

chloroform, then once from chloroform-alcohol. The melting point was 242°.

Anal. Calcd. for $C_{25}H_{22}N_2$: N, 8.0. Found: N, 7.95.

5,6-Dibromo-3-ethylpseudocumene (VIII).—The hydrocarbon (3 g.) was sulfonated by shaking it vigorously with sulfuric acid (8 cc.) and 20% oleum (4 cc.) for three minutes. The clear solution was poured over ice (300 g.) and bromine (10 g.) was added in 0.5-g. portions with shaking. A yellow emulsion formed immediately, which after standing for three hours at room temperature deposited a yellow solid. Excess bromine was removed by sodium bisulfite, and the solid (6 g.) was then washed thoroughly with alkali. The solid was crystallized twice from alcohol, when it formed white needles which melted at 65–66°.

Anal. Calcd. for $C_{11}H_{14}Br_2$: C, 42.25; H, 4.57; Br, 52.18. Found: C, 42.64; H, 4.68; Br, 52.28.

Prehntic Acid (IX).—The hydrocarbon (5 g.) was oxidized with 400 cc. of aqueous potassium permanganate (20 g.) containing potassium carbonate (12 g.). After shaking the mixture at room temperature for five hours, it was heated on the steam-bath for thirty hours, when the color had disappeared. The solution was made slightly acid with acetic acid, and lead acetate was added. The lead salt was removed, thoroughly washed, then suspended in water and the suspension was saturated with hydrogen sulfide. The lead sulfide was filtered off and the filtrate was concentrated to about 3 cc. After addition of a few drops of hydrochloric acid, the solution was cooled. It deposited the tetracarboxylic acid in the form of needles. These were removed, dried, extracted with ether and the ether soluble part was recrystallized from a little water containing a few drops of hydrochloric acid. The yield of pure material was only about 100 mg. The acid melted

at 236°. In a similar fashion, pyromellitic acid was obtained from 5-ethylpseudocumene, although the yield (about 100 mg. from 5 g. of the hydrocarbon) was again poor. It melted at 270–272°.

Mellophanic acid could not be obtained from ethylmesitylene by the above procedure. The hydrocarbon (10 g.) was heated for two weeks on the steam-bath with a solution of potassium permanganate (50 g.) and sodium hydroxide (10 g.) in water (1500 cc.). Sodium bisulfite (about 1 g.) was added, the solution was neutralized with acetic acid, and filtered to remove manganese dioxide. The organic acid was then isolated via the lead salt as described above. The yield was about 5 g. After drying at 110° for an hour, the acid melted at 238–267° with anhydride formation. When crystallized from acetone, the acid melted at 238–240°. Neutral equivalent: calcd., 80; found, 80.

Summary

1. Three ethyltrimethylbenzenes, namely, 3- and 5-ethylpseudocumenes and ethylmesitylene, have been prepared in quantity and certain of their physical properties have been determined.

2. Since 3-ethylpseudocumene is a new hydrocarbon, its structure has been proved and several derivatives of it have been described.

3. Each of the hydrocarbons has been oxidized to a benzenetetracarboxylic acid. Each gives only the one tetracarboxylic acid to be expected from its structure, though the yield is poor.

(20) Smith and Byrkit, *THIS JOURNAL*, **55**, 4305 (1933).

MINNEAPOLIS, MINNESOTA RECEIVED NOVEMBER 28, 1938

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

Prehntic Acid (Benzene-1,2,3,4-tetracarboxylic Acid)

BY LEE IRVIN SMITH AND EARL J. CARLSON¹

Some time ago, Smith and Byrkit² prepared two of the isomeric benzene tetracarboxylic acids, namely, pyromellitic acid (I) (1,2,4,5) and mellophanic acid (II) (1,2,3,5) and definitely established the orientation of the carboxyl groups in these acids by relating them to compounds of known orientation. This work also established by difference the orientation of the carboxyl groups in the third acid, prehntic (III) as 1,2,3,4. In the previous work, satisfactory methods were developed for the preparation of acids I and II in quantity from readily accessible ma-

terials; these methods consisted largely of improvements in procedures already described in the literature.

