tallized from ethanol, giving 3.3 g. of crude, yellow-brown *s*-bis-(β-phenylethyl)-urea. After several crystallizations from absolute ethanol, colorless crystals were obtained, m.p. 140–141° (Curtius and Jordan[9] report a m.p. of 138.0–138.5° for a sample crystallized from benzene).

Anal. Calcd. for $C_{17}H_{20}N_2O$: C, 76.06; H, 7.53; N, 10.44. Found: C, 76.18; H, 7.62; N, 10.55.

Ethyl β-(Homoveratrylimino)-glutarate (VII).—On mixing 21.0 cc. of β-3,4-dimethoxyphenylethylamine and 19.0 cc. of ethyl acetonedicarboxylate, heat was evolved, and a colorless solid formed. This was crystallized from absolute ethanol, 37.1 g. of colorless leaflets being obtained, m.p. 68–73°. After several additional crystallizations from absolute ethanol, the m.p. was raised to 79.2–79.8°.

Anal. Calcd. for $C_{19}H_{27}NO_6$: C, 62.47; H, 7.40; N, 3.85. Found: C, 62.84, 62.87; H, 7.38, 7.58; N, 4.02, 4.31.

The use of a 2:1 or 3:1 molar ratio of the amine to the keto-ester gave the same product. Heating the reaction mixture to over 100° resulted in the formation of the same product.

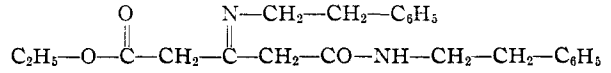
Reaction of β-Phenylethylamine with Ethyl Acetonedicarboxylate at Room Temperature and at 100°.—A mixture of 6.0 g. of β-phenylethylamine and 5.0 g. of ethyl acetonedicarboxylate was heated at 100° for 20 hours in a pressure bottle. The dark red liquid which resulted was dissolved in 300 cc. of ether. The ether solution was washed with dilute hydrochloric acid solution, water, and then dried over anhydrous calcium chloride. On concentrating the ether solution to 50 cc. and cooling, 1.20 g. of colorless solid crystallized. The m.p. varied from 112 to 119°, depending on the solvent from which the substance was crystallized (ether, ligroin, ethanol), and the rate of heating during the m.p. determination.

Anal. Calcd. for $C_{23}H_{28}N_2O_3$[11]: C, 72.60; H, 7.42; N, 7.36. Found: C, 72.85, 72.84; H, 7.39, 7.18; N, 7.80.

The same product was obtained by allowing a mixture of 10.0 g. of the keto-ester and 18.0 g. of the amine to stand at room temperature for 11 days, 12.3 g. of crude product being obtained.

Bischler–Napieralski Reaction of *s*-Bis-(β-phenylethyl)-urea.—To a solution of 6.0 g. of *s*-bis-(β-phenylethyl)-urea in 100 cc. of dry xylene was added 15.0 g. of phosphorus pentoxide, and the mixture was refluxed for four hours. After cooling, the xylene layer was decanted and the residue washed with water, leaving about 5 g. of dark, viscous material. On mixing this with 20 cc. of ethanol, 0.90 g. of colorless solid was obtained, m.p. 237–240°. Its properties were similar to those of 1-(β-3′,4′-dimethoxyphenylethylamine)-6,7-dimethoxy-3,4-dihydroisoquinoline phosphate dihydrate, but no attempt was made to further characterize the material.

(11) Probable structure:



LAWRENCE, KANSAS RECEIVED JANUARY 5, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE MOUNT SINAI HOSPITAL]

Betaine Hydrazone of Aminochromes[1]

BY HARRY SOBOTKA AND JOHN AUSTIN

A number of cyclic oxidation products of the adrenochrome type have been prepared from epinephrine homologs: epinochrome, 2-iodoepinochrome and 2-carbethoxyepinochrome. The following water-soluble betaine hydrazones of aminochromes have been prepared: the trimethylammoniumacethydrazones of adrenochrome, epinochrome, 2-carbethoxyepinochrome, and the piperidiniumacethydrazone of adrenochrome. The 2-iodoaminochromes are deiodized during this condensation reaction.

