
D. OTHER AMINOCHROMES

Noradrenaline is attacked by oxidizing agents in aqueous solution at a slower rate than adrenaline at acid pH, but at about the same rate in neutral solution (*cf.* 56, 83, 161, 189). Noradrenochrome (XIII) appears to be considerably less stable than adrenochrome, as its aqueous and alcoholic solutions decompose rapidly with the formation of dark-colored insoluble products (47, 56). However, noradrenochrome has been obtained in the solid state as "red rings on the sides of the flask" from the silver oxide oxidation of noradrenaline in dry acetonitrile (170). *N*-Isopropylnoradrenochrome (XIV) is described as a purplish-red crystal-



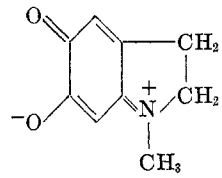
XIII

Noradrenochrome



XIV

N-Isopropylnor-adrenochrome



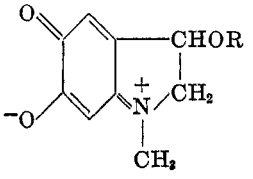
XV

Epinochrome

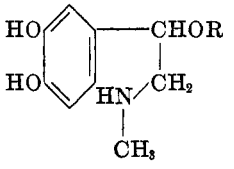
line solid obtained from the silver oxide oxidation of *N*-isopropylnoradrenaline

in a mixture of methanol and 2-propanol (230). Epinochrome (XV) has been obtained as violet rods from the oxidation of epinine in methanol (232); it is, however, not a stable substance and even in the solid state at room temperature rearranges spontaneously to 5,6-dihydroxy-*N*-methylindole (8, 9). As yet, no other unhalogenated aminochromes have been prepared in the solid state. The iodo derivatives are much less soluble in water and separate readily as brown-violet crystalline solids during the oxidation of the catechol amine with excess iodate in aqueous acid solution (20, 39, 47, 212, 232). (The aminochromes that have been isolated in the solid state, although many have not been adequately characterized, are listed in table 1.) Potassium ferricyanide in aqueous sodium bicarbonate buffer solution is a very good oxidizing system for preparing the aminochromes in solution (*cf.* 47, 48, 55, 109, 132, 137, 247, 248, 249, 260). These solutions are quite suitable for conversion to a derivative such as the semicarbazone, or for rearrangement to a 5,6-dihydroxyindole derivative. The catechol amines (Nos. 1, 2, 3, 4, 5, 6, and 8 in table 1 and Nos. 1, 3, 4, 5, 6, 9, 10, 11, 13, 14, 15, and 16 in table 2) have been converted to corresponding 2,3-dihydroindole-5,6-quinones (sometimes only as transient intermediates) by this method. Oxidation with silver oxide in aqueous suspension has also been frequently employed for preparing aqueous solutions of various aminochromes (*cf.* 25, 73, 113). The aminochromes that have been prepared in solution only (some only as transient intermediates), but have been identified by conversion to a stable derivative by an established method, are listed in table 2.

E. ADRENOCHROME *O*-ALKYL ETHERS



XVI



XVII

Adrenochrome 3-*O*-alkyl ethers (XVI: R = CH_3, C_2H_5, *i*-C_3H_7) have recently been isolated as the semicarbazones from the oxidation of the corresponding "adrenaline ethers" [*β*-alkoxy-*β*-(3, 4-dihydroxyphenyl)-*N*-methylethylamines (XVII: R = CH_3, C_2H_5, *i*-C_3H_7)] with silver oxide in methanol (133).

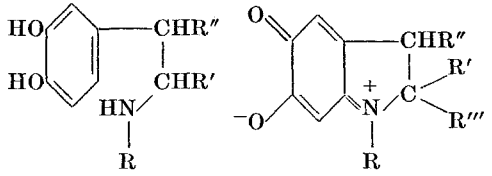
F. POLYCYCLIC SUBSTANCES CONTAINING THE 2,3-DIHYDROINDOLE-5,6-QUINONE RING SYSTEM

1. Rubreserine and brucinequinone

The substances mentioned in this section are not strictly aminochromes, since they are not derived from the oxidation of catechol amines, but they do contain the 1-alkylamino-3,4-benzoquinone group. Rubreserine (VII) is obtained by the oxidation of eseroline (XVIII) in alkaline solution by air or oxygen (221). This

TABLE 1

Aminochromes that have been isolated in the solid state



Catechol amine Aminochrome

Catechol Amine Oxidized				Aminochrome						
Name	R	R′	R″	Name	R	R′	R″	R‴	Melting point (with decomposition)	References
									°C.	
1. Adrenaline	CH_3	H	OH	Adrenochrome	CH_3	H	OH	H	Values quoted in region 100 to 135	(See text)
	CH_3	H	OH	2-Iodoadrenochrome	CH_3	I	OH	H	120	(232) (33, 212)
	CH_3	H	OH	2-Bromoadrenochrome	CH_3	Br	OH	H	90	(232) (109, 113)
2. Noradrenaline	H	H	OH	Noradrenochrome*	H	H	OH	H	Not stated	(170)
	H	H	OH	2-Iodonoradrenochrome	H	I	OH	H	Not stated	(20, 47)
3. α-Methylnoradrenaline	H	CH_3	OH	2-Iodo-2-methylnoradrenochrome	H	I	OH	CH_3	Not stated	(47)
4. *N*-Isopropylnoradrenaline	*i*-C_3H_7	H	OH	*N*-Isopropylnoradrenochrome†	*i*-C_3H_7	H	OH	H	Not stated	(230)
	i-C_3H_7	H	OH	2-Iodo-*N*-isopropylnoradrenochrome	*i*-C_3H_7	I	OH	H	Not stated	(20, 47)
5. Epinine	CH_3	H	H	Epinochrome	CH_3	H	H	H	78	(232)
	CH_3	H	H	3-Iodoepinochrome	CH_3	H	I	H	85–87	(48)
									120	(232) (20)
6. 3-Hydroxytyramine ("dopamine")	H	H	H	3-Iodonorepinochrome‡	H	H	I	H	Not stated	(20)
7. β-(3,4-Dihydroxyphenyl)alanine ethyl ester ("dopa ester")	H	$COOC_2H_5$	H	2-Carbethoxy-3-iodonorepinochrome	H	$COOC_2H_5$	I	H	127	(48)
8. β-(3,4-Dihydroxyphenyl)-*N*-methylalanine ethyl ester ("*N*-methyldopa ester")	CH_3	$COOC_2H_5$	H	2-Carbethoxy-3-iodoepinochrome	CH_3	$COOC_2H_5$	I	H	80	(140)

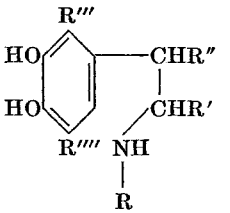
* Noradrenochrome was obtained in the solid state, but no physical constants or analysis were quoted (170). However, noradrenochrome has been identified spectroscopically (25), although as yet no derivatives (such as the semicarbazone) have been prepared.

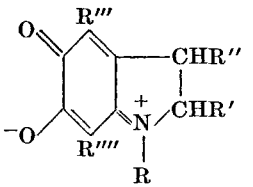
† *N*-Isopropylnoradrenochrome was described as a purplish-red crystalline substance, but no physical constants or analyses were quoted (230). It has been prepared in solution and identified spectroscopically (25), and the semicarbazone has been prepared (25, 47).

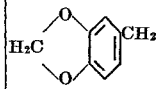
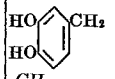
‡ 3-Iodoepinochrome was described as a violet crystalline solid, but no physical constants or analyses were given (20). The position of the iodine atom was not defined, but it has been assumed to be in the 3-position, by analogy with the epinochrome derivative.

TABLE 2

Aminochromes that have been prepared in solution only





Catechol Amine Oxidized	R*	R'	R''	R'''	R''''	Aminochrome	How Identified	References
1. α-Methylnoradrenaline	H	CH_3	OH	H	H	2-Methylnoradreno-chrome	Semicarbazone isolated	(47)
2. α-Ethylnoradrenaline	H	C_2H_5	OH	H	H	2-Ethylnoradrenochrome	Spectroscopic identification	(25)
3. 2-Methylnoradrenaline	H	H	OH	CH_3	H	4-Methylnoradreno-chrome	Semicarbazone isolated	(132)
4. *N*-(β-Hydroxyethyl)noradrenaline	$HOCH_2CH_2$	H	OH	H	H	*N*-(β-Hydroxyethyl)nor-adrenochrome	4-β-Hydroxyethylsemi-carbazone isolated	(137)
5. 3-Hydroxytyramine ("dopamine")	H	H	H	H	H	Norepinochrome	Red solution on oxidation†	(48)
6. 3-Hydroxy-α-methyltyramine	H	CH_3	H	H	H	2-Methylnorepinochrome	Red solution on oxidation†	(48)
7. β-(3,4,5-Trihydroxyphenyl)ethyl-amine	H	H	H	H	OH	7-Hydroxynorepino-chrome	Spectroscopic identification	(32)
8. β-(3,4-Dihydroxy-5-methoxyphenyl)-ethylamine	H	H	H	H	OCH_3	7-Methoxynorepino-chrome	Spectroscopic identification	(32)
9. β-(3,4-Dihydroxyphenyl)-*N*-(3,4-methylenedioxybenzyl)ethylamine		H	H	H	H	*N*-(3,4-Methylenedioxy-benzyl)norepinochrome	Rearrangement product isolated	(99)
10. *N*-(3,4-Dihydroxybenzyl)-β-(3,4-dihydroxyphenyl)ethylamine		H	H	H	H	*N*-(3,4-Dihydroxybenzyl)-norepinochrome	Rearrangement product isolated	(99, 114)
11. Sodium 1-methylamino-2-(3,4-dihydroxyphenyl)ethanesulfonate	CH_3	SO_3Na	H	H	H	Sodium epinochrome-2-sulfonate	Semicarbazone isolated	(248, 249)
12. β-(3,4-Dihydroxyphenyl)alanine ("dopa")	H	COOH	H	H	H	Dopachrome	Spectroscopic identification	(48, 73, 174)‡

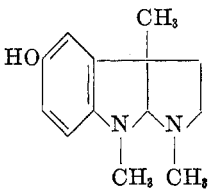
13. β-(3,4-Dihydroxyphenyl)-*N*-methylalanine ("*N*-methyldopa")	CH_3	COOH	H	H	H	*N*-Methyldopachrome	Rearrangement product isolated	(48)
14. β-(3,4-Dihydroxyphenyl)-*N*-methylalanine ethyl ester ("*N*-methyldopa ester")	CH_3	$COOC_2H_5$	H	H	H	2-Carbethoxyepinochrome	Semicarbazone isolated	(140, 232)
15. β-(3,4-Dihydroxy-2-methylphenyl)-alanine	H	COOH	H	CH_3	H	4-Methyldopachrome	Rearrangement product isolated	(70)
16. β-(3,4-Dihydroxy-5-methylphenyl)-alanine	H	COOH	H	H	CH_3	7-Methyldopachrome	Rearrangement product isolated	(70)
17. β-(3,4-Dihydroxyphenyl)-β-methoxyethyl-*N*-methylethylamine	CH_3	H	OCH_3	H	H	3-Methoxyadrenochrome	Semicarbazone isolated	(133)
18. β-Ethoxy-β-(3,4-dihydroxyphenyl)-ethyl-*N*-methylethylamine	CH_3	H	OC_2H_5	H	H	3-Ethoxyadrenochrome	Semicarbazone isolated	(133)
19. β-(3,4-Dihydroxyphenyl)-β-isopropoxy-*N*-methylethylamine	CH_3	H	$OC_3H_7(i)$	H	H	3-Isopropoxyadrenochrome	Semicarbazone isolated	(133)

* Since no halogenoaminochromes are included in this series, R, R′, R″, R‴, and R⁗ represent the same groups in the general formula for both catechol amine and aminochrome.

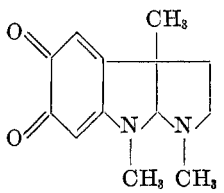
† Ether extracts of the rearranged (zinc acetate) oxidation products gave positive indole reactions with Ehrlich's reagent, but no 5,6-dihydroxyindoles were isolated.

‡ These references are only to oxidations with inorganic oxidizing agents; enzymatic oxidations are not included.

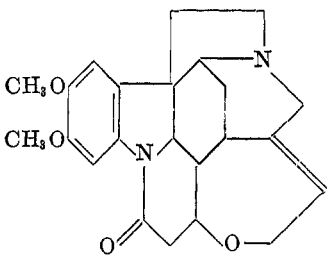
reaction initially involves the hydroxylation of the phenol ring to give a catechol derivative and its subsequent oxidation to the quinone (80, 177, 221). Rubreserine has been obtained in deep red-violet needles (with one molecule of water



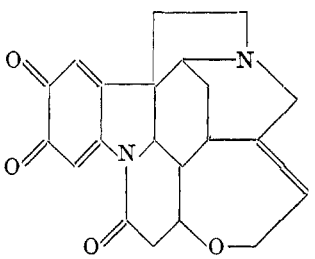
XVIII
Eseroline



VII
Rubreserine

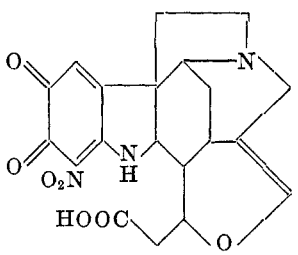


XIX
Brucine



XX
Bis-desmethylbrucine

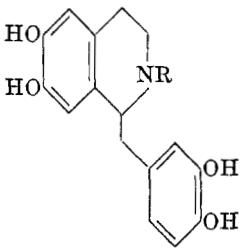
of crystallization) on crystallization from water (melting point indefinite, but in the region of 113–125°C. (221)). The melting point was considerably higher after vacuum drying [152°C. (221); 144–145°C. (80)]. Rubreserine recrystallized from benzene contains benzene of crystallization and melts at 100°C. (221). Brucine (XIX) on oxidation with 5 *N* nitric acid or chromic acid gives a red solution from which the red brucinequinone (bis-desmethylbrucine) (XX) can be isolated as its perchlorate (154, 156). If the oxidation is carried out with concentrated nitric acid, the nitrate of bis-desmethylnitrobrucine hydrate (cacotheline) (XXI) is obtained (155, 183). Several other brucine derivatives have been oxidized to corresponding quinones (129, p. 420).



