[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF DUKE UNIVERSITY AND FROM THE LABORATORY FOR ORGANIC CHEMISTRY, LEIDEN]

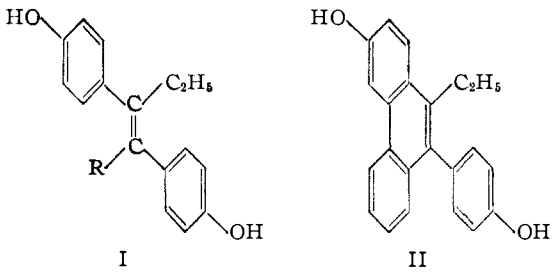
# Aromatic Cyclodehydration. XXVI.[1] 3-Hydroxy-9-(*p*-hydroxyphenyl)-10-ethylphenanthrene, an Estrogen Analog

BY CHARLES K. BRADSHER[2] AND WINSTON J. JACKSON, JR.[3]

RECEIVED FEBRUARY 18, 1952

It was found that α-(5-methoxy-2-biphenylyl)-4-methoxybutyrophenone may be cyclized and demethylated to yield the weakly estrogenic 3-hydroxy-9-(*p*-hydroxyphenyl)-10-ethylphenanthrene. The ketone was prepared in a nine-step synthesis starting from 2-amino-5-nitrobiphenyl.

Following the discovery by Dodds, *et al.*,[4] of the potent estrogenic activity of diethylstilbestrol (I, R = $C_2H_5$), a great many analogs[5] including numerous triphenylethylene derivatives were synthesized. It is reported[5,6] that the most active triphenylethylene derivative prepared, the 1-phenyl-1,2-bis-(*p*-hydroxyphenyl)-butene-1 (I, R = $C_6H_5$), was not less than one-twelfth as active as diethylstilbestrol. As a closed model of this compound, 3-hydroxy-9-(*p*-hydroxyphenyl)-10-ethylphenanthrene (II) appeared of interest in that the molecule is fixed in a pattern corresponding to the *trans* configuration of stilbestrol. Further, II contains the phenanthrene nucleus which is present, albeit in the reduced form, both in the natural estrogens and in the very active doisynolic acids.[7]



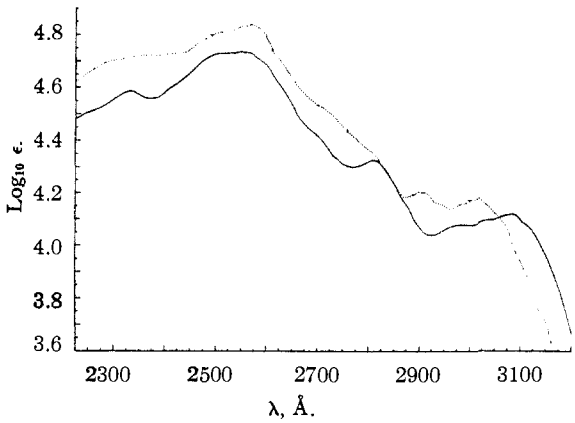
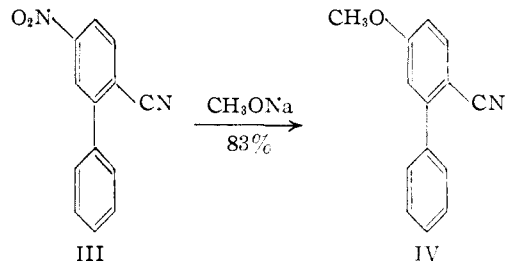




Fig. 1.—3-Hydroxy-9-(*p*-hydroxyphenyl)-10-ethylphenanthrene, —; 3-benzoxy-9-(*p*-benzoxyphenyl)-10-ethylphenanthrene, .....