At the time of the earlier work, there was no satisfactory method for preparing prehntic acid (III) in any quantity from common materials. The oxidation of prehntene—itsself rare and costly—was extremely difficult and produced the acid in minute amounts only, and it is generally true that oxidation of the polymethylbenzenes does not constitute a good preparative method for obtaining the corresponding polycarboxylic acids. Freund and Fleischer³ prepared III in good yield (60%) from 4,7-dimethyl-2,2-diethyl-

(1) Abstracted from a thesis by Earl J. Carlson, presented to the Graduate Faculty of the University of Minnesota, in partial fulfillment of the requirements for the M.S. degree, June, 1938.

(2) Smith and Byrkit, *THIS JOURNAL*, **55**, 4305 (1933).

(3) Freund and Fleischer, *Ann.*, **411**, 14 (1916).

indandione-1,3, but the preparation of the indandione required pure *p*-xylene and diethyl malonyl chloride. A similar preparation was that of Fleischer, Hittel and Wolff,⁴ who replaced the *p*-xylene by acenaphthene, but the yield of prehnitic acid obtained on oxidation of the condensation product was considerably lower. Fieser and Peters⁵ prepared the acid in 25% yield by oxidation of the product obtained by condensing acenaphthene with succinic anhydride.

In this work, the first experiments were directed toward a synthesis based upon a Diels–Alder reaction between muconic acid and acetylenedicarboxylic acid, or between derivatives of these two acids, since muconic acid is easily prepared from dioxane, chlorine, and malonic acid^{6,7} or from adipic acid,⁸ while acetylenedicarboxylic acid is readily prepared from succinic acid.^{9,10} But all attempts to bring about a Diels–Alder reaction between muconic acid or its ester and acetylenedicarboxylic acid or its ester resulted in failure. It is peculiar that this condensation should fail, since Farmer and Warren¹¹ obtained 20% yield of condensation product from dimethylmuconate and maleic anhydride, although they were unable to dehydrogenate this product beyond the dihydro stage. Hoping to obtain a product that could be dehydrogenated, the addition of dibromofumaric acid to diethyl muconate was tried, but again there was no evidence of any addition. Nor could dimethyl muconate be added to quinone.

The failure of these syntheses based upon the Diels–Alder reaction caused attention again to be directed to the oxidative degradation of readily available materials as a preparative source for III. Bamford and Simonsen¹² had oxidized 1,4-dimethylnaphthalene and had obtained III with some difficulty and in very poor yield. But it seemed likely that if a 1,2 or a 1,4-disubstituted naphthalene could be found in quantity, in which the substituents were oxygenated, it readily might undergo further oxidation to III, since it was known from the earlier work that the introduction of even one carboxyl group into a poly-

methylbenzene facilitated immeasurably further oxidation to the polycarboxylic acid. Such a derivative of naphthalene, namely, naphthalene-1,4-dicarboxylic acid, has recently been made readily available through the work of Scott and his associates.¹³ Sodium naphthalene, when carbonated, yields a mixture of dihydronaphthalene dicarboxylic acids 1,2 and 1,4. Bromine in acetic acid dehydrogenates the 1,4-acid, but only converts the 1,2-acid into a dibromide. Naphthalene-1,4-dicarboxylic acid is much the less soluble of the two resulting acids, and can be obtained easily from the mixture of dihydro acids in 50% yield. Through the courtesy of Dr. Sterling Temple, Dr. O. W. Cass and Dr. N. D. Scott of the Roessler and Hasslacher Chemicals Division of E. I. du Pont de Nemours & Co., a generous supply of the mixed dihydronaphthalenedicarboxylic acids was made available; this was converted into naphthalene-1,4-dicarboxylic acid as described by Walker and Scott.¹³

Oxidation of naphthalene-1,4-dicarboxylic acid by nitric acid was slow and indefinite and yielded no prehnitic acid. When chromic acid was used, the oxidation was vigorous but the only isolable product was a small amount (about 6%) of a yellow substance having a high melting point (>280°). But oxidation of the naphthalene diacid by permanganate, under carefully controlled conditions, combined with the isolation of the prehnitic acid as the barium salt led to prehnitic acid in yields of 33–40%.

