THE CHEMISTRY OF THE "AMINOCHROMES"*

PART I. THE PREPARATION AND PAPER CHROMATOGRAPHY OF PURE ADRENOCHROME[1]

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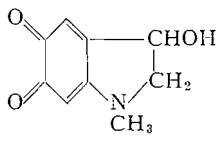
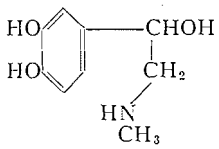
ABSTRACT

The preparation of adrenochrome in a pure stable crystalline form has been carried out by the silver oxide oxidation of adrenaline in methanol with the use of an anion-exchange resin (Dowex-1(Cl^-)) to remove heavy metal ions from the reaction mixture prior to the isolation of the product. Its paper chromatographic behavior together with that of three derivatives (adrenolutin, adrenochrome monosemicarbazone, and adrenochrome monoisonicotinic acid hydrazide) in six different solvent systems has been examined. Water was found to be the best paper chromatographic solvent so far examined for this series of compounds.

Adrenochrome (I), the substance mainly responsible for the red colors produced during the mild oxidation of adrenaline (II), was first isolated from the products of an enzymatic oxidation by Green and Richter (1). Subsequently, inorganic oxidizing agents, particularly

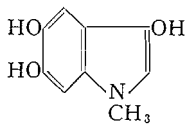


I



II

silver oxide, were successfully employed and it was observed that adrenochrome could be crystallized from methanol (containing a little formic acid) at −80° (cf. Veer (2); MacCarthy (3); Harley-Mason (4); Sobotka and Austin (5)). Green and Richter reported that adrenochrome was unstable both in the solid state and in solution (1); however, Sobotka and Austin claim that the dry crystalline material can be kept indefinitely at 0° (5). The stability of aqueous solutions of adrenochrome has been shown to be markedly affected by pH (cf. Zambotti and Moret (7)); under alkaline conditions and in the presence of certain metallic cations, particularly zinc, adrenochrome readily rearranges into adrenolutin (*N*-methyl-3,5,6-indoletriol, III) (cf. Lund (8); Fischer, Derouaux, Lambot, and Lecomte (9); Harley-Mason and Bu'Lock (10); Fischer and Lecomte (11)).



III

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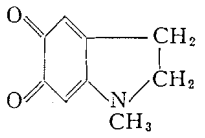
**The term "aminochromes" was suggested by Sobotka and Austin (5) for the highly colored cyclic oxidation products of β-(3,4-dihydroxyphenyl)-ethylamines and related compounds. These substances are often formulated as substituted 2,3-dihydroindole-5,6-quinones, although there is some uncertainty as to the existence of a true o-quinonoid structure (4, 6).*

Many samples of adrenochrome, prepared by existing methods, that were available to the authors appeared to contain varying amounts of adrenolutin (shown paper chromatographically by a method to be discussed later) and black water-insoluble melanin-like compounds. It appeared not improbable that contamination of the products by metal in a colloidal form or metallic ions, derived from the oxidizing agent, might be responsible for the deterioration of the samples and for the apparent instability of their aqueous solutions. In order to eliminate or drastically reduce the contamination by silver ions, the adrenaline – silver oxide reaction mixture in methanol was filtered through an anion-exchange resin bed in the chloride form prior to crystallization (at $-20°$) in order to remove ionic silver from solution as silver chloride. In this manner a highly crystalline product containing only traces of silver was obtained ($\gtrless 0.01\%$).

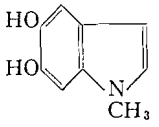
The paper chromatographic behavior of adrenochrome and related compounds has recently been studied by Fischer using *n*-butanol:acetic acid:water (8:2:2) and *i*-propanol: 0.880 ammonia:water (8:1:1) solvent systems (12). The results reported were unsatisfactory, since some of the substances apparently decomposed in these solvents. This is not altogether surprising in view of the known sensitivity of these compounds toward acids and alkalies (7, 8, 9), and furthermore Pavolini, Gambarin, and Godenigo in a study on the action of ammonia on some 5,6-indolequinones, including adrenochrome, reported a definite reaction in this case with the formation of an unstable substance, exhibiting a yellow-green fluorescence, which rapidly turns brown (13). We have repeated Fischer's work and observed with the *i*-propanol–ammonia system that the bright red non-fluorescent adrenochrome spot rapidly turned brownish-yellow in color and exhibited a yellow-green fluorescence, even on exposure to ammonia fumes, and in contact with the solvent the spot soon became dark brown indicating that marked decomposition had occurred. In the acid solvent system, a slower but definite decomposition of the adrenochrome spot was observed during the chromatography. We have found that dilute aqueous solutions of adrenochrome prepared by our method are relatively stable and that distilled water and 2% acetic acid in water are the most suitable solvents for paper chromatography. Pure adrenochrome gave a single spot $R_f 0.8 \pm 0.02$ in water on previously washed Whatman No. 1 paper. Adrenolutin had an R_f of *ca.* 0.4 under these conditions. Contamination of adrenochrome samples with adrenolutin can easily be observed paper chromatographically, since the characteristic yellow adrenolutin spot showing a strong yellow-green fluorescence is soon visible behind the red adrenochrome spot; this can be observed very quickly as it is only necessary to allow the chromatogram to run a few inches in order to see the two spots. The adrenolutin spot is quite distinct and not a diffuse "tail" to the adrenochrome spot, indicating that the impurity was present initially and not formed by continuous isomerization of the starting material. Polymeric melanin-type impurities stay in the position of the original spot. An interesting phenomenon was observed on drying of the paper chromatograms; as the paper was allowed to dry at room temperature, the color of the adrenochrome spot gradually changed from red to yellow-brown and the spot began to exhibit the characteristic adrenolutin fluorescence. The partial formation of adrenolutin was confirmed by two-dimensional chromatography, using water as running solvent in both directions, although only one spot $R_f 0.8$ was observed in the initial run; after drying and rerunning at 90° to the original direction two distinct spots with the characteristic R_f's for adrenochrome and adrenolutin appeared.