The method employed in the synthesis of II is essentially that described earlier for the preparation of 9-(*p*-hydroxyphenyl)-10-alkylphenanthrenes,[1] but some details, especially those concerned with the preparation of the necessary intermediates, warrant special mention. The conversion of 5-nitro-2-cyanobiphenyl (III) (best prepared by the Rosenmund–von Braun reaction) to the corresponding methoxy nitrile (IV) was effected by the action of sodium methoxide.[8] Reduction of the nitrile was carried out in 80% yield by the use of lithium aluminum hydride.[9] There were indications that when the resulting amine (V) in acetic acid was treated with sodium nitrite solution, the resulting alcohol (VI) was contaminated by its acetate.




The bromo derivative (VII) with potassium cyanide in alcohol–water gave only 35% yield of the desired nitrile (X), the major product being the ethyl ether (VIII). The remaining steps in the synthesis (X → XI → XII → II) are shown by accompanying formulas.

The ultraviolet absorption spectra of the dihydroxy compound (II) and of the dibenzoate (Fig. 1) show very definite similarities to those of other 9-phenylphenanthrene derivatives.[1,10] It appears that the dihydroxyphenylphenanthrene (II) has only weak estrogenic activity.[11]

An alternative approach to the synthesis of X *via* a Willgerodt reaction on 2-aceto-5-methoxybiphenyl (IX) proved unpromising.

(1) For the preceding communication of this series see C. K. Bradsher and W. J. Jackson, THIS JOURNAL, **73**, 3235 (1951).

(2) Fulbright Lecturer in Organic Chemistry, 1951–1952.

(3) Public Health Service Research Fellow of the National Cancer Institute (1950–1952).

(4) E. C. Dodds, L. Goldberg, W. Lawson and R. Robinson, *Nature*, **141**, 247 (1938).

(5) For a review on this subject see U. V. Solmssen, *Chem. Revs.*, **37**, 537 (1945).

(6) E. C. Dodds, L. Goldberg, E. I. Grunfeld, W. Lawson, C. M. Saffer and R. Robinson, *Proc. Roy. Soc. (London)*, **B132**, 83 (1944).

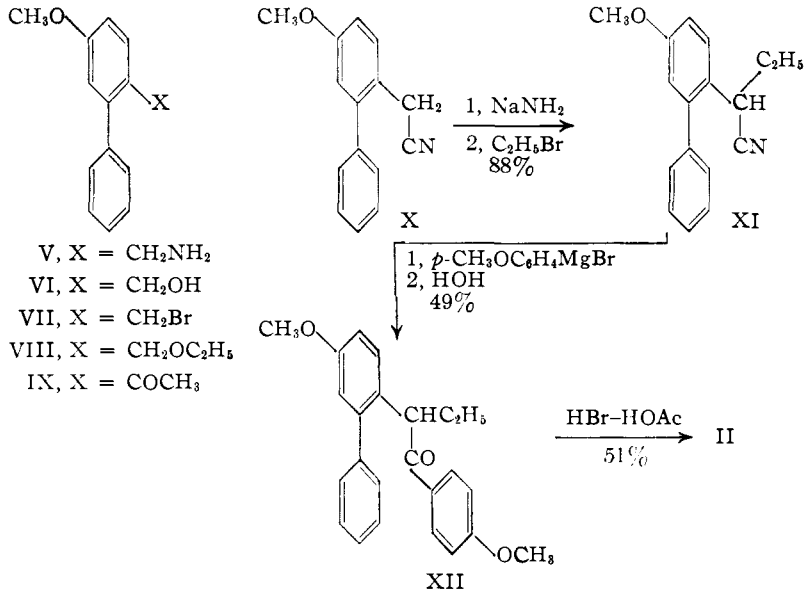
(7) K. Miescher, *Chem. Revs.*, **43**, 367 (1948).

(8) *Cf.* W. Reinders and W. F. Ringer, *Rec. trav. chim.*, **18**, 326 (1899).

(9) *Cf.* L. H. Amundsen and L. S. Nelson, THIS JOURNAL, **73**, 242 (1951).

(10) C. K. Bradsher and L. J. Wissow, *ibid.*, **68**, 2150 (1946).