The procedures from the dihydro acids to prehnitic acid are simple throughout and are well adapted to fairly large scale preparations of the final product (50 g.) and it is felt that the method described represents at the present time the simplest and cheapest method available for preparing specimens of III quickly and in quantity. The crude acid III so prepared melts at 225–228°; one recrystallization from dilute hydrochloric acid or acetic acid gives a product which melts at 232–236°. The identity of the product was proved by converting the acid to the tetramethyl ester (m. p. 131–133°) by the action of diazomethane. The tetramethyl ester so prepared showed a peculiar property which has been observed before in this Laboratory in connection with specimens of this ester. The substance crystallizes nicely from methanol in colorless needles which, when exposed to light, acquire a beautiful purple color

(4) Fleischer, Hittel and Wolff, *Ber.*, **53**, 1847 (1935).

(5) Fieser and Peters, *THIS JOURNAL*, **54**, 4347 (1932).

(6) Behrend and Koolman, *Ann.*, **394**, 228 (1912).

(7) Butler and Cretcher, *THIS JOURNAL*, **54**, 2987 (1932).

(8) Guha and Sankaran, *Ber.*, **70**, 2110 (1937).

(9) Backer and van der Zanden, *Rec. trav. chim.*, **47**, 777 (1928).

(10) *Org. Syntheses*, **18**, 3 (1938), John Wiley & Sons, Inc., New York.

(11) Farmer and Warren, *J. Chem. Soc.*, 897 (1929).

(12) Bamford and Simonsen, *J. Chem. Soc.*, **97**, 1904 (1910).

(13) Walker and Scott, *THIS JOURNAL*, **60**, 951 (1938).

with no visible change in crystalline form. When melted or dissolved, these purple crystals give colorless liquids from which a colorless solid deposits; this again acquires the purple color when exposed to light. This loss and gain of color has been repeated with the same sample as many as five times with no apparent diminution in the intensity of the color developed.

Experimental Part

A. Diene Syntheses

Glyoxal Bisulfite Addition Compound.—Dichlorodioxane was prepared from dioxane (230 g.) by the method of Böeseken, Tellegen and Henriquez.¹⁴ The yield was 125 g. (31%) of material boiling at 110–115° under 60 mm. This product was converted to glyoxal bisulfite addition compound by the method of Butler and Cretcher.⁷

Muconic acid was prepared in 35–40% yields from glyoxal bisulfite compound by the method of Behrend and Koolman⁸ and from adipic acid by the method of Guha and Sankaran,⁸ although the latter method was found to be very tedious and time consuming and did not give as good yields as the method of Behrend and Koolman. The acid usually melted with decomposition over the range 280–290°; the methyl ester (from the acid, methanol and hydrochloric acid) melted at 155–156°; the ethyl ester (from the acid chloride and ethanol) melted at 61–63°.

Acetylenedicarboxylic acid, m. p. 165–168°, was made by the method described in "Organic Syntheses."¹⁰

Muconic and acetylenedicarboxylic acids failed to react, at room temperature or at the boiling point of any of the solvents used, which included acetic acid, acetone, ether, chloroform, carbon tetrachloride, ethanol, benzene and water. In every case the only products isolated were the starting materials. Likewise ineffective was any combination of the methyl or ethyl esters of the acids, or any combination of one acid and the methyl or ethyl ester of the other. Hoping to drive the diene reaction to completion by forming the anhydride of the product, dimethyl muconate and acetylenedicarboxylic acid were refluxed in acetic anhydride, but again only the starting materials were recovered.

