Anal. Calcd. for $C_{21}H_{23}O_3(OCH_3)_3$: C, 71.67; H, 7.13; OCH_3, 20.6. Found: C, 71.89; H, 7.03; OCH_3, 20.2.

Alkaline Degradation of Pomiferitin Trimethyl Ether to Homoveratric Acid.—Pomiferitin trimethyl ether (6 g.) was treated with alcoholic potassium hydroxide as described in procedure C of the preceding communication and the homoveratric acid isolated in the same manner; yield 1.8 g., m. p. 93–94°. Pure material was obtained on further crystallization from hot water (decolorizing charcoal) and from benzene–petroleum ether; yield 0.5 g., m. p. 97–98° (mixed m. p. unchanged). The substance was further characterized by preparation of its phenacyl ester as described above; m. p. 66.5–67° (mixed m. p. unchanged).

2-Hydroxy-2,3-dihydropomiferin Trimethyl Ether and its Conversion to Pomiferin Trimethyl Ether.—Pomiferitin trimethyl ether (400 mg.) was treated with sodium and ethyl formate as described in procedure D of the preceding communication. The reaction product was isolated in the same manner and was obtained crystalline from ethanol–water; yield 270 mg., m. p. 98–105°. Pure material was obtained on further crystallization (decolorizing charcoal) from benzene–petroleum ether; yield 170 mg., m. p. 143–144°.

The substance crystallized as fine, colorless prisms. It gave no coloration with ferric chloride–alcohol and reduced Tollens reagent (pyridine solution) only slowly on heating. This reduction was in contrast to the rapid reduction of Tollens reagent shown by pomiferitin trimethyl ether. The Wilson boric acid test was negative. The substance was unchanged on heating at 100° under reduced pressure.

Anal. Calcd. for $C_{28}H_{32}O_7$: C, 69.98; H, 6.73. Found: C, 69.78; H, 6.68.

The above substance (50 mg.) was refluxed for thirty minutes with glacial acetic acid (15 cc.). Water was added (incipient opalescence) to the cooled solution and the material crystallized on standing overnight at icebox temperature; yield 45 mg., m. p. 137–138°. Pure material was obtained on one further crystallization from ethanol–water and this was identified as pomiferin trimethyl ether by melting point (139–139.5°) and mixed melting point (139–139.5°) with an authentic specimen of pomiferin trimethyl ether (m. p. 139.5°).

We are indebted to Mr. Gail Clark and to Messrs. Bernard S. Wildi and Joseph Tracht (N. Y. A. Projects O. S. U.-166 and O. S. U.-169) for assistance in preparing the plant material.

Summary

1. Pomiferin trimethyl ether (and tetrahydropomiferin trimethyl ether) was degraded by mild alkali to one mole of formic acid and pomiferitin trimethyl ether (and tetrahydropomiferitin trimethyl ether).

2. Pomiferitin trimethyl ether (and tetrahydropomiferitin trimethyl ether) reacted with sodium and ethyl formate to produce an intermediate which on mild acid treatment (glacial acetic acid) produced pomiferin trimethyl ether (and tetrahydropomiferin trimethyl ether).

3. Evidence is presented that the above intermediate is 2-hydroxy-2,3-dihydropomiferin trimethyl ether (and V, 2-hydroxyhexahydropomiferin trimethyl ether).

4. Tetrahydropomiferitin trimethyl ether was characterized as an oxime.

5. Pomiferitin trimethyl ether (and tetrahydropomiferitin trimethyl ether) was degraded by strong alkali to homoveratric (3,4-dimethoxyphenylacetic) acid.

6. The above evidence establishes the isoflavone nature of pomiferin (VI).

COLUMBUS, OHIO RECEIVED FEBRUARY 17, 1941

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

3,7-Dihydroxy-1,2,5,6-dibenzanthraquinone

BY JAMES CASON[1] AND LOUIS F. FIESER

In an earlier paper on the subject of carcinogen metabolism,[2] we described the synthesis of 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene and reported that the substance very probably is identical with the product of the metabolism of dibenzanthracene by rats and mice.[3] There remains for identification the rabbit metabolite[3,4] isolated by Levi and Boyland[4] and characterized as a dihydroxydibenzanthracene having the hydroxyl groups at some positions other than 4, 8, 9 and 10. Although we are led by certain speculative considerations to believe that the hydroxyl groups are most likely to be found at the 3′ and 7′ positions[2] and are attempting (with D. M. Bowen) to synthesize this isomer, we have in the meantime investigated one other possibility.

