[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE RADIATION LABORATORY OF THE UNIVERSITY OF CALIFORNIA, BERKELEY]

The Synthesis of 5-Hydroxy-1,2-naphthalic Anhydride

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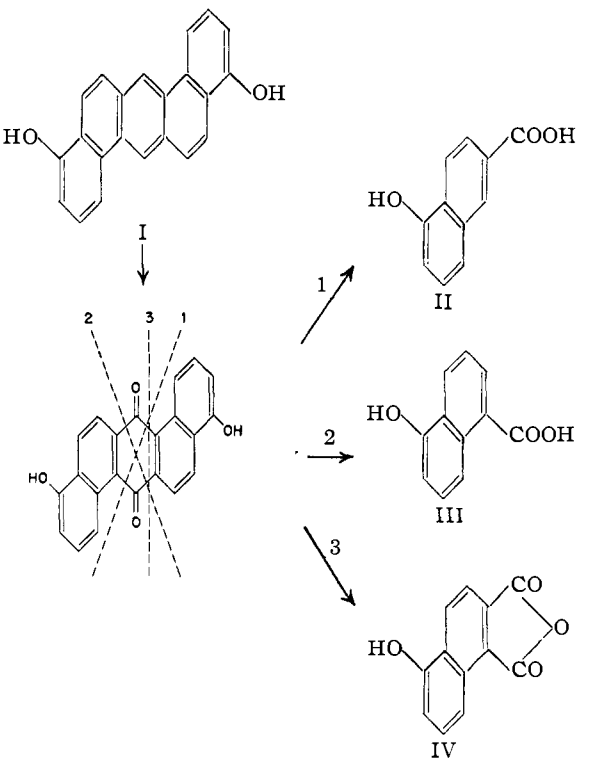
The metabolism of the carcinogenic hydrocarbon, 1,2,5,6-dibenzanthracene, has been investigated by numerous workers.[1] Until last year, however, only two metabolic products had been isolated, one from the rabbit[2] and one from the rat or mouse.[3] Both compounds were shown to be dihydroxydibenzanthracenes and Cason and Fieser[4] showed that the product from the latter animals was 4',8'-dihydroxydibenzanthracene. Recently, Heidelberger, Jones and coworkers[5] reinvestigated this problem of metabolism using C^{14}-labeled dibenzanthracene.[6]

These workers have reported that the carcinogenic hydrocarbon is metabolized by the mouse into at least four distinct substances. They found that one acidic fraction yielded a phenol which was thought to be the previously isolated dihydroxydibenzanthracene. In the same acidic fraction, however, they also found water-insoluble acids which could be extracted with aqueous 0.5 *N* sodium bicarbonate. It was deemed of interest to investigate the structure of the possible components of this new acidic metabolite. A reasonable assumption as to the identity of this acid was suggested by the observation of Cason and Fieser,[4] who found that the diacetate of their 4',8'-dihydroxydibenzanthracene (I) upon oxidation to the 9,10-quinone and subsequent alkali fusion yielded 5-hydroxy-2-naphthoic acid (II) (path 1).

It would also be possible but less probable for this symmetrical cleavage to occur by another route (path 2) and to yield 5-hydroxy-1-naphthoic acid (III). Heidelberger,[7] using carrier technique has shown, however, that neither of these two acids is identical with the new acidic metabolite.

If an unsymmetrical cleavage occurred (path 3) or if the dihydroxydibenzanthracene (I) was oxidized at one end of the molecule, another possibility would be 5-hydroxy-1,2-naphthalic anhydride (IV). To test this hypothesis, the naphthalic anhydride has been prepared and is now being tested by Dr. Heidelberger.

The 6-methoxy-1,2-naphthalic anhydride has



been prepared by Fieser and Hershberg[8] from ethyl γ-(*m*-methoxyphenyl)-butyrate by the Bougault reaction. This same method was employed to prepare the desired 5-hydroxy compound. The starting acid (VIa) has been prepared previously by Lockett and Short[9] in a yield of 16% from coumarin. Recently, Hardegger and his associates[10] synthesized the acid in an over-all yield of 40% by alkylating diethyl malonate with β-(*o*-methoxyphenyl)-ethyl bromide, the parent alcohol of which had been prepared from *o*-bromoanisole and ethylene oxide. In the present investigation, it was found that γ-(*o*-methoxyphenyl)-butyric acid (VIa) could be easily prepared in two steps from *o*-bromoanisole through its cadmium derivative and the subsequent reduction of β-(*o*-methoxybenzoyl)-propionic acid (V) in a yield of 28%. Ethyl γ-(*o*-methoxyphenyl)-butyrate (VIb) was allowed to react with diethyl oxalate in the presence of potassium ethoxide and the resulting product VII was cyclized with concentrated sulfuric acid. The 5-methoxy-3,4-dihydro-1,2-naphthalic anhydride (VIII) was obtained in an over-all

(1) Berenblum and Kendal, *Brit. J. Exper. Path.*, **15**, 366 (1934); Chalmers, *Biochem. J.*, **28**, 1214 (1934); Hieger, *Am. J. Cancer*, **28**, 522 (1936); Berenblum and Kendal, *Biochem. J.*, **30**, 429 (1939); Chalmers and Peacock, *ibid.*, **30**, 1242 (1936); Jones, *Cancer Research*, **2**, 237 (1942); *ibid.*, **4**, 209 (1944).