Dimethyl muconate and maleic anhydride were heated at 130–150° for five hours. The product was crystallized from chloroform or methanol, but repeated crystallization from these solvents failed to give a pure product and the melting point of different preparations varied considerably. For the most part, the products melted over a 3 or 4° range between 166 and 174°; in one case only a melting point of 176–177° was obtained. The yield of crude product was about 20% and much loss resulted in the crystallization. In alcohol the two substances failed to react. Since Farmer and Warren¹¹ were unable to dehydrogenate their product completely by addition of bromine and removal of hydrobromic acid, an attempt was made to dehydrogenate the above product by allowing it to stand with an excess of quinone. The result was a dark, tarry residue from which no solids could be obtained.

(14) Böeseken, Tellegen and Henriquez, *Rec. trav. chim.*, **50**, 910 (1931).

Dibromofumaric acid¹⁵ was heated at 150° with ethyl muconate for two hours. The only product isolated was unchanged dibromofumaric acid, m. p. 215°.

B. Oxidation of Naphthalenedicarboxylic Acid-1,4

The acid was prepared in 50% yield from the mixture of dihydro-1,2 and -1,4 acid obtained from sodium naphthalene.¹³

Oxidation with Nitric Acid.—The naphthalene acid (1.07 g.) was refluxed for thirteen hours in an all-glass apparatus with dilute nitric acid (300 cc., 1:3). The mixture was cooled and the solid was removed. It melted with decomposition over the range 255 to 290°. The filtrate from this material was evaporated to dryness, leaving a solid residue which decomposed at 200° and was molten at 230–232°. Recrystallization from concd. nitric acid gave a product which darkened at 210° and decomposed at 235–238°. This was undoubtedly impure prehnitic acid, but the yield was very small and this procedure was abandoned.

Oxidation with Chromic Acid.—The naphthalene acid (0.7 g.) was dissolved in acetic acid (45 cc.) and to it was added chromic anhydride (1.5 g.) in small portions. Following the initial reaction, which was quite vigorous, the mixture was heated on the steam-bath for forty-five minutes. It was diluted with twice its volume of water and thoroughly extracted with ether. Evaporation of the ether left a small amount of a yellow solid melting above 280°.

Oxidation with Permanganate.—After several small scale experiments had shown that the method was feasible, oxidations were carried out on a larger scale, of which the following is a typical example. The naphthalene acid (50 g.) was suspended in water (3000 cc.) to which had been added potassium hydroxide (7 g.). Potassium permanganate (325 g.) was then added gradually with occasional shaking. The mixture became hot, but did not boil. After standing for an hour, it was heated on the steam-bath for two hours. Alcohol was then added slowly until the permanganate color disappeared. The sludge of manganese dioxide was filtered off and washed thoroughly with water. Barium chloride dihydrate (130 g.) was added to the combined filtrate and washings. After digestion on the steam-bath for an hour, the precipitate of barium prehnitate was filtered and washed with water to remove any barium chloride. The filtrate and washings were combined and an excess of sodium carbonate was added. The barium carbonate was filtered, washed, and combined with the barium prehnitate; the solids now contained all of the barium used initially. This mixture of solids was digested on the steam-bath with an amount of dilute sulfuric acid (29 cc. in 200 cc. of water) slightly less than that required to precipitate all of the barium. The precipitate was filtered, washed well with water, and the combined filtrates and washings were transferred to a large porcelain dish and evaporated slowly on the steam-bath until the crust which formed around the edges would not dissolve when wet with the solution. At this point a small amount of hydrochloric acid was added and the mixture was cooled. Prehnitic acid crystallized slowly. This was removed, washed with a little

(15) Treibich, *Ann.*, **348**, 325 (1906).

dilute hydrochloric acid (1:1) and the mother liquor and washings were evaporated again. This process was repeated until nearly all of the solvent was gone. The crude prehnitic acid weighed 19 g. (33%), was white, and melted at 225–230°. One recrystallization from a little dilute hydrochloric acid (1:1) gave a product melting at 236–238°.