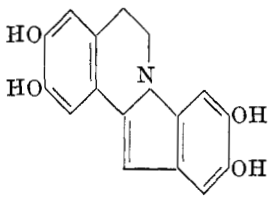
XXI
Cacotheline

2. Pyrrocoline derivatives

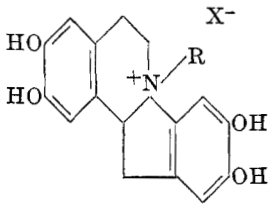
Another interesting example of the formation of an "aminochrome" type intermediate in synthetic work in the field of natural products is the oxidation of tetrahydropapaveroline (XXII: R = H) with potassium ferricyanide in bicarbonate buffer with subsequent rearrangement of the intermediate quinone to the 5,6-dihydroxyindole derivative, 5,6-dihydro-2,3,9,10-tetrahydroxydibenzo-



XXII



XXIII

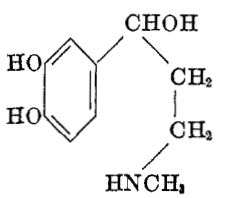


XXIV

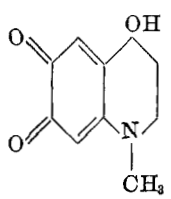
[*b*,*g*]pyrrocoline (XXIII) (115). Oxidation of laudanosoline (XXII: R = CH_3) to quaternary salts of the form shown in formula XXIV was effected by heating with chloranil in aqueous alcohol (213).

G. 1,2,3,4-TETRAHYDROQUINOLINE-6,7-QUINONES

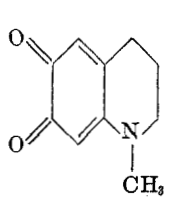
The monosemicarbazones (monoximes and mono-4-β-hydroxyethylsemicarbazones) of 1,2,3,4-tetrahydro-*N*-methylquinoline-6,7-quinone (XXV) and 1,2,3,4-tetrahydro-4-hydroxy-*N*-methylquinoline-6,7-quinone (XXVI) have been pre-



XXVII



XXVI

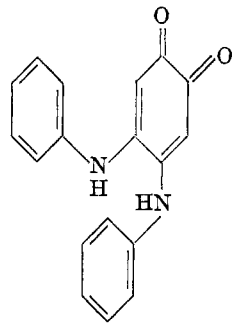


XXV

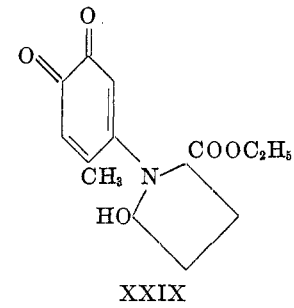
pared recently, although the isolation of the free quinones has not been reported (141). Only in the case of the 4-hydroxy compound was the method of preparation strictly analogous, i.e., the ferricyanide oxidation of α-(2-methylaminoethyl)-protocatechyl alcohol hydrobromide (XXVII) (141).

H. 1-AMINO-3,4-BENZOQUINONES

It is well known that amines can react with quinones by nuclear substitution e.g., aniline can react with *o*-benzoquinone to give 4,5-dianilino-1,2-benzoquinone (XXVIII) (149). This type of compound has the basic 1-amino-3,4-benzoquinone nucleus that is present in the aminochromes. A red pigment was isolated in crystalline form from the interaction of 1-methyl-3,4-benzoquinone and 4-hydroxyproline and identified as 4-(4′-hydroxy-2′-carbethoxy-1′-pyrrolidyl)-5-methyl-1,2-benzoquinone(XXIX)(143). The physical and chemical properties of this and similar pigments show a close resemblance to those of adren-

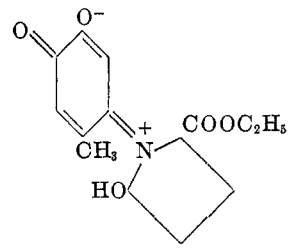


XXVIII

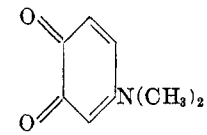


XXIX

ochrome. Thus in all probability they should be formulated as zwitterions (*cf.* XXX) (143). The deep red colors produced when amino acids are oxidized by a catechol–phenolase system are probably due to substituted 1-amino-3,4-benzoquinones similar to those formulated above (143).



XXX



XXXI

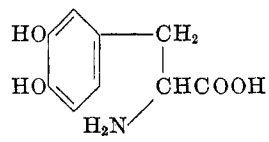
1-Dimethylamino-3,4-benzoquinone (XXXI) has recently been isolated as the monosemicarbazone (and monoxime) from the oxidation of *N*,*N*-dimethylamino-3,4-dihydroxyaniline with potassium ferricyanide (139).

I. HALLACHROME

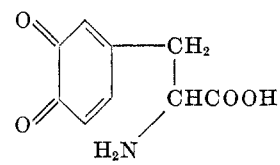
Hallachrome was the name given to a red pigment isolated from the marine worm *Halla parthenopaea* Costa (179). The substance was said to be identical with Raper's "red pigment" ("dopachrome," 2-carboxynorepinochrome, XXXII) (209). However, this identity has subsequently been disproved, since the two substances exhibit many significant differences in chemical and physical properties (49, 150). The structure of hallachrome is still unknown.

IV. Mechanism of Aminochrome Formation

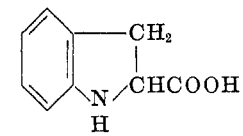
Raper postulated the following scheme for melanin formation from dopa (XXXIII) (73, 209). Dopa is first oxidized to the open-chain quinone (XXXIV),



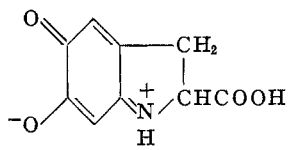
XXXIII
Dopa



XXXIV

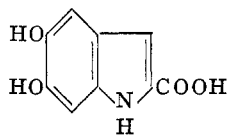


XXXV
Leucodopachrome

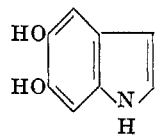


XXXII

Dopachrome

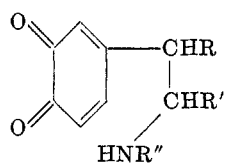


XXXVI



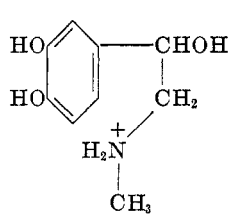
XXXVII

which cyclizes to "leucodopachrome" (XXXV); this substance is in turn oxidized to "dopachrome" (XXXII), which can then undergo further transformations by internal oxidation-reduction mechanisms to 5,6-dihydroxyindole-2-carboxylic acid (XXXVI) or 5,6-dihydroxyindole (XXXVII) (see Section VI,A).

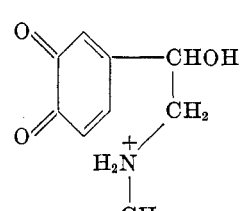


XXXVIII

In the case of adrenaline, the open-chain quinone is the hypothetical "adrenaline-quinone" (XXXVIII) (R″ = CH_3 ; R′ = H; R = OH). Neither this nor any other 1-(β-aminoethyl)-3,4-benzoquinones have been isolated as yet. The oxidation of adrenaline with lead dioxide in strongly acid solutions (pH 0–1) gives rise to a yellowish-orange solution which does not give a positive test for phenolic hydroxyls with ferric chloride and may be reduced back to adrenaline (47, 207, 216, 217). Other catechol amines (e.g., noradrenaline, α-methylnoradrenaline, *N*-isopropylnoradrenaline, 3-hydroxytyramine, epinine, and dopa) on oxidation under similar conditions give yellow solutions which probably contain the open-chain quinones (47). If these solutions (after filtration) are made basic or treated with an acetate or bicarbonate buffer adrenochrome is rapidly formed and the solution turns red; the color can be further intensified by the addition of silver oxide (47). In highly acid solutions adrenaline exists in the cationic form (XXXIX) and is oxidized to the quinone, also in the cationic form (XL). Whilst the nitrogen is protonated, intramolecular cyclization does not occur, since this involves a nucleophilic attack by the nitrogen on the 6-position of the quinone ring. On reduction of the acidity the free base is formed and cyclization occurs



XXXIX



XL

(47), as manifested by the formation of the deep red color of adrenochrome. It appears that half of the "adrenaline-quinone" cyclizes to adrenochrome and half is reduced back to adrenaline; the intensification of the red color by the addition of silver oxide arises from the direct oxidation of this re-formed adrenaline. All the open-chain quinones investigated so far cyclize readily at a pH greater than 2 (47), so it is not surprising that these quinones are not observed in the normal preparative procedures, since these methods are carried out under only faintly acid conditions (12). From a study of the redox potentials of several catechol amines, it was concluded that the first step was a two-electron change. However, the half-life of the primary oxidation product was only 0.06 sec., i.e., this substance, presumably the open-chain quinone, only has a very transitory existence (18). The evidence for and against the existence of "adrenaline-quinone" has been reviewed, and it was concluded that it exists only as a very transient intermediate between adrenaline and adrenochrome and that any physiological activity attributed to it was due to unchanged adrenaline (12).

V. Physical Properties

A. Solubility

Aqueous solutions of the unsubstituted aminochromes are deep red in color, whilst those of the halogenoaminochromes are violet. Adrenochrome is very soluble in water, methanol, and ethanol, and fairly soluble in acetone, acetonitrile, nitromethane, and dimethylformamide, but it is virtually insoluble in nonpolar solvents (122). Adrenochrome and rubreserine form yellow solutions in concentrated sulfuric acid (80, 122). The solubility of adrenochrome (from either D- or L-adrenaline) is reported to be 1.78 mg./cc. at −10°C. in methanol; the solubility of the racemate is 2.37 mg./cc. (23). 2-Iodo- and 2-bromoadrenochrome are much less soluble in water than is adrenochrome, and their solutions are violet in color. Although pure adrenochrome is relatively stable in the solid state, its aqueous solutions, together with those of other aminochromes, decompose rapidly with the formation of black precipitates (see Section VI,D).

B. Spectroscopy

1. Ultraviolet and visible absorption spectra

The ultraviolet and visible absorption spectra of adrenochrome and related aminochromes have been measured in aqueous or alcoholic solutions by various workers (*cf.* 25, 26, 32, 36, 77, 80, 82, 88, 109, 121, 159, 170, 174, 215, 232, 233, 240, 246). In general the curves show two maxima in the ultraviolet region, one in the region 205–215 mμ and a second near 300 mμ; in the visible region they exhibit a broad flat maximum in the region 470–490 mμ. With the halogenated aminochromes, the first maximum is observed at ca. 230 mμ and the long wavelength absorption shifts to ca. 520 mμ. The ultraviolet and visible spectroscopic characteristics of several aminochromes have been listed elsewhere (25, 232, 233). In concentrated sulfuric acid the 300 mμ maximum of adrenochrome is still observed but the 487 mμ maximum is shifted to ca. 380 mμ (122).

2. Infrared spectrum

The infrared spectrum of adrenochrome in the solid state (Nujol mull) has recently been reported (120). The main peaks occur at 3295 cm.$^{-1}$ (O—H stretching), and in the "carbonyl" region at 1682, 1672, 1622, and 1575 cm.$^{-1}$ (see Section V,E).

C. OPTICAL ACTIVITY

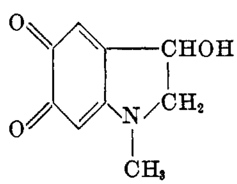
Owing to the intense color of the aminochrome solutions, measurement of their rotation is very difficult; however, the rotation of adrenochrome, freshly prepared from L-adrenaline, is said to be $[\alpha]_{630}^{25°}$ = ca. +150° (233). The sign of the rotation is apparently changed during the oxidation.

D. CHROMATOGRAPHY

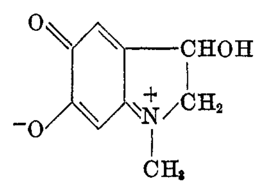
The use of 1-butanol–acetic acid–water and 2-propanol–aqueous ammonia systems (96) for the examination of mixtures containing adrenochrome or adrenolutin is quite unsatisfactory, since the compounds decompose under these conditions, but in distilled water or 2 per cent acetic acid perfectly good chromatograms can be obtained on acid-washed paper. Adrenochrome has R_f's of 0.8 and 0.82, respectively, in these solvents (121). However, on drying in air the red color of the adrenochrome spots slowly changes to brownish-yellow and the spots exhibit a typical adrenolutin fluorescence in ultraviolet light (121). Adrenochrome is adsorbed onto charcoal from aqueous solutions and can be eluted from the charcoal with methanol (72).

E. MOLECULAR STRUCTURE

Green and Richter formulated adrenochrome as 2,3-dihydro-3-hydroxy-*N*-methylindole-5,6-quinone (II) (109), but in view of many of its physical properties (e.g., color, solubility in polar solvents) and some of its chemical properties (i.e., its failure to produce a phenazine derivative readily with *o*-phenylenediamine and the consistent formation of mono derivatives with the typical ketone



II



IV

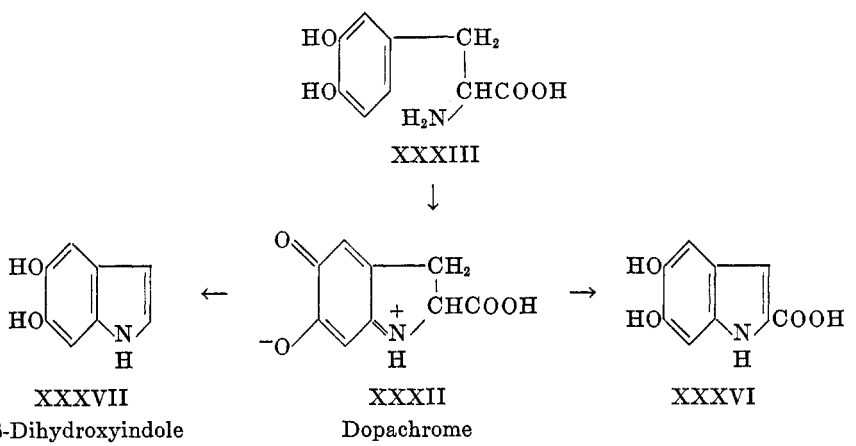
reagents), Harley-Mason suggested that the facts were better explained by the zwitterionic form (IV) (112). The infrared evidence also supports this formula; the band at 1575 cm.$^{-1}$ in the spectrum can be assigned to the $\gg C-O^-$ group and those at 1682, 1672, and 1622 cm.$^{-1}$ to the $>C=\overset{+}{N}<$, and $>C=O$ and conjugated double bonds in the six-membered ring, respectively (120).