(11) Tests on this compound for estrogenic activity have been conducted at the Lilly Research Laboratories. The new compound (pyridine solvate) is inactive in mice at 8 mcg. per mouse (100 × the effective dose of diethylstilbestrol) but elicited a good estrogenic response at 5 mg.




## Experimental

**Absorption Spectra.**—The ultraviolet absorption spectra were measured in 95% ethanol solution using a Beckman model DU quartz spectrophotometer and 1-cm. silica cells.

**5-Nitro-2-(*p*-toluenesulfonamido)-biphenyl.**—Like Smith and Brown,[12] we found that the nitration procedure of Ray and Barrick[13] gave large quantities of undesired dinitro compound. Our modification of the procedure is as follows:

To 1100 g. of 2-(*p*-toluenesulfonamido)-biphenyl (produced from the amine in 97% yield) in 2.6 l. of acetic acid at 60°, concentrated nitric acid (240 ml.) was added and the mixture warmed to 65° after which it was removed from the hot-plate. During the next two minutes the solution turned from orange to red and the temperature began to rise quickly. The reaction flask was immediately surrounded by an ice-bath, whereupon the product began to crystallize as tan granules. It was collected, washed with water, and dried, yielding 1086 g. (87%), m.p. 165.5–168.5°. The sulfonamide was converted to the free amine (IV) in 95% yield by the method of Ray and Barrick.[13]

**5-Nitro-2-cyanobiphenyl** (III). **(a) By the Sandmeyer Method.**—The procedures of both Ray and Barrick[13] and Jones and Braker[14] were tried and found to give 24 and 23%, respectively, of purified product. The melting point previously reported[13] (133–134°) was significantly lower than that observed by us (139.5–142°).

(b) **By the Rosenmund–von Braun Method.**—Essentially the procedure of Case[15] was used in the preparation of 5-nitro-2-bromobiphenyl from 600 g. of 5-nitro-2-aminobiphenyl. It was found, however, that purification of the crude product was simplified by taking it up in 1 l. of methylene chloride and filtering with suction to remove copper salts. Concentration of the solution and distillation of the residue under reduced pressure (b.p. 182–194° (4 mm.)) yielded 629 g. (81%) of yellow needles, m.p. 74–79°. This material was pure enough for the following reaction.

A mixture containing 380 g. of the crude 2-bromo-5-nitrobiphenyl above, 100 ml. of dry pyridine, 180 g. of cuprous cyanide and a little anhydrous copper sulfate in a 1-l. von Braun flask was mechanically stirred while the flask was heated in a metal-bath maintained at 220°. After an induction period of a few minutes, the mixture began to boil vigorously and it was necessary to raise the flask from the bath. When the vigor of the reaction decreased, the mixture was heated for one hour in the metal-bath at 220–230° with continued stirring. At the end of this period, the product was distilled under reduced pressure (4 mm.), heating being effected by the use of a metal-bath. It was not necessary to use a capillary to prevent bumping, but the product tends to sublime and great care had to be exercised to prevent clogging of the vacuum lines. Toward the end of the distillation, the residue sometimes decomposed rapidly. It was necessary to watch the manometer carefully and, at the first sign that the pressure in the system had risen, to remove the flask from the metal-bath and stop the distillation. Failure to observe this precaution sometimes resulted in a black brittle material being blown from the flask.

After the distillation was complete, the solid distillate was dissolved in about 2 l. of methylene chloride and filtered from a small amount of copper salts. Removal of the solvent yielded 231–256 g. (76–84%) of yellow needles, m.p. 133–140°. This material was pure enough for further reactions, but a recrystallized sample melted at 141.5–143° and gave no depression of melting point with a sample prepared by procedure (a).