A specimen of the tetramethyl ester was prepared from the acid and diazomethane in ether. The crude ester melted at 122–127°; when recrystallized once or twice from methanol, it melted at 131–133°. ¹⁸

On exposure to light, this ester acquires a purple color, which is lost on fusing or dissolving the substance and which reappears when the solid is exposed to light.

(16) Cf. ref. 12, where the m. p. of this ester is given as 135°.

Anal. Calcd. for $C_{14}H_{14}O_8$: C, 54.17; H, 4.55. Found: C, 54.19; H, 4.70.

Summary

1. A convenient, rapid method for preparation of moderate amounts of prehnitic acid (benzenetetracarboxylic acid-1,2,3,4) from easily accessible materials, is described.

2. The results—all negative—of several attempts to bring about a diene reaction between muconic acid (or its esters) and acetylenedicarboxylic acid (or its esters) are described.

MINNEAPOLIS, MINNESOTA RECEIVED DECEMBER 2, 1938

[CONTRIBUTION FROM THE FLEISCHMANN LABORATORIES, STANDARD BRANDS INCORPORATED]

Fermentation of Maltose

BY ALFRED S. SCHULTZ AND LAWRENCE ATKIN

Fermentation of maltose by yeast has been the subject of considerable study. It has been claimed by Willstätter and Bamann¹ and Sobotka and Holzman² that yeast is capable of directly fermenting maltose. They claim that direct fermentation is likely even when the yeast employed contains demonstrable amounts of maltase. Direct fermentation is inferred by the disparity between the rate of maltose fermentation and the maltase content. This would be a convincing argument if it could be shown that maltase can be recovered completely from yeast and tested at maximum activity. Kriebel, Skau and Lovering³ have studied maltase extraction and have shown that Willstätter's method of extraction is by no means quantitative. Thus it can be seen that the theory of direct fermentation of maltose rests on uncertain ground. More recently Blish and Sandstedt⁴ have undertaken the study of maltose fermentation. They have called attention to the fact that it is possible to stimulate the fermentation of maltose by the addition of various substances. They conclude that their activators are not connected with maltase activity and therefore must be considered as activating the direct fermentation of maltose.

The most active of Blish's activators is a dried

baker's yeast. The addition of this material to a mixture of pure maltose and baker's yeast markedly reduces the induction period. Blish, however, reports no test for maltase in this activator. The senior author of the present communication had found (unpublished data obtained in 1928) that an extract of dried yeast would reduce the maltose induction period. His extract was made according to the method of Kriebel, Skau and Lovering³ for making a maltase extract. If maltase is responsible for the remarkable decrease in the induction period, we might then expect the fermentation technique to provide a very sensitive test for the enzyme. To test this hypothesis a sample of baker's yeast was dried and an extract of it made. This extract was very active in promoting the fermentation of maltose. A series of experiments was then undertaken to prove that the responsible agent or activator was maltase. Other points of interest were discovered as a result of this work and are herein reported.

Experimental

Maltase in Dried Yeast.—Baker's yeast was passed through a coarse sieve and then dried before a fan at room temperature. Following the procedure recommended by Kriebel, *et al.*,³ 50 g. of the dried yeast was thoroughly suspended in 300 cc. of 5% $Na_2HPO_4 \cdot 12H_2O$. After storing at 18° for twenty hours the supernatant fluid was separated by means of the centrifuge and filtered. This yielded an opalescent solution which was neutralized to pH 6.5–6.7 by means of a 4% solution of potassium dihydrogen phosphate. Forty ml. of this extract represented 3 g. of the original dried yeast.

(1) R. Willstätter and E. Bamann, *Z. physiol. Chem.*, **152**, 202 (1926).

(2) H. Sobotka and M. Holzman, *Biochem. J.*, **28**, 734 (1934).

(3) V. K. Kriebel, E. L. Skau and E. W. Lovering, *THIS JOURNAL*, **49**, 1728 (1927).

(4) M. J. Blish and R. M. Sandstedt, *J. Biol. Chem.*, **118**, 765 (1937).