the separation and detection of a number of related compounds. This method[1a] was found to give better resolution of these compounds than conventional paper chromatography. The spots are very well-defined and there is no "trailing." The technique is especially advantageous in biochemical work since the presence of extraneous material appears to have little or no effect on the resolution of the compounds. Color reactions based on the presence of functional groups were employed for the localization of the fluorene and biphenyl derivatives. General color tests which involve the methylene carbon of the fluorene ring system[2-4] were not sufficiently sensitive for the detection of microgram quantities.

Procedure and Results

In the present work a Precision Ionograph (Precision Scientific Co.) was used. The following procedure was employed. The solutions containing the compounds are applied to circular areas (diameter 1.0 cm.) located at the geometric centers of paper strips (Whatman No. 1) which are 47 cm. long and 3.7 cm. wide. The solvent is then evaporated with the aid of an infrared lamp or a hot air blower. Since the compounds are concentrated in a smaller area than in the usual technique in which the solutions are spread across the entire width of the paper[1a] the sensitivity is increased. In addition, retention of the compounds, which to a slight extent always occurs at the point of application, is minimized by this modification. Twenty per cent. acetic acid (v./v.) was found to be the most satisfactory electrolyte for these compounds. It is allowed to travel along the paper by capillary action until the electrolyte fronts meet at the areas of application (about 2 hours), at which time the current is turned on. A potential gradient of 15.1 volts/cm. length was used by applying 650 volts across the paper. Runs of 4 to 6 hours are usually sufficient for adequate separation of the compounds. The temperature is maintained at 23 ± 0.5° by circulating water from a constant temperature bath. The papers are dried in air prior to spraying with the color reagents.

Table I shows the mobilities (cm./sec. per volt/cm.) of pure compounds. Under the experimental conditions these compounds migrate toward the cathode. The distance traveled is measured from the center of the area of application to the leading edge of the developed spot. Colors are obtained by spraying the papers with a solution of 100 mg. of *p*-dimethylaminobenzaldehyde in 50 ml. of ethanol acidified with 1.0 ml. of concentrated hydrochloric acid.[5] Alternatively, phenolamines and diamines may be located by exposing the paper to chlorine vapors. These compounds give dark brown or dark green spots. The diamino compounds appear on the developed papers as narrow, symmetrical bands. The bands are curved with the convexity directed toward the cathode. The other compounds appear as round or elliptical spots after color development.

Neither 2-acetylaminofluorene nor 2-hydroxy-7-acetylaminofluorene give colors with these reagents. The phenolic derivative (2-hydroxy-7-acetylaminofluorene) is detected by using 100 ml. of a saturated solution of sulfanilic acid in 50% ethanol to which 1.0 ml. of concentrated hydrochloric acid is added. The solution is diazotized with 50 ml. of a 0.7% solution of sodium nitrite and the diazotized mixture is applied to the paper. The paper is dried and the color is developed by spraying with a 1% solution of sodium hydroxide.[6] All color reagents should be freshly prepared. The mobility of 2-acetylaminofluorene for which a color test is not yet available was determined with the aid of radioactive 2-acetylaminofluorene labeled in the 9-position with carbon 14. The radioactivity was located on the paper with a windowless scanner.[7] Both 2-acetylaminofluorene and 2-hydroxy-7-acetylaminofluorene are isoelectric under the experimental conditions and do not move. They may be further characterized on the same paper by chromatography as follows. After completion of the electrophoretic run the paper is cut at a distance 2.5 cm. from the area of application toward the cathode. The segment containing the spot of application is then chromatographed by the ascending technique and the compounds are detected as described above. R_f values for 2-acetylaminofluorene and 2-hydroxy-7-acetylaminofluorene in butanol saturated with 3 *M* ammonium hydroxide are 0.90 and 0.88. R_f values for the same compounds developed with the aqueous layer, obtained by shaking 8 parts of butanol and 1.5 parts of ethanol with 12 parts of water, are 0.22 and 0.21, respectively.

When the method is applied to tissue extracts it is the practice to add the compounds under study to aliquots of control extracts and to run the "marked" samples and the unknown simultaneously. The results of these experiments will be reported elsewhere.