VI. Chemical Properties

A. Rearrangement

1. General introduction

One of the most characteristic reactions of adrenochrome in solution is its rapid conversion by alkali into a substance with an intense yellow-green fluorescence. This is a general reaction, since all aminochromes in solution are decolorized by alkalis with the formation of colorless or yellow solutions, exhibiting various degrees of fluorescence. In 1927 Raper introduced the concept of an internal "autoreduction" reaction to explain the mechanism by which the facile decolorization of solutions of the red pigment [postulated to be 2-carboxy-2,3-dihydroindole-5,6-quinone (XXXII); obtained from the enzymatic oxidation of tyrosine] occurred. Dopachrome (XXXII) undergoes a spontaneous change *in vacuo* to 5,6-dihydroxyindole (XXXVII), which could be isolated from the products as the dimethyl ether (209). When the red substance was decolorized by sulfur dioxide the product eventually isolated was the dimethyl ether of 5,6-dihydroxyindole-2-carboxylic acid (XXXVI) (209). These observations on the oxidation of dopa and the subsequent rearrangement of the products have been confirmed spec-

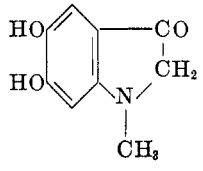


troscopically and manometrically (174, 176). It was shown that the 2-carboxyindole (XXXVI) predominated when the rearrangement was carried out in acid solution and that decarboxylation occurred as well in the pH range 5.6–8.0 (174). In the latter case the rearrangement and decarboxylation presumably occur simultaneously, since the 2-carboxy derivative is stable under the conditions of rearrangement (233). The red solutions obtained from the oxidation of related catechol amines were observed to behave similarly (51, 73).

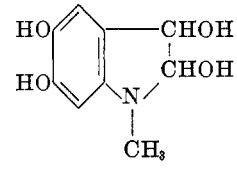
2. Rearrangement with alkali

The formation of the intense yellow-green fluorescence during the alkaline oxidation of adrenaline was noted as early as 1918 (155). Thirty years were to elapse before the correct structure was assigned to the fluorescent substance (76) [i.e.,

5,6-dihydroxy-*N*-methylindoxyl or adrenolutin (V)] and a further year before it was isolated in crystalline form (158, 159). It was demonstrated that the substances responsible for the fluorescence of alkali-oxidized adrenaline and of the anaerobic alkaline rearrangement product of adrenochrome were the same (90). Some confusion arose during the early stages of the isolation work from the fact that adrenolutin (V) crystallizes from water with one molecule of water of crystallization, and at first the fluorescent material was erroneously considered to be

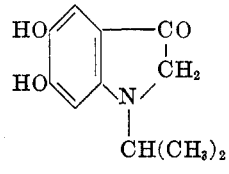


V
Adrenolutin

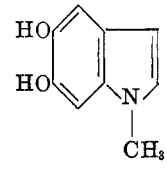


XLI

2,3,5,6-tetrahydroxy-2,3-dihydro-*N*-methylindole (XLI) (89, 112). The structure of the fluorescent material (V) was later confirmed by an elegant synthesis starting from 3,4-isopropylidenedioxyaniline (19). Adrenolutin is prepared either from adrenochrome (89, 113, 158) or directly from oxidized adrenaline (47, 120) by treatment with aqueous sodium hydroxide, followed by acidification with acetic acid. The product is oxidized readily in solution, but can be recrystallized from aqueous solutions containing some sodium hydrosulfite (47, 113). The anhydrous substance is best prepared by the high-vacuum sublimation of the hydrate (120). The hydroxyl ion-catalyzed rearrangement is a quite general reaction and 5,6-dihydroxy-*N*-isopropylindoxyl (XLII) and 5,6-dihydroxy-*N*-methylindole (XLIII)



XLII



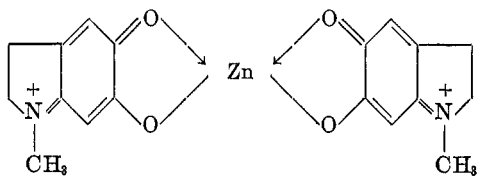
XLIII

have been obtained in the solid state from the corresponding aminochromes (8, 9, 47, 51).

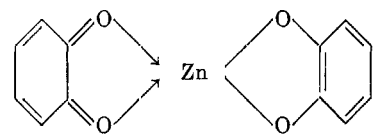
3. Rearrangement with zinc or aluminum cations

The rearrangement of aminochromes to 5,6-dihydroxyindoles or 5,6-dihydroxyindoxyls is catalyzed by zinc salts and (less readily) by aluminum salts (48, 91, 92, 116, 151). The product is the same as that derived from the alkaline rearrangement (92) but owing to the relatively mild character of zinc acetate it is often preferred to alkali, especially when dealing with halogenated aminochromes (48, 116). Decarboxylation occurs during the zinc-catalyzed rearrangement of dopa and its *N*-methyl derivative (48). The rearrangement may proceed by way of an intermediate zinc complex (XLIV); the two charges would solubil-

ize the indole complex, which decomposes by spontaneous release of protons from the 2- and 3-positions on the indole rings. Insoluble, dark green zinc complexes



XLIV

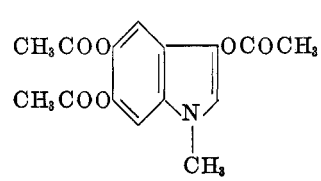


XLV

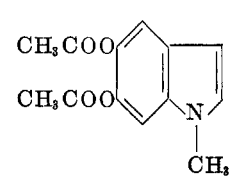
of this type have been isolated from the oxidation of catechol in the presence of zinc salts (*cf.* XLV) (48, 116). It is interesting to note that some years previously it was reported that the solutions of adrenaline oxidized at pH 1.2 with lead dioxide and possibly containing "adrenaline-quinone" gave, on neutralization with zinc oxide, a highly fluorescent product which was almost certainly adrenolutin but was incorrectly considered to be leucoadrenochrome (207). Also, the attempted purification of crude adrenaline oxidation mixtures on alumina columns had been observed to give rise to the formation of strongly fluorescent substances (251). This may have been due to the aluminum cation-catalyzed rearrangement of adrenochrome to adrenolutin.

4. Rearrangement of aminochromes with acetic anhydride and pyridine

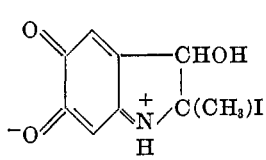
The treatment of an aminochrome with acetic anhydride in the presence of pyridine leads to the formation of the corresponding 5,6-diacetoxy compounds, i.e., rearrangement probably occurs prior to acetylation; e.g., adrenochrome gives 3,5,6-triacetoxy-*N*-methylindole (XLVI) (113) and epinochrome gives 5,6-diacetoxy-*N*-methylindole (XLVII) (9). Under these conditions 2-methyl-2-iodonoradrenochrome (XLVIII) is converted to 5,6-diacetoxy-1-acetyl-2,3-dihydro-2-methyl-2-iodo-3-ketoindole (XLIX) (47). The known 5,6-diacetoxyindoles are listed in table 5.



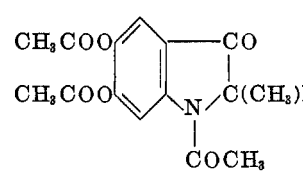
XLVI



XLVII



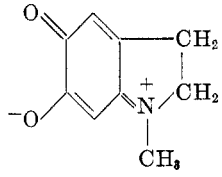
XLVIII



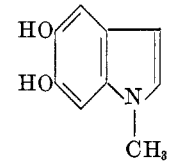
XLIX

5. Effect of 3-hydroxyl groups on ease of rearrangement

Aminochromes without a 3-hydroxyl substituent undergo rearrangement with greater facility than those with one. For instance, attempted catalytic hydro-

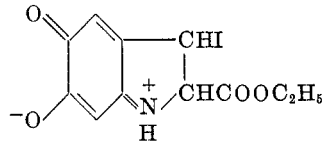


XV

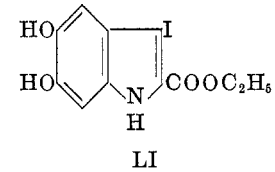


XLIII

genation of epinochrome (XV) invariably leads to the isolation of the rearrangement product 5,6-dihydroxy-*N*-methylindole (XLIII), whereas under the same conditions adrenochrome is reduced (8, 9). Ethyl 2,3-dihydro-3-iodoindole-5,6-quinone-2-carboxylate (L) undergoes rearrangement spontaneously in the solid state or in solution to ethyl 5,6-dihydroxy-3-iodoindole-2-carboxylate (LI) (48).



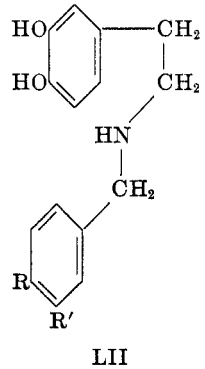
L



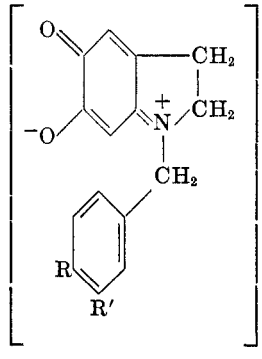
LI

6. Synthesis of 5,6-dihydroxyindoles

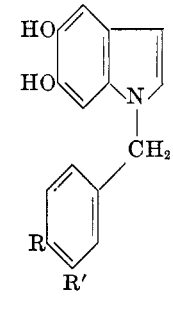
The unambiguous syntheses of several substituted 5,6-dihydroxyindoles by the rearrangement of the quinonoid oxidation products of the requisite catechol amines have been described (70; *cf.* 247). An interesting application of this reaction has been in synthetic studies on the lycorine problem. *N*-(3,4-Dihydroxybenzyl)-3,4-dihydroxyphenylethylamine (LII: R = R′ = OH) gives *N*-(3,4-dihydroxybenzyl)-5,6-dihydroxyindole (LIV: R = R′ = OH) on oxidation with potassium ferricyanide in bicarbonate buffer after the rearrangement of the intermediate quinone (LIII: R = R′ = OH) with aqueous sulfur dioxide (99, 114).



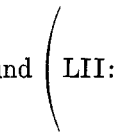
LII



LIII

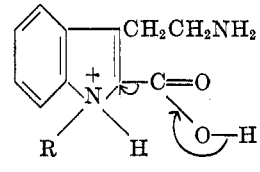


LIV

The analogous 3,4-methylenedioxybenzyl compound (LII: R + R′ = ) behaves similarly. The structures of the products (LIV), which were isolated as their polymethyl ethers, were confirmed by independent syntheses (99).

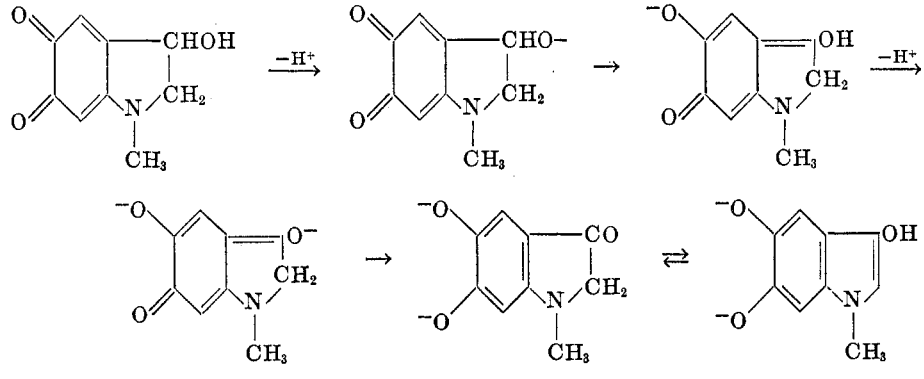
7. *Mechanism of rearrangement*

The overall reaction consists of the removal of two hydrogen atoms from the pyrrole moiety of the system and the addition of two hydrogen atoms to the benzoquinone nucleus. The mechanism of the isomerization has been tentatively formulated by Trautner and Bradley (250) as shown in Scheme A. A slightly different mechanism has recently been suggested by Sobotka, Barsel, and Chanley (233) (Scheme B). The first stage of Scheme A, i.e., the removal of a proton from what is in effect a secondary alcohol group, does not seem very probable. Scheme B appears to be the more likely of the two. This presumably initially involves an attack by a hydroxyl ion on the 3-position, with the loss of a proton from this position, followed by rearrangement of the electrons within the intermediate, and the subsequent loss of a proton from the 2-position. A slight modification of Scheme B has been advanced (232) to explain the decarboxylative rearrangement of dopachrome under neutral or slightly alkaline conditions (Scheme C). The formation of intermediates of the form LV, i.e., involving coördination of the indole nitrogen with a proton, has been suggested to explain the effect of 5-substituents on the ease of decarboxylation of 5-substituted tryptamine-2-carboxylic acids (5).

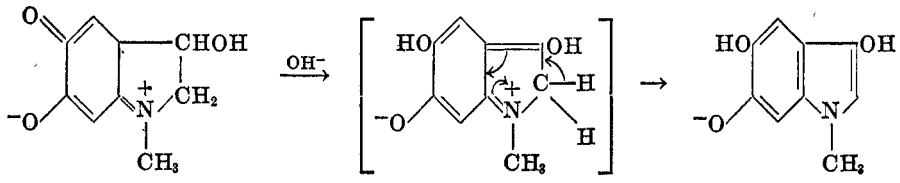


LV

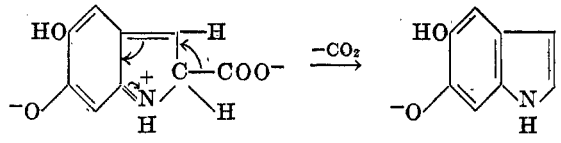
Scheme A



Scheme B



Scheme C



8. Physical properties of adrenolutin

When pure, both anhydrous adrenolutin and the monohydrate are bright yellow crystalline solids, slightly soluble in water and other polar solvents giving yellow solutions exhibiting the characteristic yellow-green fluorescence. These solutions oxidize very rapidly in air; a brown turbidity develops and the fluorescence disappears.