**5-Methoxy-2-cyanobiphenyl** (IV).—To a solution of sodium methoxide prepared by dissolving 17.5 g. of sodium metal in 1.5 l. of pure methanol, 148 g. of 5-nitro-2-cyanobiphenyl (III) was added and the resulting suspension stirred and refluxed for 20 hours. During this period the nitro compound went slowly into solution and a small quantity of a flocculent yellow compound separated. The solid phase was filtered off and the filtrate poured into 2 l. of ice-water. The resulting product, which separated as a cream-colored powder, was collected, m.p. 81–83°; yield 130 g. (94%). Recrystallization from methanol gave 114 g. (83%) of yellow needles, m.p. 84–85.5°. The analytical sample melted at 84.5–85.5°.

*Anal.*[16] Calcd. for $C_{14}H_{11}ON$: C, 80.36; H, 5.30. Found: C, 80.45; H, 5.36.

The yellow flocculent sparingly soluble material first filtered off weighed 5.7 g. (4%) and melted at 266–268° with preliminary decomposition. Two recrystallizations from acetic anhydride gave an analytical sample, m.p. 274–275° (dec.). The analysis corresponds to that expected for **3,3'-diphenyl-4,4'-dicyanoazoxybenzene.**

*Anal.* Calcd. for $C_{26}H_{16}ON_4$: C, 77.98; H, 4.03. Found: C, 77.83; H, 4.22.

**2-Phenyl-4-methoxybenzylamine** (V).—To 1 l. of anhydrous ether in a 3-necked flask fitted with a stirrer and soxhlet extractor, 28.8 g. of lithium aluminum hydride was added. After the suspension had been stirred for a few minutes, the extraction thimble was filled with 5-methoxy-2-cyanobiphenyl (IV) and refluxing commenced. During a period of eight hours, a total of 105 g. of the sparingly soluble nitrile was extracted from the thimble. The mixture was then stirred overnight, cooled in an ice-bath, and hydrolyzed[9] by the very slow addition of first 24 ml. of water, followed by 18 ml. of 20% sodium hydroxide solution, and finally by 84 ml. of water. The ethereal solution was then decanted from the pasty residue (through a filter) and the residue washed several times with ether. The combined ethereal solution was concentrated and the residue distilled under reduced pressure. The yield was 85 g. (80%) of a colorless oil, b.p. 152–153° (1 mm.), $n^{25}D$ 1.6047. A satisfactory analysis could not be obtained. On exposure to air the liquid was converted to a white granular solid which is probably the carbonate since in boiling water, it evolved a gas and yielded an insoluble oil. Addition of hydrochloric acid to the solid likewise caused the evolution of gas.

**The benzamide** was formed by refluxing the amine (V) with benzoyl chloride, pyridine and benzene. The analytical sample crystallized from ethanol–cyclohexane as lustrous white plates, m.p. 134.5–135.5°.

*Anal.* Calcd. for $C_{21}H_{19}O_2N$: C, 79.47; H, 6.03. Found: C, 79.57; H, 6.12.

**The *p*-toluenesulfonamide**, prepared by refluxing the amine in pyridine with *p*-toluenesulfonyl chloride, crystallized from ethanol as thin white needles, m.p. 127.5–128°.

(12) P. A. S. Smith and B. B. Brown, THIS JOURNAL, **73**, 2438 (1951).

(13) E. R. Ray and J. G. Barrick, *ibid.*, **70**, 1492 (1948).

(14) W. S. Jones and W. Braker, U. S. Patent 1,922,207 (August 15, 1933).

(15) F. H. Case, THIS JOURNAL, **65**, 2137 (1943).

(16) All analyses are by the Micro-Tech Laboratories, Skokie, Illinois.

*Anal.* Calcd. for $C_{21}H_{21}O_3NS$: C, 68.64; H, 5.76. Found: C, 68.75; H, 5.62.

**The hydrochloride** formed white silken needles, m.p. 214–214.5° (dec.).

*Anal.* Calcd. for $C_{14}H_{16}ONCl$: C, 67.33; H, 6.46. Found: C, 67.32; H, 6.41.

**The picrate** crystallized from ethanol as flat rectangular yellow plates which decomposed at 210–212°.

*Anal.* Calcd. for $C_{20}H_{18}O_8N_4$: C, 54.30; H, 4.10. Found: C, 54.18; H, 3.92.

