[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC., AND FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Mold Metabolites. II. The Structure of Sorbicillin, a Pigment Produced by the Mold *Penicillium notatum*

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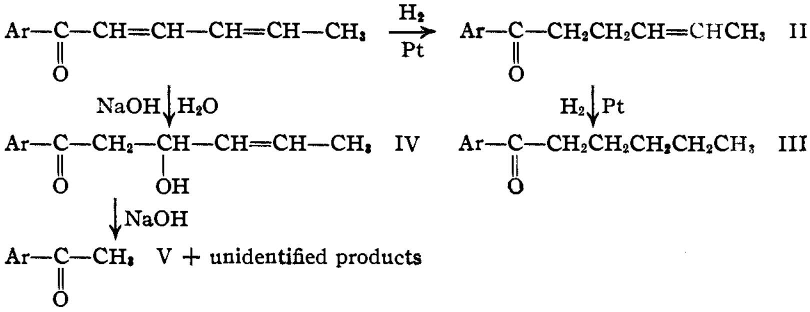
In the first paper of this series[2] there was reported the isolation of several compounds from clinical penicillin, among which was a substance designated as pigment III. A sufficient quantity (4.5 g.) of this compound was obtained to carry out degradative experiments which have led to an elucidation of its structure. The name "sorbicillin" is suggested for the mold metabolite.

This pigment ($C_{14}H_{16}O_3$, I) has previously been shown[2] to contain one phenolic hydroxyl group (sorbicillin forms a monomethyl ether, contains two active hydrogens and titrates as a monobasic acid of a strength comparable to phenol). The presence of a second was suggested by the acidity of the monomethyl ether and by the two active hydrogens present in sorbicillin. Although the pigment gave none of the reactions characteristic of an aldehyde or ketone, this fact did not eliminate the presence of a ketonic group that was strongly chelated with one of the hydroxyl groups.

Oxidation of sorbicillin with hydrogen peroxide in alkaline solution (Dakin reaction) gave as products sorbic acid and unidentified phenolic residues. This type of reaction[3] is characteristic of *o*- or *p*-hydroxyacylbenzenes, and in the present case it seemed probable that one of the hydroxyl groups was ortho to the acyl groups (this hypothesis explained the lack of reactivity of both the carbonyl and one of the hydroxyl groups).

Sorbicillin when reduced with hydrogen (platinum catalyst) in an alkaline medium produced two white products, a dihydro derivative ($C_{14}H_{18}O_3$, II) and a tetrahydro derivative ($C_{14}H_{20}O_3$, III). The pigment proved to be stable to acid hydrolysis, but treatment with boiling aqueous alkali gave a mixture of two white compounds, IV ($C_{14}H_{18}O_4$) and V ($C_{10}H_{12}O_3$). The ultraviolet absorption spectra of II, III, IV and V were determined, and Table I records the maxima and the logarithms of the molecular extinction coefficients of these substances in both neutral and basic medium. Figure 1 records the detailed spectrum of V.

The marked similarity of spectra of II, III, IV and V indicated that the conjugated system responsible for the absorption was the same in each compound, and suggested that the reactions giving rise to these compounds involved the destruction of the conjugated system of double bonds in the side chain of sorbicillin. This hypothesis led to the following formulations of the hydrogenation and hydrolysis of sorbicillin.



$$\mathrm{Ar{-}\underset{\underset{O}{\|}}{C}{-}CH{=}CH{-}CH{=}CH{-}CH_3 \xrightarrow[Pt]{H_2} Ar{-}\underset{\underset{O}{\|}}{C}{-}CH_2CH_2CH{=}CHCH_3 \quad II}$$

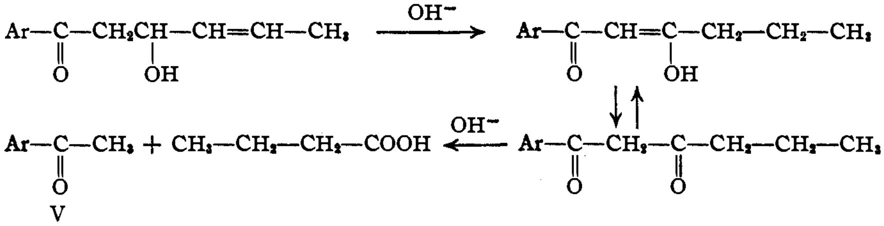
$$\mathrm{NaOH}\downarrow\mathrm{H_2O} \qquad\qquad \mathrm{H_2}\downarrow\mathrm{Pt}$$