The ultraviolet and visible absorption spectra have been measured in water (120, 159) (λ_{max} = 220; 255; 285 and 410 mμ); in aqueous buffer at pH 4.5 (113) (λ_{max} = 286 and 305 mμ); in dilute aqueous sodium hydroxide (57) (λ_{max} = 275; 325 and 408 mμ); and in ethanol (19) (λ_{max} = 229 and 289 mμ). The values quoted for the absorption maxima are shown in parentheses, and it can be seen that the nature of the solvent affects their position. The infrared spectrum of anhydrous adrenolutin in the solid state has a peak at 1647 cm.$^{-1}$ probably due to the indoxyl 3-carbonyl group (120). The fluorescence characteristics have recently been measured; the combinations of excitation and emission wavelengths showing the greatest intensity of fluorescence vary slightly with the solvent, but in general excitation is effected at 400 $\pm$ 10 mμ and the emission measured at 505 $\pm$ 20 mμ to obtain the maximum intensity of fluorescence (57, 120, 124). The x-ray diffraction spectra of adrenolutin prepared from adrenochrome by rearrangement with both alkali and zinc ions were identical (92).

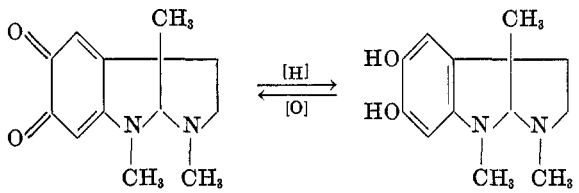
Owing to the ease with which adrenolutin is oxidized in solution and its sensitivity to acids and alkalis, its paper chromatography must be carried out in neutral (oxygen-free) solvents and on acid-washed paper (120, 121). On prewashed Whatman No. 1 paper, adrenolutin has an R_f of ca. 0.45 in water and 0.60 in methanol (120).

B. REDUCTION

1. General introduction

In the course of investigations into the extraction of physostigmine nearly a century ago, it was noticed that the red color obtained by the action of alkali on the alkaloid was discharged by hydrogen sulfide and that the color returned on removal of the reducing agent (147). This observation, which was later con-

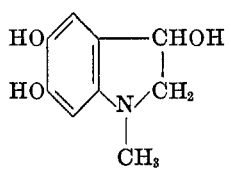
firmed with authentic rubreserine (VII) solutions (221), was no doubt due to the initial reduction of the quinone to "leucorubreserine" (LVI) and its subsequent reoxidation. The decolorization of solutions of adrenochrome and 2-iodo-



VII
Rubreserine

LVI
Leucorubreserine

adrenochrome by reducing agents [e.g., sulfur dioxide, hydrogen sulfide, hydrogen (catalytic)] was reported concurrently with their isolation (109, 212), and it was suggested that adrenochrome was reduced directly to a colorless substance called "leucoadrenochrome" [2,3-dihydro-3,5,6-trihydroxy-*N*-methylindole (LVII)]. The leuco compound, which was not isolated, gave typical catechol

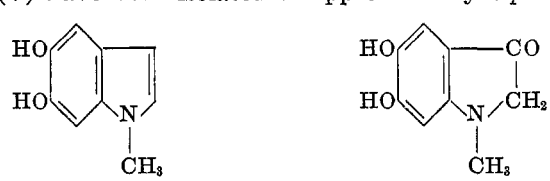


LVII
Leucoadrenochrome

reactions and its solution was optically active; $[\alpha]_D^{18°} = +79.2°$ (109). This suggested that the asymmetric carbon atom, derived from adrenaline, had not been affected by the initial oxidation or subsequent reduction. 2-Iodoadrenochrome behaved similarly with reducing agents; the optical rotation of "leuco-2-iodoadrenochrome" prepared by reduction with sodium sulfite (*cf.* Section VI,G) was quoted as $[\alpha]_D^{18°} = +154°$ (212).

2. Hydrogenation of adrenochrome

The catalytic hydrogenation of adrenochrome has been studied fairly extensively, but there is not complete agreement on (*1*) the amount of hydrogen absorbed, (*2*) the nature of the products, (*3*) the optical activity, if any, of the products, and (*4*) the mechanism of the reaction that has taken place. However, most workers are of the opinion that the reduction occurs irreversibly (23, 24, 112, 113, 246). 5,6-Dihydroxy-*N*-methylindole (XLIII) and 5,6-dihydroxy-*N*-methylindoxyl (V) have been isolated in approximately equal quantities after



XLIII

V

the uptake of hydrogen ceases at approximately one atom of hydrogen per molecule of adrenochrome (23, 112, 113). A crude mixture of 5,6-diacetoxy-*N*-methylindole (XLVII) and 3,5,6-triacetoxy-*N*-methylindole (XLVI) had probably been obtained previously by acetylation of the crude hydrogenate (33). Alternatively, the absorption of 1.5 atoms of hydrogen per molecule with the irreversible formation of two or more products (not isolated) that have no effect in raising blood pressure has been claimed (246). In very dilute solution at pH 5.61–8.08, two hydrogen atoms are said to be absorbed per molecule. In this case, the hydrogen uptake was followed polarographically and the initial change was shown to be a reversible two-electron addition (151).

3. Reduction of adrenochrome with other reducing agents

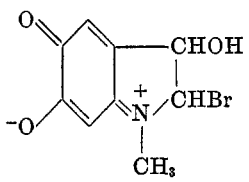
Sodium hydrosulfite ($Na_2S_2O_4$) has found widespread use as a reducing agent for the aminochromes (80, 89, 95, 112, 113, 119, 246), and as in the case of hydrogenation 5,6-dihydroxy-*N*-methylindole (XLIII) and 5,6-dihydroxy-*N*-methylindoxyl (V) have been isolated in approximately equal quantities from the reduction of adrenochrome with this reagent (112, 113). Paper chromatographic studies indicate that there are two major primary reduction products; one is 5,6-dihydroxy-*N*-methylindole and the other appears to be the adrenochrome–sodium bisulfite complex (this would eventually give V on treatment with alkali). Lesser amounts of other water-soluble indoles are formed at the same time (119). Other reagents reported to decolorize adrenochrome solutions are ascorbic acid (28, 89, 118, 119, 250), leucomethylene blue (250), lithium aluminum hydride (121), sodium borohydride (118, 119), potassium borohydride (180), cysteine (118, 119), glutathione (118, 119), glycine (15, 246), dihydroxymaleic acid (118, 119), dihydroxyfumaric acid (118, 119), thioglycolic acid (59, 119), 2,3-dimercapto-1-propanol (250), sodium phosphite (121), sodium hypophosphite (121), thiourea dioxide (119), zinc (or magnesium) in dilute acetic acid (119), sulfur dioxide (109, 119), sodium bisulfite (28, 36, 80, 82, 119, 207, 250), sodium sulfite (119, 250), sodium formaldehyde sulfoxylate (119), and sodium glyoxal bisulfite (119).

Crystalline reduction products (as described above) have been isolated after catalytic hydrogenation (113) and treatment with sodium hydrosulfite (95, 113); 5,6-dihydroxy-*N*-methylindole has been isolated from the products of reduction with ascorbic acid and sodium borohydride (118). Definite products have not been isolated in the other cases; however, spectroscopic studies showed that the mechanism of reduction and products formed may vary with the reducing agent employed; e.g., the "leuco" derivatives given by ascorbic acid and sodium bisulfite were markedly different (28, 89).

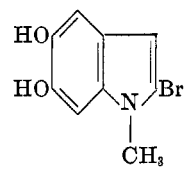
The reduction by ascorbic acid has been shown by paper chromatography to give 5,6-dihydroxy-*N*-methylindole and at least two other water-soluble products showing the chromogenic reactions of indole and catechol (118, 119). This technique has indicated that 5,6-dihydroxy-*N*-methylindole is also formed by the action of sodium hydrosulfite, sodium borohydride, cysteine, glutathione, dihydroxymaleic acid, dihydroxyfumaric acid, thioglycolic acid, thiourea dioxide, and zinc and dilute acid (119). Other unidentified water-soluble products giving

indole color reactions are also formed in each case (119). No appreciable amounts of 5,6-dihydroxy-*N*-methylindole appear to be derived from the action of sulfur dioxide, sodium bisulfite, sodium sulfite, sodium glyoxal bisulfite, and sodium formaldehyde sulfoxylate. With these reagents the main product appears to be the adrenochrome–sodium bisulfite addition complex; small amounts of unidentified by-products, probably indoles, are formed at the same time (119).

The halogen-substituted adrenochromes behave similarly on reduction. 2-Bromoadrenochrome (XII) gives 2-bromo-5,6-dihydroxy-*N*-methylindole (LVIII) on reduction with hydrosulfite (113) and the 2-iodo derivative has been obtained similarly (as the diacetyl derivative) (33).



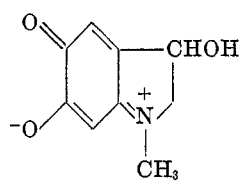
XII



LVIII

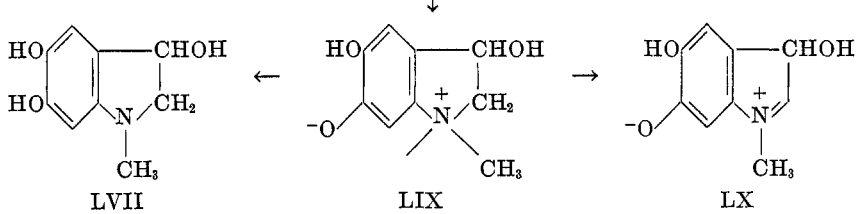
4. Mechanism of reduction

Harley-Mason proposed that the addition of one atom of hydrogen to the adrenochrome (IV) molecule results in the formation of an unstable zwitterion



IV

Adrenochrome

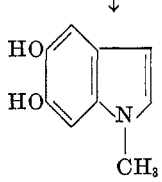


LVII

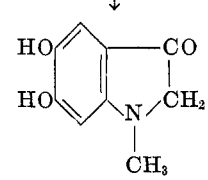
"Leucoadrenochrome"

LIX

LX



XLIII



V

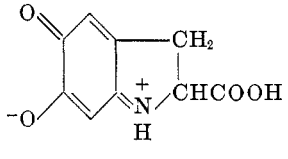
Adrenolutin

(LIX) which disproportionates into a more stable zwitterion (LX) and the hypothetical "leucoadrenochrome" (LVII). The former (i.e., LX) rearranges in the presence of alkali to adrenolutin (V) and the latter (i.e., LVII) rapidly undergoes an intramolecular dehydration to form 5,6-dihydroxy-*N*-methylindole (XLIII) (112, 113). This mechanism was proposed to explain both the hydrogenation and the hydrosulfite reductions. The above mechanism does satisfactorily account for the products isolated and does explain the described optical activity of the reduction mixture (109, 113), since LX has an asymmetric carbon atom. Also, the reduction of adrenolutin (V) with Raney nickel alloy in alkali leads to the isolation of 5,6-dihydroxy-*N*-methylindole (XLIII), presumably from the spontaneous dehydration of the intermediate (LVII) (48). Harley-Mason measured the rotation of the hydrosulfite reduction product ($[\alpha]_D^{18°}$ = 106°), but Beaudet states that the product obtained from hydrogenation is optically inactive (23). The conversion of the zwitterion LX to adrenolutin (V) was followed spectroscopically on a solution obtained from the hydrosulfite reduction (113). It is not inconceivable that different mechanisms may be operative in the cases of reduction by hydrogen or hydrosulfite. In the latter case, all the properties ascribed to LX could equally well be explained by the bisulfite derivative (see Section VI,G for the properties of this substance): e.g., (*a*) same ultraviolet spectrum; (*b*) optical activity; (*c*) formation of adrenolutin by the action of alkali; (*d*) paper chromatographic behavior.

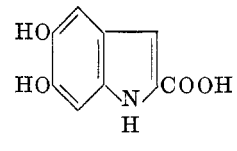
Polarographic studies in dilute solution indicated that the initial reduction product was the product of a reversible two-electron change (153, 266) but that it was rapidly transformed into a product that could not be reoxidized (266, 269). This may be due to the initial formation and rapid dehydration of "leucoadrenochrome" (LVII).

5. Reduction of aminochromes without a 3-hydroxyl group

The behavior, on attempted reduction, of aminochromes without the 3-hydroxyl group is somewhat different from that of those with it. Raper in 1927 noticed that red solutions of dopachrome (XXXII) readily rearranged to 5,6-dihydroxyindole-2-carboxylic acid (XXXVI) under reducing conditions (sulfur

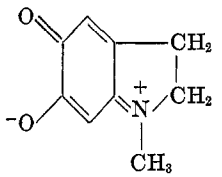


XXXII
Dopachrome

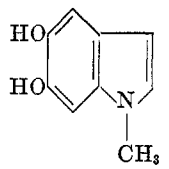


XXXVI

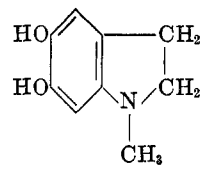
dioxide) (209). Solutions of epinochrome (XV) are instantly decolorized by the addition of zinc dust (51), and on attempted hydrogenation epinochrome rearranges to 5,6-dihydroxy-*N*-methylindole (XLIII). This reaction occurs with extreme ease and takes place prior to reduction. Traces of 2,3-dihydro-5,6-dihydroxy-*N*-methylindole ("leucoepinochrome") (LXI) have occasionally been obtained (8, 9). Continued hydrogenation of epinochrome solutions, after the



XV
Epinochrome

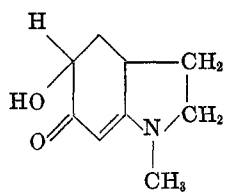


XLIII

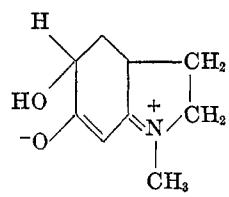


LXI
"Leucoepinochrome"

initial rearrangement stage, leads to the formation of a zwitterionic compound, probably a 5-hydroxy-6-keto-1-methyl-2,3,4,5,6,9-hexahydroindole (LXIIa and b) (8, 9). Complete hydrogenation may be achieved in acetic acid over



LXIIa

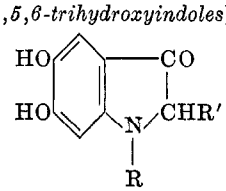


LXIIb

platinum oxide to give a 5,6-dihydroxy-*N*-methyloctahydroindole (9). However, polarographic studies on the hydrogenation of very dilute solutions of epinochrome suggested that a reversible addition of two atoms of hydrogen occurred initially (153). Ethyl 2,3-dihydro-5,6-dihydroxy-3-iodoindole-2-carboxylate

TABLE 3

5,6-Dihydroxyindoxyls (i.e., 3,5,6-trihydroxyindoles) prepared from aminochromes



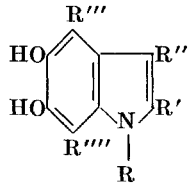
R	R′	Method of Preparation	Melting Point (with Decomposition)*	References
			°C.	
CH_3	H	Rearrangement (alkali- and Zn^{++}-catalyzed)	236 ($1H_2O$)	(120)
			230–235 ($1H_2O$)	(89)
			230–232 ($1H_2O$)	(47, 113)
			220–230 ($1H_2O$)	(91, 92)
			Turned black at ca. 100 without melting ($1H_2O$)	(158, 159)
			285	(113)
			245	(120)
			195†	(19)
i-C_3H_7	H	Rearrangement (alkali)	214–216	(25, 47)
CH_3	I	Reduction of 2-iodoöxoadrenochrome	116	(163, 164)

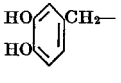
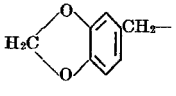
* Number of molecules of water of crystallization given in parentheses (where applicable).