A few years ago, Oster and Sobotka[2] discovered the antipressor action of adrenochrome derivatives. The great instability of the earlier preparations of adrenochrome itself and the limited solubility in water of this substance and of its 2-iodo- and 2-bromo- derivatives, which we had studied in our original experiments, impeded the therapeutic application of our observations. Attempts at a complete synthesis of more water-soluble derivatives led to the preparation of 5,6-methylenedihydroxindoxylic acid, to be described in another communication, but were given up because of the difficulties inherent in the polyfunctional nature of the desired product. This research was resumed after the end of the war with the idea of preparing water-soluble adrenochrome derivatives by condensation with water-soluble reagents for carbonyl such as the betaine hydrazides of Girard.

The preparation and isolation of adrenochrome has been facilitated by Veer[3a] and by Buchnea,[3b] who oxidized adrenaline with silver oxide (in 50% excess) in dilute methanol–formic acid solution. We have improved the yield and purity of the reaction product by operating in two stages, as described in the Experimental Part. By this procedure, we have prepared L-adrenochrome from L-epinephrine and DL-adrenochrome from DL-epinephrine. The corresponding cyclic oxidation product which we have obtained from epinine, we have designated as epinochrome. We have also prepared β-(3,4-dihydroxyphenyl)-N-methylalanine (= N-methyl-"dopa"). From its ethyl ester we have prepared by oxidation with silver oxide a solution of 2-carbethoxyepinochrome. We have also improved the preparation of 2-iodo- and 2-bromoadrenochrome and have synthesized the new 2-iodoepinochrome.

The class of cyclic oxidation products with two oxygen functions in positions 5 and 6 of a dihydroindole system and including variants in the heterocyclic moiety has been tentatively designated as "aminochromes," especially as the uncertainty of the orthoquinoid structure makes a systematic nomenclature appear premature. The formulation of adrenochrome as a 5,6-orthoquinone of 1-methyl-3-hydroxydihydroindole is not in full accord with all its chemical properties; an isomeric formulation has been considered by Harley-Mason,[4] which accounts well for the limitation of reactivity to one carbonyl group in condensations with carbonyl

(1) This work was carried out under a grant from the Life Insurance Medical Research Fund.

(2) Oster and Sobotka, *J. Pharm. Exp. Ther.*, **78**, 100 (1943).

(3) (a) Veer, *Rec. trav. chim.*, **61**, 638 (1942); (b) Office of Publication Board, U. S. Dept. of Commerce, Report No. 47 (1945).

(4) Harley-Mason, *Experientia*, **4**, 307 (1948); *J. Chem. Soc.*, 1276 (1950).

reagents. This formulation is, moreover, supported by the peculiar reactions which take place in attempts to produce leucoderivatives by hydrogenation, on which we shall report separately.[5]

The spectral data for these compounds are briefly recorded in Table I. A steep maximum of absorption between 300 and 305 mμ is shown both by the non-halogenated and the halogenated compounds. A second absorption maximum of flatter shape about 470 mμ is given by adrenochrome and epinochrome, but in the 2-halogeno derivatives the secondary maximum is shifted to the region around 520 mμ. On the short wave side, all absorption curves pass through a sharp minimum at 270 mμ. The halogenated compounds display a third maximum on the upward slope at about 230 mμ. The interpretation of these data in respect to the various structural alternatives awaits the preparation and study of additional functional derivatives.

TABLE I

ABSORPTION SPECTRA OF AMINOCHROMES AND SOME OF THEIR DERIVATIVES

Compounds	λ	ε	λ	ε	λ	ε
Adrenochrome	..	..	300	9,350	470	3920
2-Bromoadrenochrome	227	10,400	306	6,830	520	2270
2-Iodoadrenochrome	234	10,550	302	4,470	530	1550
Epinochrome	..	..	305	10,750	470	3900
2-Iodoepinochrome	229	19,350	305	8,900	510	2550
Trimethylaminoacethydrazone HCl of adrenochrome	..	..	362	20,200		
Piperidiniumacethydrazone HCl of adrenochrome	260	9,350	350	23,900		
Semicarbazone of adrenochrome	226	14,000	355	27,200		
Trimethylaminoacethydrazone HCl of epinochrome	217	11,400	350	23,950		
Trimethylaminoacethydrazone HCl of 2-carbethoxyepinochrome	230	9,160	358	12,600		

We have condensed these various aminochromes with trimethylammoniumacethydrazide hydrochloride (Girard's Reagent T), and with piperidiniumacethydrazide hydrochloride (Girard's Reagent P). These condensations lead to water-soluble orange or red-brown hydrochlorides of betaine hydrazones. We describe in the Experimental Part the condensation products of adrenochrome with trimethylammoniumacethydrazide and with piperidiniumacethydrazide. Their solutions are but slightly acid and may be adjusted by appropriate buffers to stable solutions of *p*H 6.5.