TABLE I

MOBILITIES OF FLUORENE AND BIPHENYL DERIVATIVES IN 20% ACETIC ACID AT 23°

Compound	Mobility × 10^5, cm./sec./volt/cm.	Color	Lower limit of detection, μg.
2,7-Diaminofluorene	6.02	Bright red[a]	1–2
2-Aminofluorene	4.58	Bright yellow[a]	0.5–1
2-Monomethylaminofluorene	4.34	Pale yellow[a]	5–6
2-Amino-7-hydroxyfluorene	4.05	Orange yellow[a]	0.5–1
2-Amino-7-chlorofluorene	4.00	Bright yellow[a]	1–2
2-Amino-7-acetylaminofluorene	3.61	Yellow orange[a]	1–2
2-Acetylaminofluorene	0.00	.....[b]	...
2-Hydroxy-7-acetylaminofluorene	0.00	Purple[c]	2–3
Benzidine (4,4'-diaminobiphenyl)	5.86	Orange[a]	1–2
4-Amino-4'-hydroxybiphenyl	4.36	Bright yellow[a]	1–2

[a] Obtained with *p*-dimethylaminobenzaldehyde as described in the text. [b] See text for method of detection. [c] Obtained with diazotized sulfanilic acid as described in the text.

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Structures of the 4a-Methyl-1,2,3,4,4a,9,10,10a-octahydro-8-phenanthrols

BY W. B. RENFROW AND ANTOINETTE RENFROW

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The configurations of the alicyclic rings in both of the racemic 4a-methyl-8-methoxy-1,2,3,4,4a,-9,10,10a-octahydro-2-phenanthrones (I) and in three of the four possible racemic 2-methoxy-4a-methyl- 1,2,3,4,4a,9,10,10a - octahydro - 8 - phenanthrols (III) have been established.[1] Furthermore, the orientation of the alicyclic rings has been found[1] to determine the configuration of products obtained by catalytic hydrogenation of III. Only the isomer with the alicyclic rings *trans* (IIIa) produces a hydrogenation product with the "natural" configuration at the 4b-position.

In order to simplify studies of methods for synthesizing compounds related to the steroids, we

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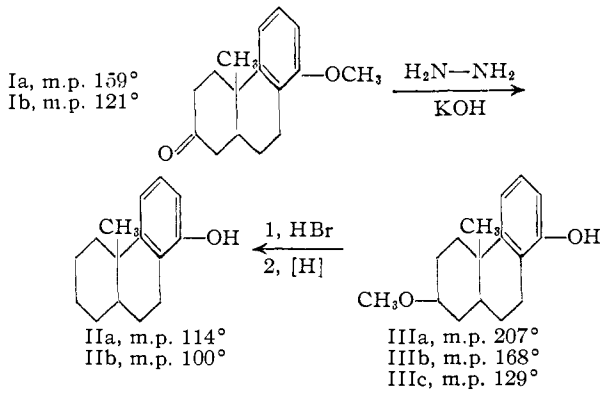

have characterized the 4a-methyl-1,2,3,4,4a,9,10,-10a-octahydro-8-phenanthrols (II). We have prepared these compounds by Huang-Minlon reduction[2] and demethylation of the methoxy ketones Ia (*trans*) and Ib (*cis*), and also by cleavage of the ether groups in the methoxyphenanthrols IIIa (*trans*), IIIb (*cis*) and IIIc (*cis*) with excess hydrobromic acid followed by dehalogenation[3] with Raney nickel alloy. The former method is more satisfactory because treatment of III with excess, concentrated hydrobromic acid leads to considerable resinification. The isomer IIa was obtained from both Ia and IIIa and must have the *trans* arrangement of the alicyclic rings. The isomer IIb was obtained from Ib, IIIb and IIIc and must have the *cis* configuration.

The phenylurethans were found to be good derivatives for further characterizing IIa and IIb, and also good derivatives for IIIa, IIIb and IIIc.

Experimental

Method A.—The methoxy ketone (Ia or Ib, 340 mg.), potassium hydroxide (270 mg.), 95% hydrazine (0.2 ml.) and diethylene glycol (2 ml.) were heated and stirred until homogeneous. A short air condenser was attached and the solution heated under nitrogen at 175–185° for 90 min., then at 195° for 2 hours. An additional 250 mg. of potassium hydroxide was added and the temperature maintained at 225° for 5 hours. The reaction mixture was diluted with water and acidified. The resulting brown solid was collected on a filter and purified by distillation at about 1 mm. followed by crystallization from ligroin; yield 70–75%.

***dl*-4a-Methyl-1,2,3,4,4a,9,10,10aα-octahydro-8-phenanthrol (IIa)**, clear, irregular plates, m.p. 114°. *Anal.* Calcd. for $C_{15}H_{20}O$: C, 83.29; H, 9.32. Found: C, 83.23; H, 9.32.