$$\mathrm{Ar{-}\underset{\underset{O}{\|}}{C}{-}CH_2{-}\underset{\underset{OH}{|}}{CH}{-}CH{=}CH{-}CH_3 \quad IV \qquad Ar{-}\underset{\underset{O}{\|}}{C}{-}CH_2CH_2CH_2CH_2CH_3 \quad III}$$

$$\downarrow\mathrm{NaOH}$$

$$\mathrm{Ar{-}\underset{\underset{O}{\|}}{C}{-}CH_3 \quad V + \text{unidentified products}}$$

Compound IV is written with the hydroxyl group on the third carbon atom because when IV was converted to V, a strong odor of butyric acid was evident. This acid probably arose from the series of steps



$$\mathrm{Ar{-}\underset{\underset{O}{\|}}{C}{-}CH_2\underset{\underset{OH}{|}}{CH}{-}CH{=}CH{-}CH_3 \xrightarrow{OH^-} Ar{-}\underset{\underset{O}{\|}}{C}{-}CH{=}\underset{\underset{OH}{|}}{C}{-}CH_2{-}CH_2{-}CH_3}$$

$$\downarrow\uparrow$$

$$\mathrm{\underset{V}{Ar{-}\underset{\underset{O}{\|}}{C}{-}CH_3} + CH_3{-}CH_2{-}CH_2{-}COOH \xleftarrow{OH^-} Ar{-}\underset{\underset{O}{\|}}{C}{-}CH_2{-}\underset{\underset{O}{\|}}{C}{-}CH_2{-}CH_2{-}CH_3}$$

Compound IV probably arose by a Michael-like 1,4-addition of water to the α,β-unsaturated ketone. The addition must have taken place 1,4 and not 1,6 because IV and V have the same absorption spectrum. Compound II is also written with the double bond out of conjugation because it also has an absorption spectrum almost identical to that of V.

In connection with the structure of V, 2,4-dihydroxyacetophenone was used as a model. The ultraviolet absorption spectra (see Fig. 2) of the two substances are quite similar, and this fact coupled with the presence of three C-methyl groups[2] in sorbicillin led to consideration of structures A, B and

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[2] THIS JOURNAL, **70**, 4238 (1948).

[3] A good example of the use of this reaction is found in the work of Cruickshank, Raistrick and Robinson [*J. Chem. Soc.*, 2057 (1938)] on the elucidation of the structure of auroglaucin.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA DATA ON DEGRADATION PRODUCTS OF SORBICILLIN

Compound	1st Maxima (mμ)	1st Maxima log ϵ_m	2nd Maxima (mμ)	2nd Maxima log ϵ_m
In Absolute Ethanol				
II	285	4.152	322	3.758
III	285	4.168	328	3.762
IV	285	4.114	321	3.741
V	285	4.126	330	3.697
In 50% Ethanol–50% 0.05 *N* Sodium Hydroxide Solution				
II	256	3.888	351	4.490
III	253	3.813	350	4.490
IV	255	3.930	352	4.476
V	255	3.824	348	4.475

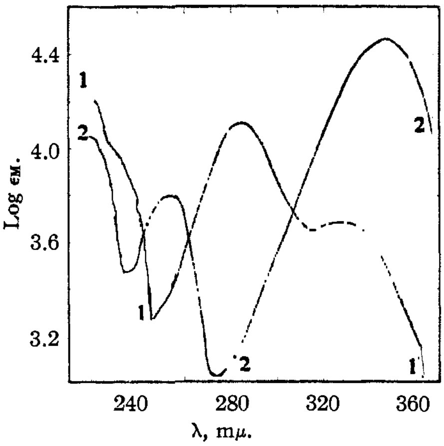
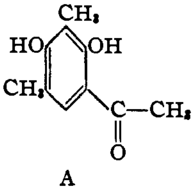
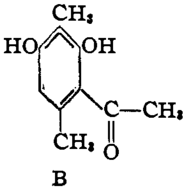


Fig. 1.—Ultraviolet absorption spectra of compound V: curve 1, in absolute ethanol; curve 2, in 50% ethanol–50% 0.05 *N* sodium hydroxide.