It seemed likely that the symmetrically substituted 3,7-isomer might be synthesized in the form of the quinone by the intermolecular con-

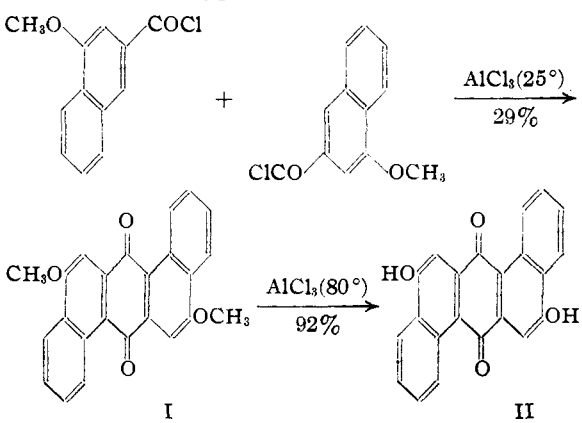
(1) Research Fellow on a grant from the National Cancer Institute (1938–1940); present address, Department of Chemistry, DePauw University, Greencastle, Indiana.

(2) Cason and Fieser, THIS JOURNAL, **62**, 2681 (1940).

(3) Dobriner, Rhoads and Lavin, *Proc. Soc. Exptl. Biol. Med.*, **41**, 67 (1939).

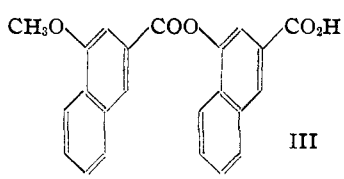
(4) Levi and Boyland, *Chemistry and Industry*, **16**, 446 (1937).

densation of two molecules of 4-hydroxy-2-naphthoic acid or a suitable derivative, for the formation of an anthraquinone would involve substitution at an α-position para to the hydroxyl-group. A convenient method of preparing the hydroxynaphthoic acid was worked out by one of us and has been reported.[5] Attempts to bring about an intermolecular condensation of 4-methoxy-2-naphthoic acid with sulfuric acid or hydrogen fluoride were unsuccessful. Since in a similar case investigated in this Laboratory[6] a condensation to 1,5-dimethyl-2,6-dimethoxyanthraquinone had been accomplished by the Friedel and Crafts reaction where other methods failed, the action of aluminum chloride on 4-methoxy-2-naphthoyl chloride was investigated. No reaction was observed in carbon bisulfide solution,[6] but with nitrobenzene as the solvent the condensation proceeded fairly smoothly at room temperature and 3,7-dimethoxy-1,2,5,6-dibenzanthracene (I) was isolated easily in a yield which is unusually favorable in this type of reaction. A small amount



I II

of a high melting acidic by-product was encountered for which the analysis indicated the formula $C_{23}H_{16}O_5$ or $C_{24}H_{18}O_5$. The substance was found to undergo hydrolysis with alkali, and two acids were isolated from the reaction mixture and identified as 4-methoxy-2-naphthoic acid and 4-hydroxy-2-naphthoic acid. The substance therefore may be assigned the structure of the ester III. It evidently arises as the result of the demethyla-



III

(5) Cason, THIS JOURNAL, **63**, 828 (1941).

(6) Fieser and Lothrop, *ibid.*, **58**, 752 (1936).

tion of a part of the starting material by the aluminum chloride.

Although the dimethoxydibenzanthraquinone did not suffer scission in the course of the low temperature reaction in nitrobenzene, the purified product was found to undergo very smooth demethylation with aluminum chloride in refluxing benzene. Sublimation gave a brick red product of the expected composition which has no characteristic point of decomposition but which gives a yellow color in aqueous alkali, in contrast to the blue coloration observed by Levi and Boyland[4] with the dihydroxydibenzanthraquinone obtained from the rabbit metabolite. Our 3,7-dihydroxy compound (II) afforded a nicely crystalline diacetate melting at 316–319°, whereas the corresponding derivative characterized by Levi and Boyland melts at 294°. The evidence from the melting points of the diacetates and from the color reactions of the dihydroxy compounds indicates that the quinone derivatives of the two series are not identical. It is concluded that the rabbit metabolite is not 3,7-dihydroxy-1,2,5,6-dibenzanthracene.[6a]