**2-Phenyl-4-methoxybenzyl Alcohol (VI).**—A solution containing 278 g. of 2-phenyl-4-methoxybenzylamine (V) in 900 ml. of acetic acid was cooled in an ice-bath, and a solution of sodium nitrite (200 g.) in water (450 ml.) was added[17] with stirring at such a rate that the temperature remained in the range 15–25°. After stirring for ten minutes longer at room temperature, the solution was diluted with an equal volume of water and extracted with 1200 ml. of methylene chloride. The extract was washed several times with water and then the methylene chloride was removed by distillation. The residue, which appeared to consist of a mixture of the desired carbinol and its acetate was refluxed for three hours with 1500 ml. of a 5% solution of alcoholic potassium hydroxide. A portion of the alcohol was removed by distillation and the residual solution was diluted with water and extracted with ether. The ethereal extract was washed with water and dilute hydrochloric acid. Upon concentration of the ethereal solution, there was obtained a clear red oil which crystallized on cooling to yield 260 g. (93%) of a yellow-orange solid, m.p. 60–63°. This material was pure enough for further reactions, but an analytical sample prepared by recrystallization from cyclohexane formed white feathery needles, m.p. 63.5–64.5°.

*Anal.* Calcd. for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59. Found: C, 78.44; H, 6.63.

**2-Phenyl-4-methoxybenzyl Bromide (VII).**—Phosphorus tribromide (50 ml.) was added slowly to a solution of 238 g. of 2-phenyl-4-methoxybenzyl alcohol (VI) in 1 l. of dry ether which was cooled in an ice-bath. After the addition of the bromide was complete, the mixture was allowed to stand for two hours at room temperature. It was next cooled in an ice-bath and 40 ml. of methanol added to assure decomposition of the excess phosphorus tribromide. The ethereal solution was washed with water and with sodium bicarbonate solution and the ether removed under reduced pressure without heating. The resulting orange oil was used directly in the following experiments without further purification.

**2-Phenyl-4-methoxybenzyl Cyanide (X). (a) Attempted Preparation by the Classical Method.**—To a suspension of 2-phenyl-4-methoxybenzyl bromide (VII) (prepared from 61 g. of the carbinol) in 120 ml. of ethanol, a solution containing 26 g. of potassium cyanide in 35 ml. of water was added. The mixture was refluxed with stirring for one hour, at the end of which only a single liquid phase was evident and a solid phase had separated. The mixture was filtered and the solution extracted with methylene chloride. The organic phase was washed, dried and concentrated. Upon vacuum distillation of the residue, 45.9 g. of a light yellow oil, b.p. 164–172° (3 mm.) was obtained. This material was shown to contain no nitrogen when treated by the sodium fusion method. The analytical sample, b.p. 167° (3 mm.), $n^{25}D$ 1.5710, had the composition expected for **2-phenyl-4-methoxybenzyl ethyl ether (VIII)**.

*Anal.* Calcd. for $C_{16}H_{18}O_2$: C, 79.31; H, 7.49. Found: C, 79.32; H, 7.58.

The higher-boiling fraction (7.9 g.) distilled at 172–182° (3 mm.), and since it gave a weak test for nitrogen, may have contained a small amount of the nitrile.

**(b) By Reaction of the Bromide with Cuprous Cyanide.**—To the bromide (VII) prepared from 6 g. of the carbinol (VI), dry benzene and an excess of cuprous cyanide were added and the mixture refluxed for two days. The yield was 1.8 g. (29%) of nitrile, b.p. 183° (3 mm.). A large undistillable residue remained.

**(c) By Reaction of the Chloride with Potassium Cyanide.**—The carbinol was converted to the chloride and allowed to react with potassium cyanide in a boiling ethanol–water medium as described by Shriner and Hull[18] for an analogous compound. The crude nitrile (b.p. 161–171° (1 mm.)) was obtained in 18% yield.