(2) Levi and Boyland, *Chem. and Industry*, **15**, 446 (1937).

(3) Dobriner, Rhoads and Lavin, *Proc. Soc. Exptl. Biol. Med.*, **41**, 67 (1939); *Cancer Research*, **2**, 95 (1942).

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

(5) Heidelberger and Jones, *Cancer*, **1**, 252 (1948); Heidelberger, Kirk and Perkins, *ibid.*, **1**, 261 (1948).

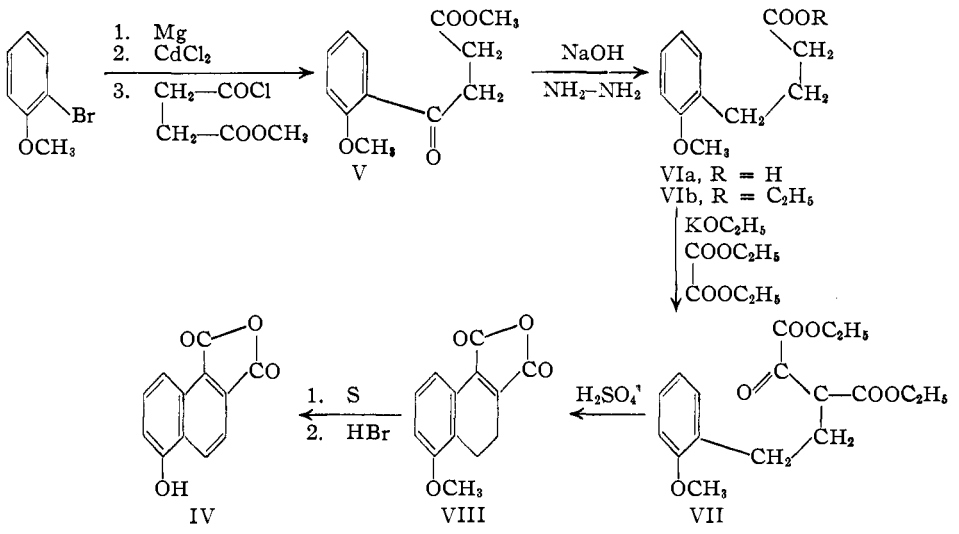
(6) Heidelberger, Brewer and Dauben, THIS JOURNAL, **69**, 1389 (1947).

(7) Heidelberger, private communication.

(8) Fieser and Hershberg, THIS JOURNAL, **58**, 2314 (1936).

(9) Lockett and Short, *J. Chem. Soc.*, 787 (1939).

(10) Hardegger, Redlich and Gal, *Helv. chim. acta*, **28**, 628 (1945).



yield of 36%. The dihydro-acid was dehydrogenated with sulfur and the resulting 5-methoxy-1,2-naphthalic anhydride was demethylated with hydrobromic acid to yield the desired 5-hydroxy-1,2-naphthalic anhydride (IV). This new anhydride was water-insoluble but soluble in aqueous 0.5 *N* sodium bicarbonate, similar to the properties shown by the new acidic metabolic fraction.

It is interesting to note that Fieser and Hershberg[8] reported that when the 6-methoxy isomer was cyclized with 75% sulfuric acid, part of the dihydronaphthalic anhydride underwent dehydrogenation. This was not observed with the 5-methoxy isomer when the cyclization was conducted at room temperature.

Experimental[11]

Methyl β-(*o*-Methoxybenzoyl)-propionate (V).—A solution of 56 g. (0.3 mole) of *o*-bromoanisole in 150 cc. of dry ether and 50 cc. of anhydrous benzene was added to 7.7 g. (0.315 mole) of magnesium turnings over a period of ninety minutes in a nitrogen atmosphere. The Grignard solution was cooled in an ice-bath, 33 g. (0.18 mole) of anhydrous cadmium chloride[12] was added and the mixture refluxed for thirty minutes at which time a negative Gilman test was obtained.[13] The ether was distilled and 200 cc. of dry benzene added. A solution of 52.5 g. (0.35 mole) of β-carbomethoxypropionyl chloride[14] in 40 cc. of dry benzene was added dropwise over the course of five minutes to the hot mixture. A vigorous exothermic reaction ensued which was easily controlled by the slow addition. A brown precipitate formed and the mixture was refluxed with stirring for one hour, decomposed with dilute hydrochloride acid and processed in the usual manner. The methyl β-(*o*-methoxybenzoyl)-propionate distils at 161–162° (1.5 mm.), $n^{27}D$ 1.5311, d^{24}_4 1.178, yield 23.8 g. (42.7%).