Euler, in describing the paper chromatography of adrenaline, states that after the chromatograms have been dried adrenaline spots show an apple-green fluorescence (14, p. 16). A possible explanation of this observation would be the air-oxidation of the

spot to adrenochrome followed by isomerization to adrenolutin on the paper. Therefore it would appear that a thin film of adrenochrome on paper will spontaneously rearrange into adrenolutin. This type of behavior has also been observed by Austin, Chanley, and Sobotka with the related compound epinochrome (IV), which even in the crystalline form undergoes a slow spontaneous rearrangement to 5,6-dihydroxy-*N*-methylindole (V) (15, 16). The paper chromatography of adrenochrome, adrenolutin, adrenoxyl (adrenochrome monosemicarbazone) (VI), and adrenochrome monoisonicotinic acid hydrazide



IV



V

(VII) has been studied in six different solvent systems: (*a*) water, (*b*) 2% acetic acid in water, (*c*) 75% methanol in water, (*d*) 75% ethanol in water, (*e*) *n*-butanol: acetic acid: water (8:2:2), and (*f*) *i*-propanol: 0.880 ammonia: water (8:1:1). Although all solvents proved satisfactory for either of the two derivatives, VI and VII, only (*a*) and (*b*) were suitable for adrenochrome and possibly only (*a*) for adrenolutin, since even in (*b*), (*c*), and (*d*) the fluorescence characteristics of the compound seem to be irreversibly altered.

An attempt to purify impure preparations of adrenochrome on cellulose columns using water as solvent was disappointing, since, although pure aqueous solutions of adrenochrome were probably obtained, it was not possible to crystallize the adrenochrome from water, and all samples prepared by freeze-drying under high vacuum (a technique which gave a very finely divided product) were shown chromatographically to be contaminated to some extent with adrenolutin (fortuitously formed during the drying process).

In view of the very considerable interest that has developed, in the physiological activity of adrenochrome and related compounds, in recent years (see Bacq (17) and Hoffer (18) for lists of references) the necessity for working with the compounds in a pure and stable form has become of paramount importance.

EXPERIMENTAL

Preparation of Adrenochrome

L-Adrenaline (9.15 g.) was suspended in methanol (360 ml.) and 90% formic acid added dropwise with stirring until a clear solution was obtained (*ca.* 3.5 ml.). Freshly prepared silver oxide (36.3 g.) was added portionwise during a period of 3 minutes, the reaction temperature being maintained between 18° and 23° during the addition of the oxidant. The reaction mixture was filtered (with suction) through a Dowex-1(Cl^-) (200/400 mesh) resin bed (diam. = 6.5 cm., height = 3.7 cm.)* and the deep red filtrate was allowed to crystallize slowly at −20°. Pure adrenochrome (1.6 g.) was obtained in deep-violet needles, m.p. 112° with decomposition. Found: C, 60.30; H, 4.69; N, 7.76. Calc. for $C_9H_9O_3N$: C, 60.33; H, 5.02; N, 7.81%. The ultraviolet and visible absorption spectra were measured in aqueous solution (λ_{max}: 301, 487 mμ; λ_{min}: 262, 361 mμ).

Samples of adrenochrome which we have prepared by repeating existing procedures contain, in our view, less well defined crystals than the product obtained as described above (rapid cooling to −80° tends to produce an amorphous product) and the products are

**The resin was prepared by extensive washing: (1) with 3N hydrochloric acid, (2) with water until neutral to litmus, and (3) finally with methanol until the washings were transparent to ultraviolet light.*

invariably more or less contaminated with adrenolutin and melanin. This has been demonstrated paper chromatographically (using Whatman No. 1 paper with water as running solvent). No particular advantage either in yield, purity, or crystalline form, appeared to attend the use of absolutely dry methanol. The silver contents of adrenochrome samples prepared in this manner and by the method of Sobotka and Austin (5) were determined by the dithizone method after complete oxidation of the organic material in the sample with a mixture of nitric and perchloric acids (cf. 19, p. 544) and were observed to be very low (not greater than *ca.* 100 parts per million) whereas preparations obtained by other routes had considerably higher silver contents (of the order of 1000 p.p.m.). Sobotka and Austin filtered the methanol reaction mixture through a sodium sulphate bed prior to crystallization. This may have fortuitously acted as an anion-exchange resin and removed silver ions from solution as the sulphate. It is conceivable that both the resin and the sodium sulphate beds would help to hold back colloidal as well as ionic silver. One possible advantage of the resin over sodium sulphate would be that it would enable the reaction to be carried out in aqueous solution if required.