**(d) From the Bromide by Reaction at 40–45°.**[19]—The bromide (VII), prepared from 238 g. of the carbinol, was stirred for 14 hours at 40–45° with a solution containing 104 g. of potassium cyanide, 240 ml. of water, and 480 ml. of ethanol. At the end of this period the mixture was refluxed for a half hour, then filtered to remove inorganic material and taken up in methylene chloride. The methylene chloride solution was washed, filtered, dried and concentrated. The residue, upon vacuum distillation, yielded 113.6 g. of a yellow oil, b.p. 148–166° (1 mm.), which consisted mainly of the ethyl ether (VIII), followed by 85.8 g. (35%) of an orange oil, b.p. 166–171° (1 mm.), which was the desired product. The analytical sample boiled at 169–171° (1 mm.), $n^{25}D$ 1.5920.

*Anal.* Calcd. for $C_{15}H_{13}ON$: C, 80.69; H, 5.87. Found: C, 80.70; H, 5.98.

A sample of the nitrile was hydrolyzed by refluxing it in 20% alcoholic potassium hydroxide solution. Acidification of the mixture yielded **2-phenyl-4-methoxyphenylacetic acid**, which crystallized from cyclohexane as clusters of thick white needles, m.p. 115–116°.

*Anal.* Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.50; H, 6.00.

Hydrolysis of the nitrile (XIV) in a hydrobromic–acetic acid mixture yielded **2-phenyl-4-hydroxyphenylacetic acid**, which crystallized from water as flat white needles, m.p. 141–142°.

*Anal.* Calcd. for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.78; H, 5.32.

**α-(5-Methoxy-2-biphenylyl)-butyronitrile (XI).**—Sodium amide[20] was prepared in liquid ammonia from 2.6 g. of sodium. The ammonia was displaced by 30 ml. of anhydrous ether, and then 20.5 g. of 2-phenyl-4-methoxybenzyl cyanide (X) in 30 ml. of anhydrous ether was added slowly. The mixture was stirred at room temperature for a half hour and then 12 ml. of ethyl bromide was added very slowly. The mixture was stirred overnight, then poured on ice, acidified with cold dilute hydrochloric acid, and extracted with ether. The extracts were washed with water and bicarbonate, dried over magnesium sulfate, and concentrated. Distillation of the residue under reduced pressure yielded 20.4 g. (88%) of a very viscous colorless oil, b.p. 172–181° (2 mm.). The analytical sample boiled at 181° (2 mm.), $n^{25}D$ 1.5683.

*Anal.* Calcd. for $C_{17}H_{17}ON$: C, 81.24; H, 6.82. Found: C, 81.35; H, 7.08.

Hydrolysis of a sample of the alkylated nitrile (XI) with 20% potassium hydroxide solution, as in the case of the homolog, yielded **α-(5-methoxy-2-biphenylyl)-butyric acid**, which crystallized from ether–petroleum ether in small white crystals, m.p. 90.5–91°.

*Anal.* Calcd. for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found: C, 75.45; H, 6.90.

**α-(5-Methoxy-2-biphenylyl)-4-methoxybutyrophenone (XII).**—To a Grignard reagent prepared from 30 g. of pure *p*-bromoanisole, 20.1 g. of the alkylated nitrile (XV) in dry ether solution was added slowly. The mixture was refluxed and stirred for nine hours and then allowed to stand overnight. It was then decomposed with 200 ml. of 2 *N* hydrochloric acid, the ether was distilled, and the acid mixture was heated, with stirring, on the steam-bath for a half-hour. The ketone thus obtained was taken up in ether and filtered from a small amount of 4,4'-dimethoxybiphenyl. The ethereal solution was washed with water and sodium bicarbonate solution, dried and concentrated. The residual oil, upon seeding, slowly crystallized. It was collected and washed with ethanol; yield 15.1 g. of yellow prisms, m.p. 94–97°. Recrystallization from ethanol yielded 14.1 g. (49%) of white prisms, m.p. 97–98°. The analytical sample crystallized from ethanol as white rectangular prisms, m.p. 98–98.5°.