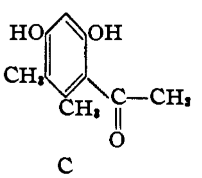
C for compound V. These three compounds were synthesized, and A proved to be identical in all



A



B



C

respects with V. It is interesting to note that A is a known compound, having been reported[4] while the synthetic work was in progress. Hassall and Todd isolated the material from the culture liquors of the mold, *Aspergillus clavatus*, and gave the compound the name of clavatol. They synthesized clavatol by a route different from that reported here.

The intermediate 2,4-dihydroxy-5-methylacetophenone was prepared by a method slightly different from that employed by Yanagita[5] for the same substance (in the present synthesis 2,4-dimethoxybenzaldehyde was refluxed with hydrazine and alkali,[6] followed by demethylation of the product to give 4-methylresorcinol, which was subsequently C-acetylated to produce 2,4-dihydroxy-5-methylacetophenone). Treatment of this substance with methyl iodide and alkali under mild conditions produced 2,4-dihydroxy-3,5-dimethylacetophenone (A).[7] Mixed melting point determinations of A and V as well as of their respective diacetates confirmed their identity.

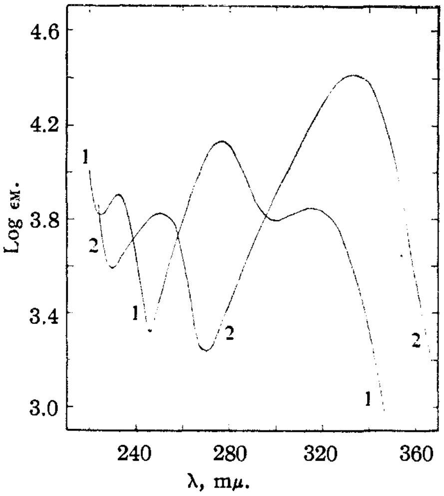
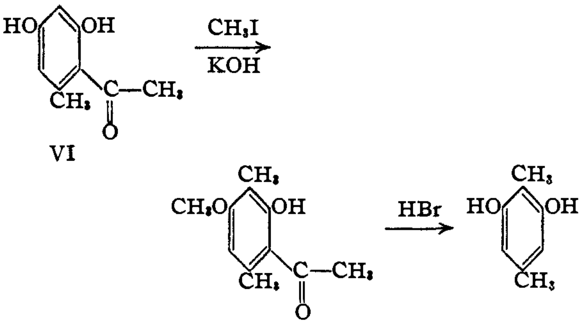


Fig. 2.—Ultraviolet absorption spectra of 2,4-dihydroxyacetophenone: curve 1, in absolute ethanol; curve 2, in 50% ethanol–50% 0.05 *N* sodium hydroxide.

Compound B was synthesized by a similar nuclear methylation of 2,4-dihydroxy-6-methylacetophenone (VI), which was prepared by the method reported by Hoesch.[8] When the Robinson[7] procedure was used for the alkylation, a low yield of 2-hydroxy-3,6-dimethyl-4-methoxyacetophenone (VII) was obtained. Attempts to cleave the methoxyl group with 48% hydrobromic acid led to deacetylation as well as demethylation of the molecule, and 2,5-dimethylresorcinol (VIII) was isolated in good yields.



VI

VII

VIII

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[4] Hassall and Todd, *J. Chem. Soc.*, 611 (1947).

[5] Yanagita, *Ber.*, **71**, 2271 (1938).

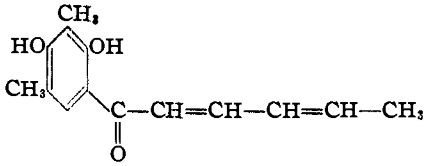
[6] This procedure is similar to the one developed by Huang-Minlon [THIS JOURNAL, **68**, 2487 (1946)] for the reduction of similar substances.

[7] Nuclear alkylations of resacetophenone-like compounds have been carried out by Robinson and Shah [*J. Chem. Soc.*, 1491 (1934)], but in their work the products were methyl ethers of the alkylated compounds. In the present synthesis the secondary O-methylation reaction was eliminated by the use of a lower reaction temperature.