The phenylurethan was prepared by heating the phenanthrol (60 mg.) with dry pyridine (2 drops) and phenyl isocyanate (4 drops) at 90° for 1 hour. Pyridine and excess phenyl isocyanate were removed by distillation at reduced pressure and the residue crystallized from ligroin; small, well-formed needles, m.p. 126–127°. *Anal.* Calcd. for $C_{22}H_{25}O_2N$: C, 78.77; H, 7.51. Found: C, 78.51; H, 7.41.

***dl*-4a-Methyl-1,2,3,4,4a,9,10,10aβ-octahydro-8-phenanthrol (IIb)**, irregular fragments, m.p. 99–100°. *Anal.* Calcd. for $C_{15}H_{20}O$: C, 83.29; H, 9.32. Found: C, 83.12; H, 9.21.

The phenylurethan, clear, irregular fragments, m.p. 147–148°. *Anal.* Calcd. for $C_{22}H_{25}O_2N$: C, 78.77; H, 7.51. Found: C, 78.47; H, 7.51.

Method B.—The methoxyphenanthrol (IIIa, IIIb or IIIc, 500 mg.) and 48% hydrobromic acid (3 ml.) were warmed to about 100° and acetic anhydride (50 drops) added cautiously. Sufficient acetic acid was added to produce a homogeneous solution and the mixture heated at 110° for 2 hours. Excess hydrobromic and acetic acids was distilled at reduced pressure, the residue washed with water and taken up in 10% sodium hydroxide. The alkaline solution was heated in a boiling water-bath and treated with Raney nickel alloy (1.5 g.). The nickel was filtered off, washed with alcoholic alkali and the filtrate acidified. The precipitated phenanthrol was extracted with ether, washed, distilled at about 1 mm. and crystallized from ligroin; yield 10–20%. The product obtained from IIIa was identical with the material obtained by method A from Ia, and the product from both IIIb and IIIc was identical with that from Ib.

The melting range of an intimate mixture of IIa and IIb was 87–96°.

The phenylurethan of *dl*-2α-methoxy-4a-methyl-1,2,3,4,-4a,9,10,10aα-octahydro-8-phenanthrol (IIIa), fine needles, m.p. 160–161°. *Anal.* Calcd. for $C_{23}H_{27}O_3N$: C, 75.60; H, 7.45. Found: C, 75.51; H, 7.55.

The phenylurethan of *dl*-2β-methoxy-4a-methyl-1,2,3,4,-4a,9,10,10aβ-octahydro-8-phenanthrol (IIIb), very fine needles from benzene–ligroin, m.p. 190–192°. *Anal.* Calcd. for $C_{23}H_{27}O_3N$: C, 75.60; H, 7.45. Found: C, 75.63; H, 7.72.

The phenylurethan of *dl*-2α-methoxy-4a-methyl-1,2,3,4,-4a,9,10,10aβ-octahydro-8-phenanthrol (IIIb), fine needles, m.p. 179–180°. *Anal.* Calcd. for $C_{23}H_{27}O_3N$: C, 75.60; H, 7.45. Found: C, 75.40; H, 7.38.

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7-Alkyl Derivatives of 2-Aminofluorene[1]

BY EUGENE SAWICKI

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4-Methylaminoazobenzene[2] and 2-aminofluorene[3] are carcinogens. A methyl group in the extended *para* position of 4-methylaminoazobenzene causes a marked decrease in carcinogenic activity[4] while an ethyl group in the analogous position causes a slight increase in activity[5] as compared to 4-methylaminoazobenzene.

Assuming that approximately the same forces are operative as in the azo dyes one could expect 7-methyl-2-aminofluorene to be, at the most, weakly carcinogenic, while the 7-ethyl analog could be strongly carcinogenic. On the other hand, any differences in activity among the analogous derivatives of the diverse groups of carcinogens would signify an important difference in chemical or physical reactivity at that level.

The nitration of 2-methyl- and 2-ethylfluorene gave a mononitro-7-methylfluorene and a mononitro-7-ethylfluorene whose almost identical ultraviolet spectra are very similar to that of 2-nitrofluorene, Table I. Comparison of the absorption spectra of the derived acetylamino-7-methylfluorene and acetylamino-7-ethylfluorene with 1-, 2- and 4-acetylaminofluorene[6,7] in Table I shows a definite

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