Anal. Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 65.09; H, 6.32.

The ester (27 g., 0.12 mole) was saponified with alcoholic potassium hydroxide and the acid distilled, b. p. 195–196° (0.5 mm.), yield 20 g. (86%). The acid solidified upon standing and was recrystallized from ligroin, m. p. 95.6–96.6°.

Anal. Calcd. for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.69; H, 5.88.

γ-(*o*-Methoxyphenyl)-butyric Acid (VIa).—A solution of 24 g. (0.108 mole) of methyl β-(*o*-methoxybenzoyl)-propionate, 14.6 cc. of 85% hydrazine hydrate, 29 g. (0.5 mole) of potassium hydroxide and 140 cc. of diethylene glycol[15] was heated for two hours at 150°, the condenser removed and the solution distilled until the temperature of the reaction mixture reached 200°. The heating was continued for four hours at this temperature, then allowed to cool, diluted with 250 cc. of water and acidified with 150 cc. of 6 *N* hydrochloric acid. The mixture was extracted three times with 100-cc. portions of ether, the ethereal extract washed with water and the ether evaporated. The residue was dissolved in a solution of 8 g. of sodium hydroxide in 150 cc. of water, 10 cc. of dimethyl sulfate added and the mixture heated for one hour on a steam-bath. The reaction was cooled, acidified and extracted with ether. The acid boils at 131–132° (0.1 mm.), $n^{27}D$ 1.5265, yield 13.7 g. (65.3%). Hardegger, *et al.*,[10] report 143–145° (0.2 mm.).

The *p*-phenylphenacyl ester melts at 88–89° (alc.).

Anal. Calcd. for $C_{25}H_{24}O_4$: C, 77.30; H, 6.23. Found: C, 77.06; H, 6.42.

The reduction of the carbonyl group was also accomplished by hydrogenolysis of sodium β-(*o*-methoxybenzoyl)-propionate over copper chromite catalyst[16] and the reduced acid was received in a yield of 58%.

The ethyl ester of γ-(*o*-methoxyphenyl)-butyric acid (VIb) was prepared by heating a solution of 13.5 g. (0.07 mole) of the acid, 8.4 cc. of ethanol, 0.5 g. of 2-naphthalenesulfonic acid in 22.5 cc. of benzene. The azeotrope containing the water formed in the reaction was removed in the usual manner. Ethyl γ-(*o*-methoxyphenyl)-butyrate boils at 101–102° (0.2 mm.), yield 12.7 g. (82.1%), $n^{29}D$ 1.4998.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.41; H, 7.95.

Ethyl α-(Ethoxalyl)-γ-(*o*-methoxyphenyl)-butyrate (VII).—The reaction was conducted following the method of Fieser and Hershberg.[17] Diethyl oxalate (13.6 g., 0.063 mole) was added to a well-stirred ethereal suspension of potassium ethoxide, prepared from 2.5 g. of potassium

(11) Analyses by The Microanalytical Laboratory of the Department of Chemistry, University of California. All melting points are corrected.

(12) Cason, THIS JOURNAL, **68**, 2078 (1946), and earlier papers.

(13) Gilman and Heck, *ibid.*, **52**, 4949 (1930).

(14) "Org. Syn.," Vol. 25, John Wiley and Sons, Inc., New York, N. Y., p. 19.

(15) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

(16) Fieser and Heymann, *ibid.*, **63**, 2333 (1941).

(17) Fieser and Hershberg, *ibid.*, **57**, 1851 (1935).

and 2.94 g. of absolute ethanol. Ethyl γ-(*o*-methoxyphenyl)-butyrate (14 g., 0.063 mole) was then added dropwise with stirring and the mixture refluxed for twelve hours. The reaction mixture was decomposed with water and extracted with ether. A red viscous oil remained after distillation of ether.

5-Methoxy-3,4-dihydro-1,2-naphthalic Anhydride (VIII).—The above oxalyl ester was added slowly to 120 cc. of concentrated sulfuric acid, the temperature being kept between 20–25°, and the mixture allowed to stand for two hours at room temperature. The solution was poured onto ice, the yellow solid filtered and recrystallized from benzene–ligroin, yield 5.2 g. (36% based on starting ester VIb), m. p. 169–170°.

Anal. Calcd. for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C, 68.17; H 4.62.