The mother liquors were examined chromatographically on paper using water as solvent and there was evidence that they contained adrenochrome R_f 0.82, a little adrenolutin R_f 0.4–0.45, a non-fluorescent dark-violet unidentified compound R_f 0.92, and a non-fluorescent colorless zone R_f 0.0–0.4. All these zones gave blue colors (i.e. reduced) with the ferric chloride – potassium ferricyanide reagent (see next section). The low R_f area probably contained some unchanged adrenaline, since it gave a red color when sprayed with potassium ferricyanide or ammonium persulphate solution.

Paper Chromatography of Adrenochrome and Related Compounds

Whatman No. 1 paper was washed with distilled water for 12 hours and dried prior to use. It was observed that unwashed paper caused some decomposition of the adrenochrome. The ascending method for paper chromatography was invariably employed, and 10–20 μg. of sample were applied to the paper in each case. The results obtained are given in Table I.

In all cases, the solvent was allowed to ascend a distance of *ca.* 9 to 12 inches. There was a considerable variability in the time required for this to occur; water and 2% acetic acid having the advantage of giving considerably quicker results than the other solvent systems. The approximate times taken by the various solvents are given in Table II.

Detection of Spots

All four compounds are colored and therefore are self-indicating. Adrenolutin (III) also exhibits a marked yellow-green fluorescence in ultraviolet light. It has been previously stated that, on drying, the spots of I undergo a slow change to III; this substance, being a catechol derivative, has reducing properties and the chromatogram can be developed in the following manner (if a permanent record is required). The developing solution was prepared immediately before use, and consisted of 3% ferric chloride solution (5 ml.), 3% potassium ferricyanide solution (5 ml.), and water (90 ml.). The papers were dipped in the reagent and the reducing compounds formed permanent blue spots. The excess reagent was washed off; the papers were dipped in 2 *N* hydrochloric acid and finally excess acid was washed off. Other chromogenic reagents for adrenochrome include: (*a*) Ehrlich's reagent (violet spot), (*b*) 3% ferric chloride solution (gray-brown spot), (*c*) diazotized *p*-nitroaniline (red-brown spot), and (*d*) zinc chloride solution (yellow-green fluorescent spot in ultraviolet light).

TABLE I

R_f IN STATED SOLVENT SYSTEMS

Compound	A	B	C	D	E	F
Adrenochrome (I)	0.8 ±0.02	0.82±0.02	S. dec. (0.7–0.8)	S. dec. (0.7–0.8)	R. dec.¶	R. dec.
Adrenolutin (III)*	0.45±0.05	0.47±0.03§	S. dec. (0.7–0.8)	S. dec. (0.7–0.8)	R. dec.	R. dec.
Adrenoxyl (VI)†	0.48±0.05	0.50±0.03	0.65±0.05	0.85±0.05||	0.57±0.05**	0.58±0.05**
Adrenochrome monoisonicotinic acid hydrazide (VII)‡	0.30	0.42	0.72	0.65	0.21	0.62**

NOTE.—*A = Water. B = 2% acetic acid in water. C = Methanol:water (3:1). D = Ethanol:water (3:1). E = n-Butanol:acetic acid:water (8:2:2). F = i-Propanol:0.880 ammonia:water (8:1:1). S. dec. = slow decomposition. R. dec. = rapid decomposition.*

* = *The adrenolutin was prepared by the method of Harley-Mason and Bu'Lock (20).*

† = *Supplied by the Labaz Co.*

‡ = *Supplied by the Pfizer Co.*

§ = *In all acidic solvents adrenolutin exhibited a pink fluorescence.*

|| = *Occasionally inexplicable lower R_f's were observed for adrenoxyl with this solvent system.*

¶ = *Differing results were obtained dependent on the time taken to run the chromatogram. The red color of the spot slowly faded with the formation of a dirty yellow diffuse spot exhibiting a pink fluorescence.*

** = *These results are different from those reported previously (12); we are unable to explain these discrepancies. The R_f values quoted in Table I were obtained consistently in our investigations. Fischer's claim that adrenoxyl resolves into adrenochrome and a fluorescing spot R_f 0.88 in the ammoniacal solvent system F (12) would appear to be due to a misinterpretation of the evidence in view of known instability of adrenochrome in ammonia.*

TABLE II

Solvent system	A	B	C	D	E	F
Approximate running time (hours)	2.5–3.0	2.5–3.0	7.5	15.5–18.0	15.5–18.0	18.0–20.0

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