† Not prepared from an aminochrome (19).

TABLE 4

5, 6-Dihydroxyindoles prepared from aminochromes

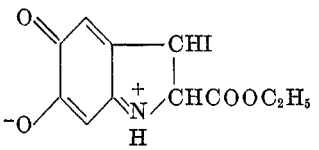


R	R′	R″	R‴	R⁗	Method of Preparation	Method of Identification	Melting Point (with Decomposition)	References
							°C.	
H	H	H	H	H	Rearrangement (sulfur dioxide)	Dimethyl ether (m.p. 154–155°C.) isolated	140*	(73, 174, 209)
H	COOH	H	H	H	Rearrangement (neutral; weak alkali)	Dimethyl ether (m.p. 202–203°C.) isolated	234†	(209)
CH_3	H	H	H	H	Reduction and rearrangement (weak alkali; Zn^{++}-catalyzed; spontaneous)	Isolated in the solid state and as dimethyl ether (m.p. 138–139°C.) and diacetyl derivative (m.p. 101°C.)	133 134–135 136	(8, 9) (118) (48, 112, 113) (73) (33)
CH_3	I	H	H	H	Reduction	Diacetyl derivative (m.p. 153–155°C.) isolated	—	(33)
CH_3	H	I	H	H	Rearrangement (Zn^{++}-catalyzed)	Crystalline solid isolated	88–90	(48)
CH_3	Br	H	H	H	Reduction	Crystalline solid isolated	121–123	(113)
i-C_3H_7	H	H	H	H	Reduction	Diacetyl derivative (m.p. 89.5–90.5°C.) isolated	—	(122)
H	$COOC_2H_5$	I	H	H	Rearrangement (weak alkali; Zn^{++}-catalyzed; spontaneous)	Crystalline solid isolated	140	(48)
H	H	H	CH_3	H	Rearrangement (Zn^{++}-catalyzed)	Crystalline solid isolated	146–149	(70)
H	H	H	H	CH_3	Rearrangement (Zn^{++}-catalyzed)	Crystalline solid isolated	108–109	(70)
	H	H	H	H	Rearrangement (sulfur dioxide)	Isolated in the solid state as the tetramethyl ether (m.p. 121–122°C.)	143–145	(114, 99)
	H	H	H	H	Rearrangement (sulfur dioxide)	Dimethyl ether (m.p. 93°C.) isolated	—	(99)

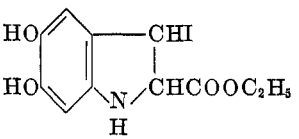
* Crystalline 5,6-dihydroxyindole obtained by an alternative route (30, 114).

† Crystalline 5,6-dihydroxyindole-2-carboxylic acid obtained by an alternative route (31).

(LXIII) has been obtained from the reduction of the corresponding quinone (L) (48). This reaction is reversible, the product undergoing rapid oxidation by air in solution (48).



L



LXIII

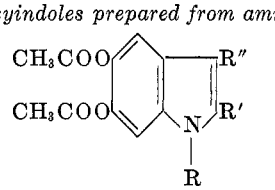
The 3,5,6-trihydroxyindoles (i.e., 5,6-dihydroxyindoxyls) and 5,6-dihydroxyindoles that have been isolated from the reduction and rearrangement of the aminochromes are listed in tables 3 and 4. The 5,6-diacetoxy- and 3,5,6-triacetoxyindoles are listed in table 5.

6. Formation of 2,3-dihydro-5,6-dihydroxyindoles (5,6-dihydroxyindolenins)

The occasional formation of traces of a true "leucoaminochrome", i.e., 2,3-dihydro-5,6-dihydroxy-*N*-methylindole (LXI), by the reduction of epinochrome has already been mentioned. A true leuco derivative, i.e., ethyl 2,3-dihydro-5,6-dihydroxy-3-iodoindole-2-carboxylate (LXIII), is produced by the reduction

TABLE 5

5,6-Diacetoxyindoles prepared from aminochromes



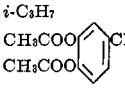
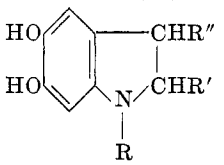
R	R′	R″	Method of Preparation	Melting Point	References
				°C.	
CH_3	H	H	Direct acetylation of hydroxy compound	110	(118)
			Acetic anhydride in pyridine	101–102	(8, 9)
			Deiodination of triacetylindole	100–101	(33)
i-C_3H_7	H	H	Direct acetylation of hydroxy compound	89.5–90.5	(122)
CH_3	I	H	Direct acetylation of hydroxy compound	153–155	(33)
CH_3	H	I	Acetic anhydride in pyridine	146–147	(48)
CH_3	Br	H	Direct acetylation of hydroxy compound	164	(113)
CH_3	H	$OCOCH_3$	Direct acetylation of hydroxy compound	111	(92)
				112	(159)
				112–113	(19, 113)
			Acetic anhydride in pyridine	112–113	(113)
CH_3	I	$OCOCH_3$	Direct acetylation of hydroxy compound	140	(163)
			Acetic anhydride in pyridine	150	(47)
i-C_3H_7	H	$OCOCH_3$	Deiodination of the triacetyliodoindole	87	(47)
$COCH_3$	H	$OCOCH_3$	Deiodination of the triacetyliodoindole	125–127	(47)
$COCH_3$	I	$OCOCH_3$	Acetic anhydride in pyridine	208–209	(47)
$COCH_3$	CH_3	OH	Deiodination of the triacetyliodoindole	185–187	(47)
i-C_3H_7	I	$OCOCH_3$	Acetic anhydride in pyridine	163–164	(47)
	H	H	Direct acetylation of hydroxy compound	194–195	(114)

TABLE 6

2,3-Dihydro-5,6-dihydroxyindoles prepared from aminochromes

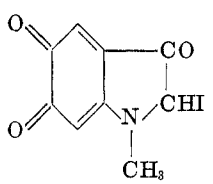


R	R′	R″	Melting Point (with Decomposition)	Reference
			°C.	
CH_3	H	H	140–141	(9)
H	$COOC_2H_5$	I	103	(48)

of the corresponding aminochrome with sodium hydrosulfite (48). Rubreserine also undergoes a reversible reduction to a true leuco compound (80). Consideration of the structural formula of rubreserine shows that it does not have hydrogen atoms in the 2- and 3-positions of the indole ring available for the intramolecular redox reaction and there is no 3-hydroxyl group to permit irreversible dehydration of the reduced form. The 2,3-dihydro-5,6-dihydroxyindoles that have been obtained this way are listed in table 6.

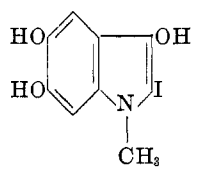
7. *Reduction of 2-iodoöxoadrenochrome*

2-Iodoöxoadrenochrome (XI) is reduced by sodium hydrosulfite, ascorbic acid, or thiourea to 2-iodoadrenolutin (LXIV) (163).



XI

2-Iodoöxoadrenochrome

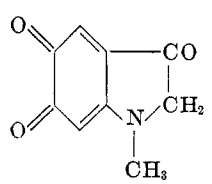


LXIV

2-Iodoadrenolutin

C. OXIDATION

Since adrenochrome contains a secondary alcohol group, it should be theoretically possible to oxidize it to a ketone, i.e., to *N*-methylindoxyl-5,6-quinone (LXV), the so-called "oxoadrenochrome." Although the preparation in solution



LXV

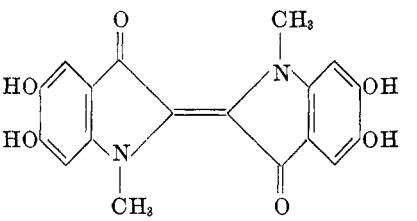
"Oxoadrenochrome"

and further reactions of this compound have been described (59, 60, 61, 62), the work has not been substantiated (113) and oxoadrenochrome has not been isolated. The 2-iodo derivative of oxoadrenochrome (XI) is obtained as a violet crystalline precipitate when adrenaline is oxidized with one molecular equivalent of iodic acid in 85 per cent methanol at 40°C. (163, 164).

The oxidation of adrenaline past the adrenochrome stage has already been mentioned in Section II, the final products being brown or black insoluble polymeric pigments of unknown composition. The rate of oxidation of adrenochrome in aqueous buffer solution increases with the pH and the temperature of the solution (182, 270). In alkaline solution it is probably the rearrangement product, i.e., adrenolutin, that is undergoing autoxidation; under these conditions four atoms of oxygen per molecule of adrenochrome are readily absorbed (158, 250, 271). Noradrenochrome appears to be oxidized to melanitic pigments faster than adrenochrome (56, 131).

All aminochromes are oxidized in solution, the final products being brown or black insoluble pigments, the melanins. The mechanisms of formation and structures of these compounds are not known with certainty. It appears probable that the aminochrome rearranges to a 5,6-dihydroxyindole which is, in turn, oxidized to a true 5,6-indolequinone, and that this substance subsequently undergoes a series of self-condensation reactions [*cf.* review by Mason (175, p. 163)]. The autoxidation and oxidation by silver oxide in acetone of a number of 5,6-dihydroxyindoles has been described as proceeding via yellow (true indolequinone) and purple (melanochrome) stages before the formation of the black melanins (29).

Acid solutions of adrenolutin take up oxygen slowly in the cold, and rapidly at 100°C., to give a dimeric oxidation product, 5,6,5′,6′-tetrahydroxy-1,1′-dimethylindigo (LXVI) (113). The melanization reaction can occur also by an



LXVI

internal oxido-reduction mechanism. The transformation of adrenochrome to black insoluble products also takes place under anaerobic conditions (24, 45, 113); at pH 5 decomposition occurs at the same rate in nitrogen as in air (24, 45). The conversion under these conditions is markedly catalyzed by acid (113).

D. STABILITY OF SOLUTIONS

An understanding of how the stability of adrenochrome solutions is influenced by various external factors is of considerable importance if systematic investigations into the physiological activity of the substance are to be undertaken. The

stability of the solutions is conditioned by chemical changes involving mainly rearrangements, oxidations, and oxido-reductions.

The decomposition of adrenochrome solutions is followed polarographically (269) or, more usually, by observing the optical density of the solution at ca. 490 mμ. The visual changes that are observed when dilute ($M/500$) solutions of adrenochrome in media of varying acidity or alkalinity undergo slow atmospheric oxidation at 23°C. have been recorded (250). The solution was "visually" stable for 48 hr. in $M/200$ buffer (pH 8) and for 2 hr. in $M/200$ sodium dihydrogen phosphate. In dilute acid slight fading was observed on mixing; the solution then became green in color and a dark precipitate formed. Only in the early stages were the changes induced by acid reversible (250). In Sörensen buffers in the pH range 0.0–2.2, adrenochrome solutions range in color from lemon-yellow to red. Initially this color change is reversed on neutralization; however, an irreversible change slowly occurs and a blue precipitate forms, the rate of this change increasing with the acidity of the medium (35). 2-Iodoadrenochrome exhibits similar changes, although its solutions at high acidity are more stable and the yellow color is only observed at pH 0–0.2 (35). In alkaline media isomerization to the fluorescent adrenolutin occurs almost instantaneously, and this is followed rapidly by a destructive oxidation. (The rates of rearrangement and oxidation increase with pH; see Section II,A.) The concentration and temperature as well as the pH of adrenochrome solutions have a marked effect on the stability, and the observed rate of disappearance of adrenochrome increases linearly with the pH (in the intermediate range) and with the concentration (78, 182, 270). The induction period before decomposition sets in is shortened by raising the temperature (logarithmic relationship) and the rate of decomposition also increases with temperature (24, 45, 182, 270). Solutions in saline (0.9 per cent) were more stable than solutions in distilled water, which in turn were more stable than solutions in methanol (88). The rate of decomposition under neutral or mildly acid conditions is as fast in nitrogen as in air (24, 45).

E. HALOGENATION

Although 2-iodo and 2-bromoadrenochrome are usually isolated from the oxidation products of adrenaline with potassium iodate or bromine, adrenochrome is the initial product of the oxidation. The monosemicarbazone can be isolated under certain conditions from the iodate oxidation (68, 163) and there is also spectroscopic evidence for the intermediate formation of adrenochrome (39, 63, 68, 163). It has been shown that the relative concentrations of the reactants and the pH of the reaction mixture are important factors in determining whether iodination occurs or not. Iodination only occurs at pH 3 and with at least a 1:1 molar ratio of iodate to adrenaline (39, 63, 64, 67, 68, 101, 163). In the case of the iodate oxidation, some of the iodate is first reduced by adrenaline to iodide, which is then oxidized to free iodine (the effective substituting agent at acid pH) by the excess iodate. Although oxidation with iodine is usually considered to give adrenochrome (*cf.* 39, 63, 74, 109, 189, 190), the 2-iodo derivative is obtained under acid conditions (63, 77).

From the reduction products of iodoadrenochrome, an iodo-5,6-diacetoxy-*N*-methylindole (m.p. 153–155°C.) has been obtained (33). However, the diacetyl derivative of the rearrangement product of iodoepinochrome melts at 146–147°C. (116). The iodine atom is assumed to be in position 3 in the latter case and in position 2 in the former case. Noradrenochrome, 2-methylnoradrenochrome, and *N*-isopropylnoradrenochrome (i.e., all aminochromes with a 3-hydroxyl group) are assumed to be iodinated in the 2-position and all aminochromes without a 3-hydroxyl group to be iodinated in the 3-position (47). By analogy, the bromination of adrenochrome is assumed to occur at position 2 (113). No chlorine-substituted aminochromes have been described as yet; the oxidation of adrenaline hydrochloride solution with chlorine water simply gives a black melanitic precipitate (219, 220).