5-Methoxy-1,2-naphthalic Anhydride.—The dihydro-acid (4.0 g., 0.017 mole) and 0.55 g. (0.017 mole) of sulfur was heated at 230–235° for thirty minutes. After cooling the solid mass was recrystallized twice from benzene and then sublimed. The yellow solid melts at 228–229°; yield 3.2 g. (80%).

Anal. Calcd. for $C_{13}H_8O_4$: C, 68.42; H, 3.53. Found: C, 68.35; H, 3.57.

5-Hydroxy-1,2-naphthalic Anhydride (IV).—The above methoxynaphthalic anhydride (1.2 g., 0.005 mole) was refluxed for seven hours with 25 cc. of glacial acetic acid and 25 cc. of 48% hydrobromic acid. Upon cooling the solution to room temperature, an amorphous tan solid formed. Further concentration of the mother liquor yielded additional material. The combined solids were sublimed and then recrystallized from water, m. p. 271–272°, yield 0.5 g. (47%).

Anal. Calcd. for $C_{12}H_6O_4$: C, 67.29; H, 2.82. Found: C, 67.11; H, 2.87.

5-Methoxy-1,2,3,4-tetrahydro-1,2-naphthalic Anhydride.—The dihydro-anhydride (VIII) was hydrogenated over platinum oxide in glacial acetic acid. The solvent was removed under reduced pressure and the residue sublimed in vacuum. The yellow solid was recrystallized from benzene–ligroin, m. p. 144–146°.

Anal. Calcd. for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found: C, 67.35; H, 5.01.

Summary

5-Hydroxy-1,2-naphthalic anhydride, a possible metabolite of 1,2,5,6-dibenzanthracene, and several of its derivatives have been prepared by means of the Bougault reaction.

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A Study of Diffusion in Agar Gels by a Light Absorption Method

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Introduction

While precision methods are now available for determination of diffusion coefficients in solution systems,[1,2,3,4] these in general require elaborate apparatus and painstaking techniques. There is still, therefore, need for a relatively simple, rapid and generally applicable procedure for evaluation of diffusion coefficients with moderate precision.

The advantages to be gained by use of gels to immobilize one or both of the initial phases are considerable. Difficulties in establishment of an initial sharp boundary are reduced and convection effects are almost eliminated. Moreover, quantitative determination of dispersion by diffusion is greatly simplified since the cell can be subjected to mechanical and thermal stress during analysis without appreciable disturbance of the established gradients. For light-absorbing substances, the analysis may be conducted by direct scanning of the cell with a slit spectrophotometer. With instruments now available concentrations may be measured for narrow bands along the axis of diffusion with a limit of error of a few tenths of one per cent. This compares favorably with refractive index gradient measurements and obviates objections which have been raised to older light absorption methods.[2]

Since uncertainty exists regarding the effects of gels on the diffusion process[5,6] we have undertaken a study of these factors in conjunction with techniques for conducting the determination of diffusion coefficients. Our study of agar in lieu of other gel forming substances was prompted from consideration of its moderately good optical transparency in the ultraviolet and the lack of chemical interaction between it and acidic or neutral substances. While agar gels are considerably more opaque than gelatin gels, this optical density due to light scattering is quite uniform and reproducible. It has been studied in considerable detail by Donnan and Krishnamurti[7] and by Hatschek.[8]

Studies by Sabin and Sobotka[9] indicate that the interstitial fluid in an agar gel contains appreciable amounts of dissolved substances which can cause a "barophoresis" effect in diffusion measurements.

To obtain further information regarding the nature of the gel fluid a series of extraction experiments on agar gels prepared from "Difco Bacto-agar" have been conducted. These indicate that one-quarter to one-third of the agar can be extracted by cold water. The amount of

(1) J. W. McBain and T. H. Liu, THIS JOURNAL, **53**, 59 (1931).

(2) H. Neurath, *Chemical Revs.*, **30**, 357 (1942).

(3) H. S. Harned and D. M. French, *Ann. N. Y. Acad. Sci.*, **46**, 267 (1945).

(4) O. Lamm and A. Polson, *Biochem. J.*, **30**, 528 (1936).

(5) (a) L. Friedman and E. O. Kraemer, THIS JOURNAL, **52**, 1295 (1930); (b) L. Friedman, *ibid.*, **52**, 1305, 1311 (1930); (c) K. Klemm and L. Friedman, *ibid.*, **54**, 2632 (1932).

(6) V. Moravek, *Kolloid-Z.*, **49**, 39 (1929).

(7) F. G. Donnan and K. Krishnamurti, *Colloid Symposium Annual*, **7**, 1–16 (1930).

(8) E. Hatschek, *Kolloid Z.*, **48**, 246–248 (1929).

(9) A. B. Sabin and H. Sobotka, THIS JOURNAL, **50**, 1561–1572 (1928).