The betaine hydrazones of epinochrome and of 2-carbethoxyepinochrome have not yet been fully characterized and will be described separately.[6] Their close similarity with those of adrenochrome in respect to melting point, solubility, spectral data and circumstances of synthesis strongly supports the formulation of all these compounds as mono-hydrazones.

In the case of 2-iodoadrenochrome and 2-iodoepinochrome, the iodine atom was lost during the condensation reaction and the resulting products proved identical with the betaine hydrazones of the corresponding non-halogenated compounds. A similar dehalogenation in the case of 2-bromoadrenochrome prevented the isolation of any condensation product.

The absorption spectra of the betaine hydrazones, given in Table I, differ completely from those of the parent aminochromes. They are characterized by a sharp absorption maximum of $\epsilon = 20{,}000$ to 24,000 between 350 and 360 mμ with a "shoulder" around 440 mμ. The maximum is separated from the rise toward short wave lengths by a minimum at 270 mμ.

Adrenochrome has recently attracted attention for a variety of reasons. As a potential biological oxidation product of epinephrine, it has been studied as a component of important enzyme systems.[7] Claims of hypoglycemic effects[8] have not been substantiated.[9] Roskam and Derouaux have observed hemostatic effects with adrenochrome, which run parallel to the shortening of bleeding time by adrenaline itself and other oxidation products of adrenaline, *e.g.*, adrenalone.[10] Adrenochrome is distinguished in this case by the absence of any incubation period. These authors did not feel satisfied with the physical properties of adrenochrome; Braconier, Le Bihan and Beaudet therefore prepared for them certain keto-derivatives, namely, the oxime, the semicarbazone and the 2,4-dinitrophenylhydrazone[11]; all these derivatives are mono-derivatives. We likewise were unable to prepare a disemicarbazone.[3a,11]

In our antipressor experiments, the 2-halogenoderivatives of adrenochrome proper and of the corresponding adrenalone derivative showed no difference amongst themselves in antipressor activity. This was in contrast to the immense specific differences between adrenaline itself and related amines as pressor substances. The betaine hydrazones, described below, have been found to shorten the bleeding time in man in a striking and uniform manner.[12] Our findings on aminochromes indicate that the hemostatic action, like the antipressor effect, is not specifically linked to the adrenaline configuration in the heterocycle. Thus, we are led to believe that both antipressor and hemostatic effects reside in the constellation of the oxidized ortho-diphenol grouping common to all aminochromes. This is in accordance with Oster's concepts.[13] Therefore, these biological effects appear to be localized in the region of the molecule where condensation with carbonyl reagents takes place. The assumption, then, is plausible that these condensation products are split in the body and that the aminochromes are regenerated in this process.

Experimental

Preparation of DL-Adrenochrome from DL-Epinephrine.— To a suspension of 9.16 g. of DL-epinephrine (base) (0.05

(5) Austin, Chanley and Sobotka, THIS JOURNAL, **73**, in press (1951).

(6) Sobotka and Austin, in preparation.

(7) *E.g.*, Randall, *J. Biol. Chem.*, **165**, 733 (1946); Meyerhof and Randall, *Arch. Biochem.*, **17**, 171 (1948).

(8) Marquardt, *Z. ges. exp. Med.*, **114**, 112 (1944); *cf.* ref. 5b.

(9) Snyder, Leva and Oberst, *J. Am. Pharm. Assoc., Sci. Ed.*, **36**, 253 (1947); Ingle, Shepherd and Haines, *ibid.*, **37**, 375 (1948).