It has been suggested (233) that the fact that the iodo derivative of adrenochrome is optically active (212) illustrates that substitution occurs at atom 2 (at the moment there is no completely unambiguous proof that substitution does in fact occur at atom 2), and that the asymmetric atom 3 is not affected. However, this would only be true if the substitution occurred by an S_N1 mechanism; if an S_N2 mechanism were operative the product would be optically active if substitution occurred at either position 2 or position 3. Substitution at the 2-position would lead to the formation of another asymmetric center and the product would presumably be a diastereoisomeric mixture. Owing to the limited stability of these compounds, it has not, as yet, been possible to resolve them.

F. DEHALOGENATION

During the attempted preparation of the Girard T and P derivatives of 3-iodoepinochrome and 2-iodo- and 2-bromoadrenochrome, the halogen atom was eliminated and the derivatives derived from epinochrome and adrenochrome were obtained (232). 5,6-Diacetoxy-*N*-methylindole has been isolated from the action of acetic anhydride on 2-iodoadrenochrome in the presence of zinc dust and sodium acetate (33). If 2-iodoadrenochrome or 2-iodo-*N*-isopropylnoradrenochrome is rearranged with zinc acetate and the resulting solution treated with sodium hydrosulfite the only product isolated is the deiodinated 5,6-dihydroxyindoxyl; in this case, however, the iodine atom may be removed from the dihydroxyindoxyl (47). When 2-iodoadrenochrome is reduced by ascorbic acid or sodium hydrosulfite, there is paper chromatographic evidence that both 5,6-dihydroxy-2-iodo-*N*-methylindole and 5,6-dihydroxy-*N*-methylindole are produced (122).

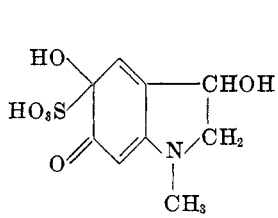
The addition complex of sodium bisulfite and 2-iodoadrenochrome gives rise to adrenochrome and not the 2-iodo derivative on cautious treatment with dilute alkali (36).

G. SODIUM BISULFITE DERIVATIVE

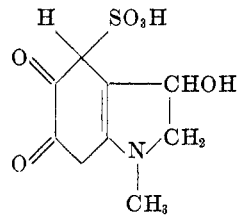
The red color of adrenochrome solutions is rapidly discharged on the addition of sodium bisulfite, with the formation of pale yellow fluorescent solutions (28, 36, 82, 250). Although the product was initially considered to be leucoadreno-

chrome, the properties of this "leuco-bisulfite" were different from those of other "leuco derivatives," e.g., that produced by ascorbic acid (28, 89). Solutions of adrenochrome decolorized by bisulfite show the properties of an addition complex rather than a reduction product, i.e., they do not give reactions of phenols and oxidants do not regenerate the red color, but cautious treatment with alkali does (28, 89). Recently the complex has been obtained in the solid state as a pale yellow solid giving strongly fluorescent solutions (82). The bisulfite derivative is optically active ($[\alpha]_D^{20°} = +50°$ in water) and its ultraviolet spectrum has absorption maxima at 247 and 350 mμ and a minimum at 275 mμ (28, 82). A semicarbazone and a *p*-nitrophenylhydrazone of this compound have been reported (although no melting points or analysis were quoted). These substances are readily converted into the corresponding adrenochrome derivatives by the action of alkali.

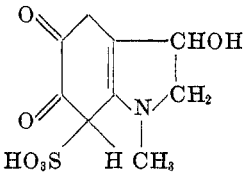
It has been suggested that the structure of this substance is not given by a simple addition to the carbonyl group (LXVII) but is of the type shown in formula LXVIII or formula LXIX (36, 82). When examined chromatographically



LXVII



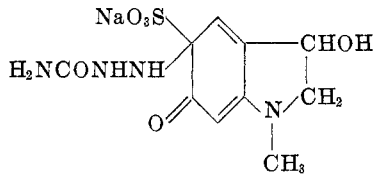
LXVIII



LXIX

on paper, this substance gives a strongly fluorescent spot with an R_f of ca. 0.04 in 1-butanol–acetic acid–water systems (82, 119) and of ca. 0.9 in 2 per cent acetic acid (119). A similar spot is also observed among the reaction products when adrenochrome is treated with sodium hydrosulfite, sulfur dioxide, sodium formaldehyde sulfoxylate, and sodium glyoxal bisulfite (82, 119).

In the patent literature it is claimed that treatment of the adrenochrome–sodium bisulfite addition product with semicarbazide or reagents that are specific for ketones gives substances of the general type shown in formula LXX, i.e., sodium 3-hydroxy-6-keto-*N*-methyl-5-semicarbazido-2,3,5,6-tetrahydroindole-5-sulfonate (236).



LXX

H. "BIURET" REACTION

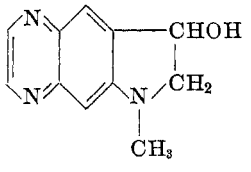
Adrenochrome gives with alkaline copper sulfate solutions a violet color resembling that of the biuret reaction. The material responsible for the violet

color is an unidentified, green, copper-free solid (probably not a single compound) (38).

I. REACTIONS WITH AMMONIA AND ORGANIC BASES

The aminochromes being superficially at least orthoquinones would be expected to condense with *o*-phenylenediamine to give phenazine derivatives; however, as yet only unidentified amorphous products have been obtained (51, 112). The fluorescent products obtained from the alkaline oxidation of adrenaline have been known for some time to be very unstable in air; however, in 1949 it was reported that comparatively stable fluorescent derivatives are produced by the action of primary amines (e.g., ethylenediamine, butylamine, benzylamine, aniline, etc.) in ammonia solution on adrenaline at elevated temperatures (i.e., 50–100°C.) (186). The fluorescent products can be extracted from the reaction mixture with the higher aliphatic alcohols. Ammonia itself reacts with adrenochrome to give a series of unstable fluorescent intermediates, leading rapidly to the formation of a dark brown melanitic precipitate (195).

The product obtained from the reaction between ethylenediamine and adrenochrome had the same fluorescence characteristics as that from ethylenediamine and adrenaline, and it appears that oxidation of adrenaline to adrenochrome is an essential prerequisite for the formation of fluorescent products (50, 262, 263, 268). Although the structure of the fluorescent product (or products) was unknown, this procedure formed the basis of a new fluorimetric assay method for adrenaline, introduced in 1952 (262, 263). Since then much work has been devoted to improving the experimental procedure (increasing the intensity of the fluorescence) and to differentiating empirically between the fluorescent products obtained from adrenaline and from noradrenaline. Variation of the reaction parameters appears to have a considerable effect on the fluorescence characteristics and absorption spectra of the products (*cf.* 7, 50, 102, 165, 185, 199, 200, 268); it was soon apparent that the reaction was not as simple as originally supposed and that variable mixtures of products were produced depending on the reaction conditions employed. Two-dimensional paper chromatography (after elimination of excess ethylenediamine, which interferes with the separation) has shown that the reaction between ethylenediamine and adrenaline gives eighteen products, while that with noradrenaline gives nineteen (184). When labelled adrenaline was used, ten of the spots were radioactive; presumably these were derived from adrenaline. A single spot, with fluorescence characteristics similar to those of the crude reaction product, proved to be a major component of the mixture in both cases (184). The isolation of the fluorescent



LXXI

compound from the reaction between adrenochrome and ethylenediamine in aqueous methanol has recently been reported. It is 2,3-dihydro-3-hydroxy-1-methylpyrrolo[4,5,*g*]quinoxaline (LXXI), i.e., the reaction involves a further oxidation stage with the loss of two hydrogen atoms (117). The yield is said to be very low and many by-products (some fluorescent) are produced.

VII. Preparation and Properties of Functional Derivatives of the 5-Carbonyl Group

A. GENERAL INTRODUCTION

One of the main pillars of evidence for the dipolar structure for adrenochrome, and for aminochromes in general, was the persistent formation of "mono" condensation products with all the typical ketone reagents (i.e., hydroxylamine, phenylhydrazine, etc.). Early workers reported that epinochrome gave a monophenylhydrazone (51), that adrenochrome gave a monoxime (109), and that dopachrome gave a monophenylhydrazone, a mono-*p*-nitrophenylhydrazone, and a mono-*p*-bromophenylhydrazone (255). These substances were stable crystalline solids and immediately attracted the attention of the physiologists, who sought a way to "stabilize" adrenochrome for use in biological work.

B. PREPARATIVE METHODS

These derivatives are readily formed in good yield, either from the aminochrome or directly from the oxidized catechol amine solution, by treatment with the desired reagent in aqueous solution in the presence of sodium acetate. They are considerably more stable than the parent aminochromes and are usually obtained as well-defined crystalline solids varying from yellow to deep red-brown in color. The known derivatives of this type derived from the aminochromes are listed in table 7.

C. PHYSICAL PROPERTIES

1. Solubility

Adrenochrome monosemicarbazone [this substance is sometimes formulated as a derivative of adrenochrome in the true *o*-quinonoid form, i.e., as LXXII, but the zwitterionic form (LXXIII) is better suited to explain many of its properties] is almost insoluble in nonpolar solvents, but it is reasonably soluble in methanol, ethanol, pyridine, and water. Its solubilities in water at 5°, 10°, 15°, and 20°C. are 0.310, 0.350, 0.365, and 0.400 mg./cc., respectively (26). In attempts to increase the solubility in water the effect of various hydrophilic groups (e.g., $-SO_3Na$; $-CH_2CH_2OH$) on the solubility has been studied (137, 138, 248, 249). The most soluble compound obtained was the sodium salt of epinochrome monosemicarbazone-3-sulfonic acid (LXXIV), which has about fifty times the solubility of adrenochrome monosemicarbazone at 0°C. (138). There are references in the patent literature as to the ability of sodium salts of

TABLE 7

Aminochrome derivatives

Aminochrome	Functional Derivative	Melting Point (with Decomposition)*	References
		°C.	
Adrenochrome†	Oxime	278 ($1.5H_2O$)‡	(109)
		192 ($1H_2O$)	(256)
		178–182 ($2H_2O$)	(135)
		172 ($1H_2O$)	(45)
	Semicarbazone	203 ($2H_2O$)	(45)
		212–213	(218)
		204	(163)
		203	(24)
		Not stated	(26, 235)
	Thiosemicarbazone	215–220	(98)
	2′-Methylsemicarbazone	Not stated	(233)
	4′-β-Hydroxyethylsemicarbazone	208	(137)
	Phenylhydrazone	Not stated	(234)
	4′-Nitrophenylhydrazone	200 ($4H_2O$)	(234, 256)
	4′-Carboxyphenylhydrazone	195–200	(233)
	Isonicotinylhydrazone	210–213	(22)
		207 ($0.75H_2O$)	(140)
	Benzylthiazylhydrazone	236–238	(233)
	Carboxyhydrazone	167–169	(233)
	Cyanoacetylhydrazone	194	(140)
	Girard P derivative	210	(232)
	Girard T derivative	160§	(232)
Epinochrome	Oxime	180–181	(140)
	Semicarbazone	225	(140)
	4′-β-Hydroxyethylsemicarbazone	198	(137)
	Phenylhydrazone	226–227	(51)
	Girard P derivative	Not stated	(230, 231, 232)
	Girard T derivative	140	(230, 231, 232)
Dopachrome	Phenylhydrazone	168 ($1H_2O$)	(255)
	4′-Nitrophenylhydrazone	190 ($2H_2O$)	(255)
	4′-Bromophenylhydrazone	174 ($2H_2O$)	(255)
2-Methylnoradrenochrome	Semicarbazone	195–196	(47)
4-Methylnoradrenochrome	Semicarbazone	197–198	(132)
2-Ethylnoradrenochrome	Semicarbazone	Not stated	(25)
N-Isopropylnoradrenochrome	Semicarbazone	204	(47)
		Not stated	(25)
	Girard T derivative	195	(230, 233)
N-(β-Hydroxyethyl)noradrenochrome	Semicarbazone	218 ($1H_2O$)	(140)
	4′-β-Hydroxyethylsemicarbazone	197–199	(137)
3-Methoxyepinochrome	Semicarbazone	217–218	(133)
3-Ethoxyepinochrome	Semicarbazone	214–215	(133)
3-Isopropoxyepinochrome	Semicarbazone	206–207	(133)
2-Carbethoxyepinochrome	Semicarbazone	212	(140)
	4′-β-Hydroxyethylsemicarbazone	165–167	(137)
	Girard T derivative	Not stated	(232)
2-Carbethoxyiodoepinochrome	Semicarbazone	149–150	(140)
Sodium 2-epinochromesulfonate	Semicarbazone	227–228 ($3H_2O$)	(248, 249)
Sodium 3-epinochromesulfonate	Semicarbazone	227–228 ($3H_2O$)	(138)
3-Epinochromesulfonic acid¶	Semicarbazone	Darkens at 130; decomposes at 195 ($1.5H_2O$)	(138)

* Number of molecules of water of crystallization given in parentheses (where applicable).

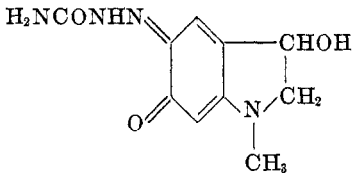
† The majority of these compounds have been prepared from adrenochrome derived from L-adrenaline, but adrenochrome monosemicarbazone has also been prepared from the D- and DL-forms.

‡ The figure 2 may have been a misprint, since a melting point of 178°C. would seem to be in closer agreement with the other figures quoted.

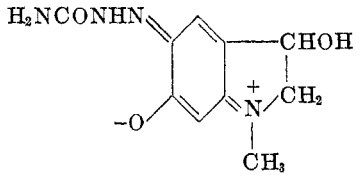
§ This melting point has recently been quoted as 260°C. (233).

¶ A number of metal salts besides the sodium salt of this derivative have been prepared and the following melting points (with decomposition) are given: calcium, barium, cupric, zinc, cobalt, and silver salts, no decomposition below 270°C.; potassium salt, 243°C.; ammonium salt, 223°C.; magnesium salt, 190°C.; aluminum salt, 130°C. (138).