Experimental Part[7]

4-Methoxy-2-naphthoic Acid.—A solution of 3.9 g. of 4-hydroxy-2-naphthoic acid[5] (m. p. 223.5–225°) in 200 cc. of water containing 1 equivalent of alkali was treated at the steam-bath temperature with 8 equivalents of dimethyl sulfate and excess sodium hydroxide, added in about fifteen minutes. Heating was continued for five minutes longer and the slightly cloudy solution was filtered with Super-Cel and acidified. The precipitated product melted at 199–201° and was satisfactory for use; yield 3.85 g. (92%). If lower melting material is obtained it should be further methylated. A sample of the acid treated with Norit in toluene solution and crystallized twice from toluene–hexane (1:1) formed snow white needles, m. p. 202–202.5°.

Anal. Calcd. for $C_{12}H_{10}O_3$: C 71.26; H, 5.00. Found: C, 71.28; H, 5.20.

3,7 - Dimethoxy - 1,2,5,6 - dibenzanthraquinone.—Attempts to condense the methoxy acid with the use of sulfuric acid resulted only in extensive sulfonation, and hydrogen fluoride at room temperature gave nothing recognizable except starting material. The acid chloride failed to react in the presence of aluminum chloride in carbon bisulfide solution. After several trials of the Friedel and Crafts reaction in nitrobenzene solution, the following conditions were adopted as the most satisfactory.

(6a) This conclusion is further substantiated by a comparison of the properties of the synthetic 3,7-dihydroxy quinone and its dimethyl ether with those recently reported for the rabbit metabolite derivatives by Boyland, Levi, Mawson and Roe, *Biochem. J.*, **35**, 184 (1941).—L. F. F., 4-22-41.

(7) Microanalyses by Lyon Southworth. All melting points are corrected; those above 300° were taken in evacuated capillaries in an aluminum block with a 500°-thermometer inserted to the immersion line.

A mixture of 1 g. of precipitated 4-methoxy-2-naphthoic acid and 1.08 g. of phosphorus pentachloride was heated for one-half hour on the steam-bath, the phosphorus oxychloride was removed at diminished pressure, and an ice cold solution of 2.62 g. of aluminum chloride in 8 cc. of nitrobenzene was added. The reaction mixture was allowed to stand at room temperature with occasional swirling for forty-eight hours and then decomposed with ice and hydrochloric acid. The solvent was removed with steam and the residual tarry cake was heated under reflux for one-half hour with 75 cc. of ether, which dissolved starting material and the acidic by-product and left the anthraquinone as a granular red powder. This was dissolved in about 75 cc. of xylene and the solution was refluxed with Norit, filtered, and concentrated to a volume of 25 cc. for crystallization. The quinone separated as bright orange needles, m. p. 344–348°; yield 265 mg. (29%). A sample crystallized twice more from ethyl benzoate formed fibrous, pale orange needles, m. p. 347–349°; this material was sublimed in high vacuum at 250 ± 5° prior to analysis. The quinone gives an intense greenish blue color in concentrated sulfuric acid.

Anal. Calcd. for $C_{24}H_{16}O_4$: C, 78.26; H, 4.37. Found: C, 78.50; H, 4.68.

When the ethereal solution remaining after collection of the quinone was shaken with sodium bicarbonate solution a fine precipitate separated, and this was collected by filtration of the two-layer system and found to consist of the sodium salt of the by-product III. The filtered ether layer was extracted again with sodium bicarbonate solution, and on acidification of the combined bicarbonate extracts there was obtained 180 mg. of crude starting material. The precipitated sodium salt was shaken with dilute acid and ether until it had been decomposed and the free acid dissolved, and the ethereal extract was washed, dried and evaporated. The residue was crystallized from benzene and afforded 65 mg. of fine, tan needles, m. p. 259–261°. After two further crystallizations, the **ester III** was obtained as small, pale yellow needles, m. p. 259.7–260.2°.

Anal. Calcd. for $C_{28}H_{16}O_8$: C, 74.19; H, 4.32. Found: C, 74.50; H, 4.69.