*Anal.* Calcd. for $C_{24}H_{24}O_3$: C, 79.97; H, 6.71. Found: C, 80.21; H, 6.89.

(17) T. A. Geissman and R. W. Tess, THIS JOURNAL, **62**, 514 (1940).

(18) R. L. Shriner and C. J. Hull, *J. Org. Chem.*, **10**, 228 (1945).

(19) We are indebted to Dr. Simon W. Kantor for this suggestion.

(20) R. Levine and C. R. Hauser, THIS JOURNAL, **68**, 760 (1946).

**3-Hydroxy-(9-*p*-hydroxyphenyl)-10-ethylphenanthrene** (II).—To 8.5 g. of the ketone (XVI) above dissolved in 170 ml. of hot acetic acid, 85 ml. of 48% hydrobromic acid was added and the solution refluxed for 72 hours under an atmosphere of nitrogen. At the end of this period, the mixture was poured into water and extracted with methylene chloride. The extract was washed with water and sodium bicarbonate solution, dried and concentrated. The residue was recrystallized several times from ethanol–water to yield 4.3 g. (51%) of small irregular light yellow plates of analytical purity. This material melted at 194.5–195.5°, but in the region 125–145° there appeared to be sintering and evolution of a volatile constituent. This was presumably due to the loss of solvent from the solvated crystals, for when the air-dried material above was dried to constant weight in vacuum, 13% of its original weight was lost.[21]

*Anal.* Calcd. for $C_{22}H_{18}O_2$: C, 84.05; H, 5.77. Found: C, 84.12; H, 6.09.

A sample of the air-dried solvated material gave analytical results in good agreement with the assumption that the solvate contained one mole of ethanol per mole.

*Anal.* Calcd. for $C_{24}H_{24}O_3$: C, 79.97; H, 6.71. Found: C, 80.13; H, 6.92.

The solvate obtained when the dihydroxy compound (II) crystallizes from pyridine–water melts at 148–150°. Analysis of an air-dried sample indicates that one mole of pyridine is combined with one mole of the dihydroxy compound (II).

*Anal.* Calcd. for $C_{27}H_{23}O_2N$: C, 82.41; H, 5.89; N, 3.56. Found: C, 82.47; H, 6.04; N, 3.60.

**The dimethyl ether** of II was prepared in sodium hydroxide solution by the action of methyl sulfate. It crystallized from acetic acid–ethanol as small flat colorless needles, m.p. 163.5–164.5°. In hot acetic acid, the product gives a pink color which disappears on cooling or on addition of ethanol.

*Anal.* Calcd. for $C_{24}H_{22}O_2$: C, 84.18; H, 6.48. Found: C, 84.14; H, 6.67.

**The dibenzoate** of II, prepared by the Schotten–Baumann procedure yielded transparent plates from acetic acid, m.p. 214–215°.

*Anal.* Calcd. for $C_{36}H_{26}O_4$: C, 82.74; H, 5.02. Found: C, 82.68; H, 4.93.

One gram of the dibenzoate was hydrolyzed by refluxing it for three hours in a mixture containing 10 ml. of purified dioxane and 15 ml. of 20% potassium hydroxide solution. The dihydroxy compound (II) was precipitated by the action of carbon dioxide and recrystallized from ethanol–water, m.p. 193.5–195.5° alone or when mixed with the analytical sample obtained from the cyclization product.

**5-Methoxy-2-acetobiphenyl**[22] **(IX).**—A Grignard reagent was prepared from 28 ml. of methyl iodide and most of the ether removed. To the concentrated Grignard reagent, 31.3 g. of 5-methoxy-2-cyanobiphenyl (IV) was added in 100 ml. of dry thiophene-free benzene. The remainder of the ether was removed and the mixture refluxed with stirring for 16 hours. At the end of this period, 250 ml. of 2 *N* hydrochloric acid was added and the mixture heated for one hour with stirring on the steam-bath in order to hydrolyze the imine to the ketone. During this period, the benzene was allowed to distil. The organic residue was taken up in methylene chloride and the solution washed with water and sodium bicarbonate solution. Concentration of the solution and fractionation of the residue under reduced pressure yielded the ketone (IX) as a light yellow oil, 23.2 g. (68%), b.p. 165–170° (2 mm.), which crystallized on standing, m.p. 67–72°. Recrystallization from ethanol gave colorless prisms, m.p. 73.5–74.5°.