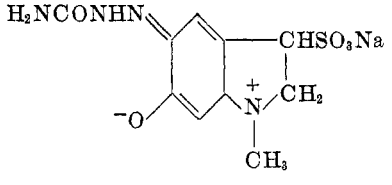
certain organic acids to increase the water solubility of adrenochrome monosemicarbazone (97, 237).



LXXII



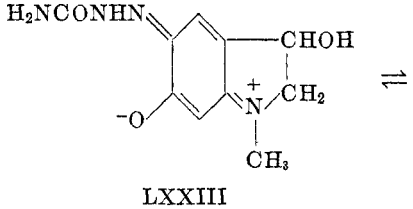
LXXIII



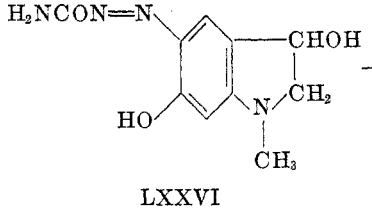
LXXIV

2. Ultraviolet and visible absorption spectra

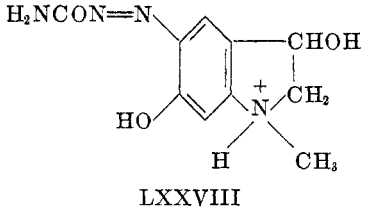
The structure of adrenochrome monosemicarbazone in solution and its variation with pH and the attendant color changes have been extensively studied spectroscopically (65, 138, 204, 233). These authors agree that in neutral solution the semicarbazone (a) has a maximum at 220 mμ (65), (*b*) has maxima in



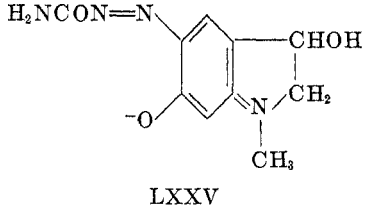
LXXIII



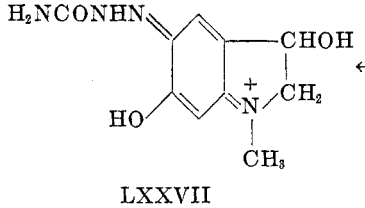
LXXVI



LXXVIII



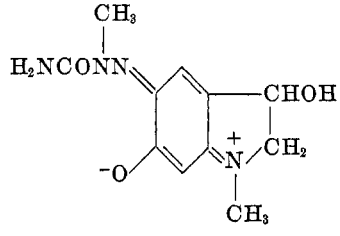
LXXV



LXXVII

the region 354–357 mμ (65, 204, 232, 233), and (*c*) has an inflection in the region 400–440 mμ (204, 232, 233). A very similar spectrum is observed under mildly acid conditions, but increasing acidity causes a shift of the 354 peak to 375 mμ (65, 204); the other maximum and the inflection disappear (65). Under alkaline conditions a different phenomenon is observed. As the pH is increased the 354–357 mμ peak disappears and the 400–440 mμ inflection transforms to a maximum in the region of 437–447 mμ (65, 204, 232, 233). In neutral solution the spectrum resembles that of benzoquinone monosemicarbazone, *p*-ethoxyphenylazoformamide, and *p*-hydroxyazobenzene; in alkaline solution it is similar to that of *p*-naphthoquinone *N*-methylphenylhydrazone (65). The shifts in maxima are ascribed to a pH-dependent equilibrium between the azophenol ion (LXXV) and a tautomeric mixture (independent of pH) of the monohydrazone (LXXIII) and undissociated azophenol (LXXVI) (204, 233).

In acid solution protonation of the neutral molecule could occur to give a cation of the type of LXXVII or LXXVIII. Isobestic points are observed at 360 mμ and 393 mμ, the former representing the equilibrium between cationic and neutral forms and the latter the equilibrium between neutral and anionic forms (204). In the case of the *N*-methylsemicarbazone (LXXIX) a hydrogen atom is not available on the 2′-nitrogen atom to migrate. Thus the number of possible molecular species is limited and no spectral changes occur with increasing pH; the substance slowly decomposes (233).

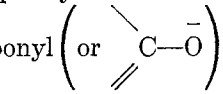
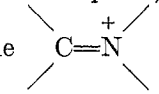
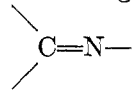
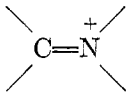
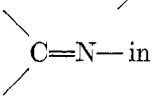


LXXIX

In general, aminochrome monosemicarbazones exhibit their main absorption maxima at ca. 360 mμ in mildly acid and neutral solutions and this shifts to 435–460 mμ in alkaline solution (138). The former peak seems to occur at somewhat shorter wavelengths in the case of the oximes and at longer wavelengths in the case of the thiosemicarbazones and the 2′-methylsemicarbazones (233). Detailed lists of absorption maxima and extinction coefficients have been given in previous papers (138, 232, 233). Measurement of the optical density of the 354 mμ peak (pH range 3.65–7.80) has been suggested for the determination of adrenochrome monosemicarbazone (66).

3. Infrared spectra

The infrared spectrum of adrenochrome monosemicarbazone in the solid state has been reported (138, 204). The main peaks in the high-frequency region occurred at 3360–3280 and 3120–3040 cm.$^{-1}$ (O—H and bonded N—H stretching

(204)). In the "carbonyl" region, there is some confusion as to the correct allocation of the various absorption frequencies. Peaks observed at 1650 and 1600 cm.$^{-1}$ have been assigned to the semicarbazide carbonyl and the conjugated C=N, respectively. The remaining strong "carbonyl" band at 1690 cm.$^{-1}$, although at a higher frequency than would be expected, would have to be assigned to the remaining carbonyl  group (204). Another worker assigns bands at 1695 and 1653 cm.$^{-1}$ to the "amide and ketone" carbonyls, respectively (138). The author has recently obtained the spectrum of this compound[1] (as a Nujol mull). The high-frequency peaks were observed at 3370 and 3120 cm.$^{-1}$ In the 1750–1550 cm.$^{-1}$ region strong peaks were observed at 1703, 1663, 1610, and 1562 cm.$^{-1}$ In a complex molecule of this nature definite assignments are difficult, since there is probably a considerable amount of mixing of the vibrations. Accepting the zwitterionic formulation (LXXIII) for the monosemicarbazone, the band at 1703 cm.$^{-1}$ could be assigned to the semicarbazone carbonyl group, and the peak at 1562 cm.$^{-1}$ to the Amide II absorption; the remaining peaks at 1663 and 1610 could be identified with the  and  groups. [The frequencies of  and  in myosmine monohydrochloride and myosmine are quoted as 1667 and 1626 cm.$^{-1}$ (267)].

4. Optical activity

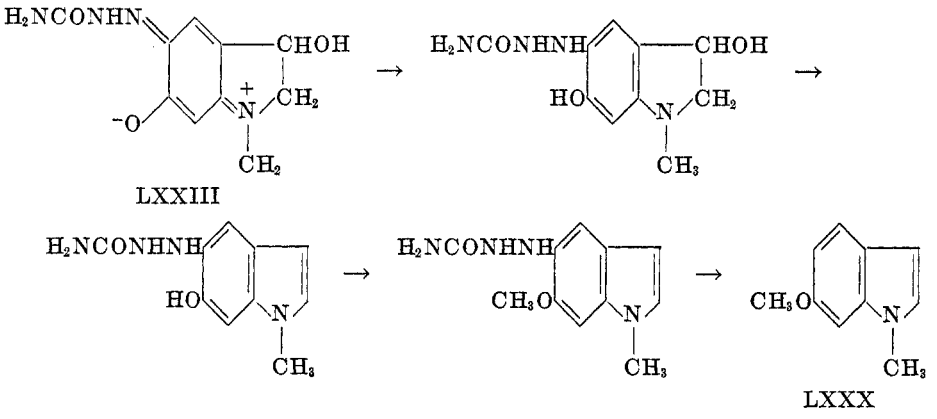
The optical activity of solutions of the semicarbazones derived initially from L- and D-adrenaline have been reported. That from L-adrenaline has $[\alpha]_{579}^{15°} = +135°$ in methanol and $+352°$ in pyridine, whereas the product derived from D-adrenaline has values of $-135°$ in methanol and $-395°$ in pyridine (26).

D. STRUCTURE

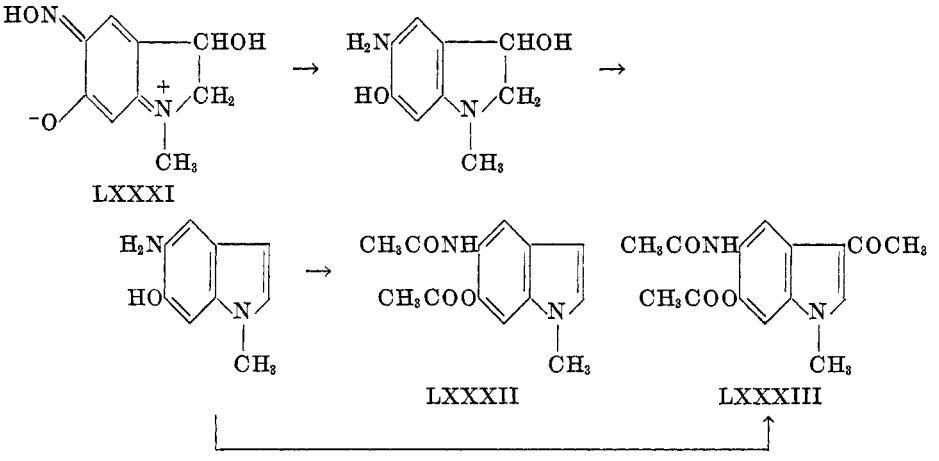
Although it seems to have been generally assumed (on theoretical grounds) that condensation with the ketone reagent occurs at the 5-position, this has only recently been proved chemically. Iwao subjected adrenochrome monosemicarbazone (LXXIII) to catalytic hydrogenation, and pyrolysis of the methylated product led to the isolation of 6-methoxy-*N*-methylindole (LXXX) (136) (*cf.* Scheme D), the semicarbazide function having been eliminated from the 5-position. (The structure of the methoxyindole (LXXX) was confirmed by unambiguous synthesis.) The same author applied a similar series of reactions to adrenochrome monoxime (LXXXI) and obtained the acetyl derivatives of 5-amino-6-hydroxy-*N*-methylindole (LXXXII and LXXXIII) (135) (*cf.* Scheme E).

[1] This spectrum was obtained through the courtesy of Dr. R. N. Jones of the National Research Council (Ottawa), who also offered much useful advice on its interpretation.

Scheme D



Scheme E



E. CHEMICAL PROPERTIES

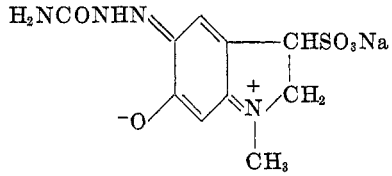
The chemical properties of these compounds have not been very widely studied, but several color reactions of adrenochrome monosemicarbazone have

TABLE 8

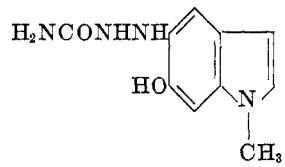
Color reactions of adrenochrome monosemicarbazone

Reagent	Color Produced	Reference
1. Zinc and acetic acid	Solution decolorized	(218)
2. Ferric chloride	Orange turning blood-red	(218)
3. Mercuric acetate	Blood-red	(218)
4. Nessler's reagent	Pinkish-red	(67)
5. Fehling's solution	Emerald-green	(67)
6. Sodium nitrite and dilute hydrochloric acid	Pale yellow	(218)
7. Sodium nitrite and 50 per cent sulfuric acid	Red	(67)
8. Concentrated sulfuric acid	Green	(67)
9. Dilute aqueous acid	Pale yellow (reversible)	(67)
10. Dilute aqueous alkali	Intense yellow (reversible)	(67)

been described (see table 8). The preparation of an unstable hydrochloride (m.p. 150°C.) of adrenochrome monosemicarbazone has recently been reported (133). The hydrogenation has already been described (see Section VII,D). Replacement of the 3-hydroxyl group in the monosemicarbazone by an —SO_3Na group to give LXXIV occurs during the interaction of the semicarbazone and sodium bisulfite in aqueous solution (containing sulfur dioxide; pH ca. 1–1.2) at 30°C. for long periods (138). Inversion of the sign of optical rotation occurs during the



LXXIV



LXXXIV

course of the reaction, indicating that the mechanism is probably a simple S_N2 substitution, rather than a dehydration of the semicarbazone followed by addition of a molecule of sodium bisulfite (138). Catalytic hydrogenation of LXXIV gives 6-hydroxy-*N*-methyl-5-semicarbazidoindole (LXXXIV), the sulfonic acid group being eliminated during the reduction (138).

F. RELATED COMPOUNDS

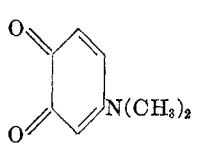
Some interesting compounds closely related to those above have been described recently showing very similar physical properties. They are the monoxime and monosemicarbazone of 1-dimethylamino-3,4-benzoquinone (XXXI) (139) and the monoximes and monosemicarbazones of 1,2,3,4-tetrahydro-*N*-methylquinoline-6,7-quinone (XXV) and 1,2,3,4-tetrahydro-4-hydroxy-*N*-methylquinoline-6,7-quinone (XXVI) (137, 138, 140). The melting points of tetrahydroquinoline derivatives are listed in table 9.

TABLE 9

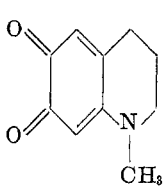
1,2,3,4-Tetrahydroquinoline-6,7-quinone derivatives

Quinone	Functional Derivative	Melting Point (with Decomposition)*	Reference
		°C.	
1,2,3,4-Tetrahydro-*N*-methylquinoline-6,7-quinone	Oxime	182	(141)
	Semicarbazone	225	(141)
	4′-β-Hydroxyethylsemicarbazone	186-187	(137)
1,2,3,4-Tetrahydro-4-hydroxy-*N*-methylquinoline-6,7-quinone	Oxime	177	(141)
	Semicarbazone	247 ($3H_2O$)	(141)
	4′-β-Hydroxyethylsemicarbazone	194 ($0.5H_2O$)	(137)
Sodium 1,2,3,4-tetrahydro-*N*-methylquinoline-6,7-quinone-4-sulfonate	Semicarbazone	Colors at 160-170 and decomposes at 210 ($4H_2O$)	(138)

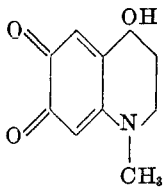
* Number of molecules of water of crystallization given in parentheses (where applicable).