(10) Derouaux, *Compt. rend. soc. biol.*, **131**, 830 (1939); Roskam and Derouaux, *Arch. Internat. Pharm. Ther.*, **69**, 348 (1944).

(11) Braconier, Le Bihan and Beaudet, *Arch. Internat. Pharm. Ther.*, **69**, 181 (1943).

(12) Sobotka and Adelman, *Proc. Soc. Exp. Biol. Med.* **75**, 789 (1950).

(13) Oster, *Nature*, **150**, 289 (1942).

mole) in 725 ml. of absolute methanol was added enough concentrated hydrochloric acid (d. 1.19) to produce a clear solution. To this solution was added 9.5 ml. of concentrated (98%) formic acid. After addition of 20 g. of anhydrous sodium sulfate, the solution was cooled to 10° and 31.0 g. of dried powdered silver oxide (0.133 mole) was added and vigorously shaken for two minutes. The suspension was filtered with suction through a mat of anhydrous sodium sulfate. To the filtrate was added 4.7 ml. of concentrated formic acid and another batch of 31.0 g. of silver oxide, again shaken for two minutes and filtered as above. The dark red clear filtrate was cooled to −80° for two hours. The adrenochrome crystallized in long reddish violet rods in a yield of 5.2 g.; m.p. 125° (dec.). It keeps indefinitely in the dry state at ice-box temperature. Spectral data for this and the subsequent compounds are summarized in Table I.

The preparation of L-adrenochrome was carried out in exactly the same manner. It formed larger crystals under these conditions than the racemate; m.p. 125° (dec.).

2-Bromoadrenochrome.—Two grams of DL-epinephrine was dissolved in 50 ml. of 1.3% acetic acid. Four-and-one-half grams of sodium acetate was added and the solution cooled to 0°. The theoretical quantity (6 atom equivalents) of bromine (5.25 g.), dissolved in 100 ml. water, was added dropwise. The solution turned red-violet at once and after standing for several hours at 0°, 0.9 g. of brownish violet platelets of 2-bromoadrenochrome were obtained in poor yield but in pure state; m.p. 90° (dec.).

Anal. Calcd. for $C_9H_8O_3NBr$: C, 41.80; H, 3.12; N, 5.43; Br, 30.97. Found: C, 41.61; H, 3.70; N, 4.44; Br, 28.37.

2-Iodoadrenochrome.—Two grams of DL-epinephrine (base) was suspended in 1600 ml. of water and enough concentrated hydrochloric acid (d. 1.19) was added to obtain a clear solution. To this was added 3.45 g. of potassium iodate (50% excess) and the mixture was shaken at room temperature until the iodate was dissolved. After a few minutes, deep red-violet rod shaped crystals of 2-iodoadrenochrome precipitated. After two hours, a crop of 3 g. (90%) was filtered off; m.p. 120° (dec.).

Anal. Calcd. for $C_9H_8O_3NI$: C, 35.43; H, 2.64; N, 4.59; I, 41.60. Found: C, 35.30; H, 2.89; N, 4.94; I, 41.83.

Epinochrome.[14]—One-fiftieth mole (4.1 g.) of epinine hydrochloride was dissolved in 650 ml. of absolute methanol, containing 2% concentrated (98%) formic acid per weight of alcohol. To this solution, 10 g. of anhydrous sodium sulfate and 14 g. of dried silver oxide (0.06 mole) were added and vigorously shaken for two minutes. To the filtered solution another 10 g. of anhydrous sodium sulfate and 14 g. of silver oxide were added and the solution again vigorously shaken for two min. After filtration, the dark-red solution was concentrated *in vacuo* under nitrogen at room temperature to 325 ml. Exactly 3 volumes of dry ether were added and the solution cooled overnight at −80°. Dark-red rods of epinochrome crystallized in a yield of 1.7 g.; m.p. 78°. The dry substance may be kept for many months in a deep-freeze box.

Anal. Calcd. for $C_9H_9O_2N$: C, 66.24; H, 5.56. Found: C, 66.25; H, 5.76.

2-Iodoepinochrome.—To a solution of 2 g. of epinine hydrochloride in 1600 ml. of water was added 3.2 g. of potassium iodate (50% excess) at 0°. After standing for two hours, 3 g. of violet-brown iodoepinochrome was obtained as a crystalline precipitate; m.p. 120°.