For saponification, 50 mg. of the ester was refluxed for two hours with 3 cc. of 1 *N* sodium hydroxide. The solution was diluted and acidified, and the light brown precipitate (50 mg.) was sublimed at 180–200° at 20 mm., leaving a small amount of dark residue. The nearly colorless sublimate on crystallization from aqueous alcohol gave 22 mg. of almost white needles, m. p. 197.5–200.5°, and this material when crystallized again from aqueous alcohol and then from benzene (3 cc.)–ligroin (1 cc.) afforded snow-white needles melting at 201.5–202.5° and giving no depression when mixed with pure 4-methoxy-2-naphthoic acid. When the aqueous alcoholic mother liquor from the first crystallization was concentrated to 1–2 cc., there crystallized 18 mg. of crude 4-hydroxy-2-naphthoic acid. After two recrystallizations from benzene the acid formed nearly white needles, m. p. 223–225.5°; mixture of this material with pure 4-hydroxy-2-naphthoic acid, 224–226°.

3,7-Dihydroxy-1,2,5,6-dibenzanthraquinone.—The dimethoxy compound I was not altered by being refluxed with hydrobromic and acetic acids but was hydrolyzed satisfactorily as follows. A mixture of 100 mg. of the dimethoxy quinone, 290 mg. of aluminum chloride, and 20 cc. of benzene was heated under reflux for five hours. The complex was then decomposed with ice and hydrochloric acid and the benzene was boiled off. The product separating as a brick red precipitate was dissolved in 75 cc. of 0.5 *N* sodium hydroxide and the opaque solution was filtered with Super-Cel and neutralized by passing in a stream of carbon dioxide at the boiling point. The precipitate was digested at the boiling point under carbon dioxide for fifteen minutes and, after cooling, the violet-black product was collected; yield 85 mg. (92%). The filtrate was dark green; the addition of hydrochloric acid largely discharged the color but gave no appreciable precipitate. A 35-mg. sample of the precipitated quinone when sublimed at 270 ± 5° and 2–3 × 10^{-4} mm. gave only 6 mg. of brick-red sublimate, and the remainder was decomposed to a dark amorphous residue.

Anal. Calcd. for $C_{22}H_{12}O_4$: C, 77.64; H, 3.55. Found: C, 77.75; H, 3.91.

The sublimed dihydroxy quinone gave a yellow color in dilute aqueous alkali, and the addition of sodium hydrosulfite gave a pink color changing to orange-red. The solution in concentrated sulfuric acid is deep indigo blue. When heated in an evacuated capillary, the substance eventually showed signs of decomposition, but remained unmelted up to 440°.

3,7-Diacetoxy-1,2,5,6-dibenzanthraquinone.—When a suspension of 50 mg. of the crude dihydroxy compound in 5 cc. of acetic anhydride containing a trace of sodium acetate was refluxed, the dark dihydroxy compound was rapidly converted into the yellow diacetate. After refluxing for one hour, the excess anhydride was decomposed with water and the product collected. The yield of material melting at 310–315° was 50 mg. (80%). Two recrystallizations from xylene (about 10 cc.) gave 40 mg. of beautiful lemon-yellow needles, m. p. 316–319°, decomp. When heated in an open capillary, the substance melted at approximately the same temperature but underwent more extensive decomposition.

Anal. Calcd. for $C_{26}H_{16}O_6$: C, 73.58; H, 3.79. Found: C, 73.57; H, 4.04.

When a small sample of the diacetate was boiled with dilute alkali for a few minutes the yellow color characteristic of the free hydroxy compound in alkali was produced, and the vat test was then the same as described above. The solution in concentrated sulfuric acid is blue.

Summary

3,7-Dimethoxy-1,2,5,6-dibenzanthraquinone has been synthesized by the action of aluminum chloride on 4-methoxy-2-naphthoyl chloride in nitrobenzene solution. The free dihydroxy compound and its diacetate differ from the corresponding derivatives of a dihydroxydibenzanthracene isolated by Levi and Boyland as a product of the metabolism of the carcinogenic hydrocarbon by rabbits, and this therefore is not the 3,7-dihydroxy derivative.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASS. RECEIVED FEBRUARY 17, 1941