XXXI



XXV



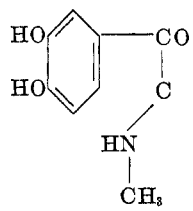
XXVI

VIII. Some Comments on the Significance of Adrenochrome Chemistry in the Medical and Biological Fields

A. The composition of "deteriorated" adrenaline solutions

In the past twenty-five years, diverse physiological effects have been ascribed to the somewhat indefinite oxidation products of adrenaline referred to by such names as "aged," "deteriorated," "oxidized," "colored," or "pink" adrenaline (*cf.* reviews in references 10 and 152). Such solutions, which may be colored pink, yellow, or brown and may contain black insoluble melanins, will often still contain unchanged adrenaline (as demonstrated by bioassay) (10, 72). Thus products representing all stages of the overall transformation of adrenaline to melanin are often present in these solutions: i.e., adrenochrome (which is probably responsible for the pink color); adrenolutin (the fluorescent product); 5,6-dihydroxy-*N*-methylindole (the stable dehydration product of the hypothetical "leucoadrenochrome"); and possibly compounds of an oxidation level between adrenochrome and melanin (e.g., true indolequinones, "oxoadrenochrome", dimeric indigoid-type molecules, "adrenoxine", etc.).

It should also be remembered that pharmaceutical preparations of adrenaline may contain "stabilizers," including reducing agents such as ascorbic acid and sodium bisulfite and in some cases other pharmacological substances (e.g., novocaine) and that the effect of these substances on the oxidation of adrenaline must be considered. The oxidation of adrenaline is retarded by ascorbic acid (257); however, the presence of adrenalone (LXXXV) and its further oxidation products has been reported in adrenaline solutions that have undergone slow



LXXXV

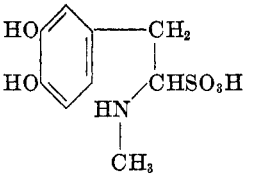
oxidation in air in the presence of ascorbic acid (27). Adrenaline and sodium bisulfite also show mutual inhibition of oxidation; oxygen uptake is exceedingly slow in neutral solution, but at pH 8–9 strongly fluorescent colorless solutions are formed with an oxygen uptake of between one and two atoms per molecule

(250). The enzymatic oxidation of adrenaline by polyphenolases in the presence of sodium bisulfite also leads to the formation of colorless fluorescent solutions (at no time was a red color observed); the oxygen uptake corresponds approximately to the adrenochrome level of oxidation being reached (250). The fluorescence mentioned above was described in both cases as persisting for a relatively long time; it was therefore unlikely to be due to adrenolutin and most probably was due to the adrenochrome–sodium bisulfite complex. This fact was probably not realized by early workers and this fluorescence was no doubt confused with that of adrenolutin. 5,6-Dihydroxy-*N*-methylindole is not present in "deteriorated" solutions containing bisulfites (171).

Increasing the pH of the solution has a deleterious effect on the stability of adrenochrome solutions (see Section VI,D), and it has been suggested that the alkalinity of the glass container may be an important factor in determining the stability of the solution (171). Traces of metallic cations present in the solution will catalyze its decomposition, and it is desirable that heavy metal salts should be excluded from pharmaceutical preparations of adrenaline (see Section II,A, 2).

Paper chromatograms of "deteriorated adrenaline" solutions using a 1-butanol–acetic acid–aqueous sodium metabisulfite solvent system often show a characteristic fluorescent spot at R_f 0.04 which is probably due to the adrenochrome–sodium bisulfite complex (81, 82, 119) formed by interaction between adrenochrome present in the solution under examination and the solvent system. This permits a rapid examination of adrenaline preparations for adrenochrome even if there are present in the mixture other colored components which would normally mask the red color (*cf.* 81).

The anaerobic degradation of adrenaline in solution by bisulfites has recently been described, and a white nonbasic crystalline product which did not contain sodium (m.p. 253–254°C.) was isolated (226). A white crystalline solid (m.p. 259°C.), identified as 1-methylamino-2-(3,4-dihydroxyphenyl)ethanesulfonic acid (LXXXVI), has been obtained from the action of sodium bisulfite on adrenaline in aqueous solution at 100°C. (249).



LXXXVI

B. ESTIMATION OF ADRENALINE

1. General comments

The majority of chemical methods available for the determination of adrenaline involve its oxidation to adrenochrome (or 2-iodoadrenochrome), which is either determined directly by colorimetry or converted into a fluorescent derivative which is determined fluorimetrically.

2. Colorimetric methods

Colorimetric methods were first employed for the chemical estimation of adrenaline. A method based on the oxidation with iodate was described in 1909 (100); subsequent developments have been adequately reviewed elsewhere (*cf.* 83, 142, 199, 228, 239). The technique has been satisfactorily extended to the differential estimation of mixtures of adrenaline and noradrenaline based on the different rates of oxidation of these bases at different pH's: e.g., at pH 4 adrenaline is completely oxidized by iodine in 90 sec.; under these conditions only 10 per cent of the noradrenaline is oxidized. At pH 6 both are completely oxidized in 3 min. (86) (see 83 for lists of references).

A method based on the colorimetric estimation of the red and purple-red products (unidentified) obtained from the condensation of adrenaline and *o*-phenylenediamine or 2,3-diaminonaphthalene in the presence of hydrogen peroxide has been suggested (242).

3. Fluorimetric ("lutin" method)

In 1947 West described an adrenaline assay method (265) based on the earlier observations that adrenaline developed a characteristic yellow-green fluorescence in alkaline solution (21, 105, 157, 194). The problems involved in the measurement of the intensity of the fluorescence owing to the transient nature of the substance responsible could be considerably alleviated by the addition of ascorbic acid, which retarded the decomposition of the fluorescent substance (77, 265). General improvements in the experimental technique were possible following the clarification of the chemical reactions involved (76, 89, 90, 112, 113, 124). It was shown that the fluorescence produced aerobically from adrenaline in alkaline solution was the same as that produced anaerobically from adrenochrome (90, 124), and the sensitivity of the method was improved by the use of oxidizing agents (other than air) to oxidize the adrenaline to adrenochrome prior to its rearrangement with alkaline ascorbic acid (76, 77, 160).

The alkaline oxidation by atmospheric oxygen of noradrenaline also produces a fluorescent derivative, although the intensity of the fluorescence is much lower than that developed by adrenaline under the same conditions (105). However, it has been possible to adapt the differential oxidation method (as used in the colorimetric methods) to assay mixtures of adrenaline and noradrenaline fluorimetrically. Manganese dioxide (69, 161, 198), potassium ferricyanide (76, 77, 85), and iodine (85, 188, 189, 239) have all been used as oxidants prior to rearrangement to the fluorescent indoxyls.

The relatively recent development of the spectrophotofluorimeter has enabled the excitation and fluorescence spectra of adrenolutin and noradrenaline to be determined accurately (57, 124). Marked differences in both the excitation and fluorescence spectra were observed, enabling a quantitative differential estimation of the two catechol amines to be made (57, 58).

Another differential estimation of adrenaline and noradrenaline has been based on the facts that the alkaline rearrangement of noradrenochrome to noradreno-

lutin is slower than the corresponding rearrangement of adrenochrome and that the fluorescent product obtained from the "nor" series is destroyed faster by ascorbic acid (214). (See references 58, 83, 199, 203 and 239 for lists of references on these methods.)

4. Fluorimetric ("ethylenediamine" method)

The formation of relatively stable fluorescent products, extractable with the higher aliphatic alcohols, during the reaction of adrenaline with ethylenediamine (186) was adapted to the determination of adrenaline and related substances in blood in 1952 (262, 263). (The chemistry of this reaction, which involves the preliminary oxidation of adrenaline to adrenochrome, is discussed in Section VI,I.) The basic procedure has been adapted to the differential determination of mixtures of adrenaline and noradrenaline, e.g., the addition of sodium thiosulfate to acid solutions of the catechol amines prior to the condensation inhibits the formation of the fluorescent derivative from noradrenaline (166). As in the case of the "lutin" method the excitation and fluorescence spectra of the two condensates are sufficiently different to permit a quantitative differentiation to be made (134, 184, 200).

The formation of fluorescent derivatives in the ethylenediamine reaction depends on the catechol moiety of the molecule, whereas the strongly fluorescent derivatives utilized in the alkaline rearrangement method are indoxyls, i.e., the presence of a 3-hydroxyl group in the aminochrome is an essential prerequisite for the production of a strongly fluorescent indoxyl. Thus a combination of the two methods of estimation permits the differential estimation of mixtures of noradrenaline, adrenaline, and 3-hydroxytyramine (261). 3-Hydroxytyramine can be converted into a weakly fluorescent derivative (presumably 5,6-dihydroxyindole) by oxidation with iodine and subsequent rearrangement of the oxidation product with alkaline ascorbic acid (239).

5. Chromogenic reactions utilized in the paper chromatography of adrenaline

Adrenaline spots on paper chromatograms can be readily shown by spraying the papers with solutions of suitable oxidizing agents which oxidize the colorless adrenaline spots to pink adrenochrome spots. Potassium ferricyanide is the most common reagent (83, p. 15; 111, 144, 145). After the developed chromatograms are exposed to ammonia fumes (225) or sprayed with methanolic ammonia (81), or the papers are simply dried (83, p. 16), the formerly colorless nonfluorescent spots of adrenaline exhibit the characteristic yellow-green fluorescence of adrenolutin. Spraying with ethylenediamine alone (83, p. 15) or in the presence of an oxidizing agent (e.g., iodine or potassium ferricyanide) (79) converts the adrenaline into fluorescent spots. This reaction is very sensitive and has been used quantitatively (79).

C. ESTIMATION OF ADRENOCHROME

Two insensitive colorimetric methods were first employed. One depended on the formation of a violet color with Ehrlich's reagent that was not extractable

with chloroform (94); the other depended on the cautious decomposition of the bisulfite complex at pH 11.5, followed by immediate stabilization of the adrenochrome liberated by acidification to pH 3.2 and direct colorimetric estimation of the product by comparison with suitable standards (37). Fischer and Lecomte introduced a fluorimetric method for the estimation of adrenochrome and adrenolutin in biological systems, based on measurement of the direct fluorescence of adrenolutin and the combined fluorescence of the adrenolutin present originally and that produced by the zinc ion-catalyzed rearrangement of adrenochrome, ascorbic acid being used to stabilize the fluorescent material (95). This method has since been employed to estimate the formation of the substances during the enzymatic oxidation of adrenaline (151) and has recently been used to follow the fate of injected adrenochrome in humans (197).

D. PHYSIOLOGICAL ACTIVITY

1. Adrenochrome

It is not the purpose of the author to enter into a detailed discussion of the considerable volume of literature on the physiological and pharmacological properties of adrenochrome and related compounds. The presence of adrenochrome in mammalian tissues has not as yet been unequivocally demonstrated, i.e., it has not been isolated. This is not altogether surprising in view of the experimental difficulties that would be associated with the isolation of such a reactive substance. However, this does not completely rule out the possibility that it may have a transient and perhaps important role to play in the metabolism of adrenaline. The presence of unidentified adrenaline "oxidation products," which exhibit a fluorescence similar to that of adrenolutin and appear to be associated with tissue proteins, has recently been claimed in rabbit tissues (190, 252). The lowering of the adrenaline level with the attendant formation of adrenochrome and other unidentified substances is said to occur in rabbits when the animals are placed in compression chambers filled with pure oxygen under a pressure of 3.5 to 6 atm. (106).

Adrenochrome has been claimed to exhibit antipressor, hemostatic, hypoglycemic, hallucinogenic, antihyaluronidase, ACTH-like, and antimitotic activity. Adrenochrome monosemicarbazone (and similar derivatives) exhibit hemostatic activity. These investigations have already been discussed in several publications (10, 126, 127, 152, 168, 192, 193, 233).

2. Adrenoerythrin

The first stage in the oxidation of adrenaline probably involves the oxidation of the catechol moiety of the molecule with the formation of "adrenaline-quinone," sometimes called "adrenoerythrin." This substance may exist under conditions of high acidity (pH ca. 1.0), but at neutral pH it undergoes a very rapid intramolecular cyclization to adrenochrome (see Section IV); therefore it is unlikely to have more than a transient existence under biological conditions. Although the production by the oxidation of adrenaline under alkaline conditions of a quinone which retains the biological activity of adrenaline has been claimed

(216, 224), other workers consider this highly improbable and point out that the physiological activity of such solutions is probably due to unchanged adrenaline (12).

3. Adrenoxine

In order to explain some aspects of the physiological activity of oxidized adrenaline solutions, the formation in solution of an unidentified further oxidation product of adrenochrome named "adrenoxine" has been postulated. The evidence on which this proposal is based has been discussed in certain reviews (10, 17, 169).

4. Metabolism of adrenochrome

The metabolism of adrenochrome, adrenolutin, "adrenochrome reduction products" (sodium hydrosulfite reduction), and adrenochrome monosemicarbazone in rabbits, cats, and dogs has been studied (93, 94, 95). Adrenochrome injected into rabbits rapidly disappears from the blood and is transformed in the liver to adrenolutin, which is removed from the system via the kidney (93). Most of it is excreted as adrenolutin (both free and sulfoconjugated), and the residual adrenochrome is excreted unchanged. The urine also contains a fluorescent brown pigment, which is hydrolyzed to a stable red-violet pigment by boiling 10 per cent hydrochloric acid. A similar pigment is obtained from glycine and adrenochrome (15), suggesting that the brown metabolite was formed by interaction with proteins or amino acids. The injected adrenochrome is destroyed by a different mechanism in cats and dogs; some (ca. 15 per cent) is excreted unchanged, and a considerable amount (ca. 70 per cent) is converted to a "reduction product" of adrenochrome, and other indoles (ca. 10 per cent) are also detected (95).

An unidentified unstable yellow pigment which rapidly decomposes in air has been observed in the urine of rats after injection of β-C^{14}-*dl*-adrenochrome. This pigment, which was derived from the injected adrenochrome, decomposed when paper chromatographic investigation was attempted unless oxygen was rigorously excluded from the system (223).

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THE THERMOCHEMISTRY AND REACTIVITY OF ALKOXYL RADICALS

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