*Anal.* Calcd. for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24. Found: C, 79.91; H, 6.35.

**The 2,4-dinitrophenylhydrazone** crystallized from ethanol as small red-orange needles, m.p. 203–204°.

*Anal.* Calcd. for $C_{21}H_{18}O_5N_4$: C, 62.06; H, 4.46. Found: C, 61.78; H, 4.43.

**Attempted Willgerodt Reaction on 5-Methoxy-2-acetobiphenyl.**—The ketone (6.8 g.) was subjected to the action of sulfur and morpholine according to the general procedure of Schwenk and Bloch.[23] On working up the product in the recommended manner, 2.4 g. of a viscous red acidic oil was obtained. This material would not crystallize even when triturated with cyclohexane and seeded with a sample of the expected acid obtained by hydrolysis of 2-phenyl-4-methoxybenzyl cyanide (X).

(21) The per cent. yield quoted above was based upon the estimated vacuum-dried weight of product.

(22) This is a modification of the procedure used by Mr. H. K. Porter of this Laboratory in the preparation of 5-methoxy-2-propiobiphenyl.

(23) E. Schwenk and E. Bloch, THIS JOURNAL, **64**, 3051 (1942).

DURHAM, N. C.
LEIDEN, THE NETHERLANDS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

# Molecular Structure of the Galactogen from Beef Lung[1]

BY M. L. WOLFROM, GORDON SUTHERLAND AND MAX SCHLAMOWITZ[2]

RECEIVED MARCH 12, 1952

The galactogen of beef lung, designated pneumogalactan, has been further purified and shown to contain no significant amount of L-galactose. As isolated, the polysaccharide is alkali-sensitive and contains one titratable acid function, probably carboxyl, per 35–40 anhydrohexose units. This allows it to move as an anion, essentially but not quite homogeneously, under electrophoresis. A search for its hydrolytic enzyme was made without significant result. Its acetate and methyl ether are described. Hydrolysis of the latter yielded the 2,4-, 2,3,4- and 2,3,4,6- methyl ethers of D-galactose identified by paper chromatograms and by chromatographic separation of their crystalline anilides. Periodate oxidation of the polysaccharide indicated an oxidant consumption of 4 moles per anhydrotrisaccharide unit with the formation of 2 moles of formic acid. Pneumogalactan therefore consists of a main chain of D-galactopyranose units linked 1 → 6. To every other unit of this backbone structure is attached one D-galactopyranose entity in the 1 → 3 position.

In some commercial processes for the preparation of heparin from beef lung, a by-product polysaccharide is obtained which has been characterized as a galactan (galactogen).[3] This substance is of interest as representing the first galactan isolated from mammalian tissue and we propose for it the name pneumogalactan. We report herein a different method of purification which resulted in a product that on acid hydrolysis indicated a content of D-galactose of 100 ± 2%. Therefore no significant amount of L-galactose is present in the polymer. An examination of the crystalline hydrolyzate material for the possible presence of D,L-galactose was made but none was found. Contrary to our previous report,[3] the presently better purified preparation exhibited a negative test with the alkaline

(1) The data herein recorded supersede those reported by M. L. Wolfrom and F. A. H. Rice, *Abstracts Papers Am. Chem. Soc.*, **113**, 3Q (1948).

(2) Postdoctoral Research Fellow of the National Institutes of Health, United States Public Health Service.

(3) M. L. Wolfrom, D. I. Weisblat, J. V. Karabinos and O. Keller, *Arch. Biochem.*, **14**, 1 (1947).