Anal. Calcd. for $C_9H_8O_2NI$: C, 37.39; H, 2.79; I, 43.90. Found: C, 36.91; H, 3.26; I, 42.63.

(14) Our first preparation of epinochrome was carried out by Dr. Anna Weizmann in November, 1947, under a fellowship from the Daniel Sieff Research Institute, Rehoboth, Israel.

The ethyl ester of DL-N-methyl-3,4-dihydroxyphenylalanine was prepared from the free acid,[15] by refluxing in absolute ethanol, saturated with HCl gas. Recrystallized from a mixture of methanol and ether, it melted at 160°.

Anal. Calcd. for $C_{12}H_{18}O_4NCl$: C, 52.27; H, 6.57; N, 5.08. Found: C, 52.31; H, 6.75; N, 5.67.

The oxidation with silver oxide, following the procedure described above for epinephrine, led to a red solution, but no crystals of the very soluble 2-carbethoxyepinochrome, the ester of the N-methyl homolog of Raper's "red pigment," were obtained.

Trimethylammoniumacethydrazone Hydrochloride of Adrenochrome.—One-fiftieth mole (3.6 g.) of DL-adrenochrome and 3.4 g. of trimethylammoniumacethydrazide hydrochloride (Girard's Reagent T) were dissolved in 200 ml. of absolute methanol to which was added 2 ml. of glacial acetic acid. The solution was left at room temperature until the color had changed to red-orange. Upon addition of three volumes of dry ether, the crude red-brown derivative was obtained in a yield of 6.5 g. It was dissolved in about 50 ml. of absolute methanol and purified through a chromatographic column of aluminum oxide which had been brought to *p*H of 6.0 by pretreatment with hydrochloric acid. The column retained some melanin-like by-products. The dark orange eluate was concentrated at room temperature *in vacuo* under nitrogen. About 3.4 g. of the purified compound crystallized from the concentrated solution in red-brown prisms. It was further purified by several recrystallizations from methanol; dec. 160°.

Anal. Calcd. for $C_{14}H_{21}O_3N_4Cl$: C, 51.14; H, 6.44. Found: C, 51.27; H, 6.65.

Aqueous solutions of more than 20% may be prepared with a *p*H of 4.0. They may be adjusted to *p*H of 6.5 by appropriate buffers.

To a boiling solution of 0.75 g. of Girard Reagent T in 150 ml. of methanol containing 1% acetic acid, was added 0.9 g. of iodoadrenochrome in small portions. The color of the solution changed from red-violet to dark orange-red on refluxing for 20 minutes. When purified and isolated, the red-brown crystals (0.25 g.) proved identical with the above compound by elementary composition and by absorption spectrum.

Piperidiniumacethydrazone Hydrochloride of Adrenochrome.—By the same method, but using piperidiniumacethydrazide hydrochloride (Girard Reagent P), we have obtained the corresponding adrenochrome derivative. The brown highly water-soluble substance melts with decomposition at 210°.

Anal. Calcd. for $C_{16}H_{23}O_3N_4Cl$: C, 54.14; H, 6.54; N, 15.79. Found: C, 54.31; H, 5.34; N, 15.50.

We have also condensed trimethylammoniumacethydrazide hydrochloride with epinochrome, iodoepinochrome and 2-carbethoxyepinochrome. As in the case of iodoadrenochrome, the iodine of iodoepinochrome was eliminated during this condensation, yielding the same betaine hydrazone as epinochrome itself. The preparation and properties of these compounds will be detailed in a subsequent report.[6]

The absorption spectra (Table I) were obtained with a Beckman quartz spectrophotometer model D.U. The micro analyses were performed by Elek Micro Analytical Laboratories, Los Angeles, California.

Acknowledgments.—We are indebted to the Wellcome Research Laboratories for a supply of epinine hydrochloride and to the Sterling-Winthrop Research Institute for DL-epinephrine hydrochloride. We wish to thank Mr. F. H. Bergmann for the spectrographic measurements.

NEW YORK, N. Y. RECEIVED SEPTEMBER 5, 1950

(15) Deulofeu, *Org. Syntheses*, **22**, 89 (1943).