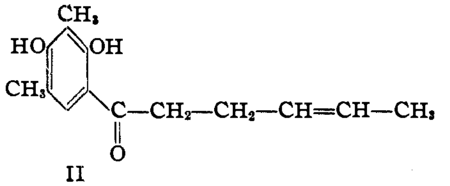
[8] Hoesch, *Ber.*, **48**, 1127 (1915).

Compound C was synthesized through the action of acetonitrile, dry hydrogen chloride and zinc chloride on 4,5-dimethylresorcinol, which had been prepared by methylation and reduction of 2,4-dihydroxy-6-methylbenzaldehyde[9] (with hydrazine and alkali) followed by demethylation of the two methoxyl groups.

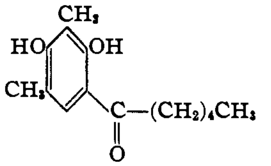
The above evidence now makes it possible to formulate with a considerable amount of assurance the structures of sorbicillin (I), II, III and IV, as well as V.



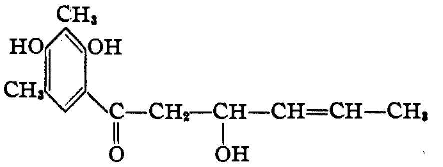
I



II

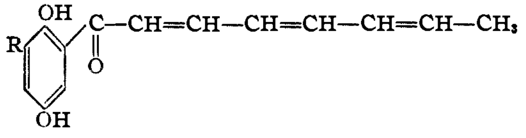


III



IV

It is of interest to note the relationship of structure between sorbicillin and the mold metabolite, auroglaucin, which Gould and Raistrick[10] isolated from the mold *Aspergillus glaucus* (the nature of R has not been definitely determined).



Experimental

Oxidation of Sorbicillin.—To a solution of 1.5 g. of sorbicillin in 10 ml. of 0.1 *N* sodium hydroxide was added dropwise a 10% solution of hydrogen peroxide until the color was discharged (a total of 3 ml. was added over a period of one-half hour). The solution was then warmed to 60°, cooled, acidified and extracted with ether. The ether solution was extracted with a dilute solution of sodium bicarbonate, which was subsequently acidified and extracted with ether. The ether extract was dried, evaporated to a small volume, passed through a small column of acid-washed alumina, again concentrated to a small volume and cooled. The product that separated was recrystallized from methanol and water to yield 200 mg. of white needles, m.p. 131–132°, not depressed by admixture with an authentic sample of sorbic acid.

Anal.[11] Calcd. for $C_6H_8O_2$: C, 64.27; H, 7.19; neut. equiv., 112. Found: C, 64.27; H, 7.24; neut. equiv., 112.

There was a considerable amount of a low-boiling acid produced in the reaction with an odor reminiscent of acetic acid. Attempts to isolate other products failed, although the reaction was run under a variety of conditions.

Reduction of Sorbicillin.—Sorbicillin (500 mg.) was dissolved in 100 ml. of 0.1 *N* sodium hydroxide solution, 50 mg. of platinum oxide was added, and the mixture was shaken in an atmosphere of hydrogen until colorless (twenty minutes). The solution was filtered from the catalyst, acidified and extracted with ether. After the ether layer was dried and evaporated to a small volume, addition of low-boiling petroleum ether produced 150 mg. of white crystals, m.p. 67–92°. This solid material was dissolved in ether and submitted to chromatographic absorption and elution on acid washed alumina. Ether was used as the developing solvent, and two fractions of the chromatogram filtrate were taken (the filtrate was tested periodically for solute with ferric chloride, which indicated where the fractions should be cut). The first fraction gave 50 mg. of solid when the solvent was evaporated. Three recrystallizations of the substance from an ether–petroleum ether mixture produced 30 mg. of white flakes (II), m.p. 146–147°. A solution of the compound in an ethanol–water mixture gave a purple-black color with a trace of ferric chloride. The substance is soluble in alkali but insoluble in water and soluble in all organic solvents except petroleum ether.

Anal. Calcd. for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74; neut. equiv., 234. Found: C, 71.86, 72.02; H, 7.67, 786; neut. equiv., 216 (potentiometric titration against a hydrogen electrode).

The second fraction from the chromatogram when evaporated produced 80 mg. of solid, which after three recrystallizations from an ether–petroleum ether mixture gave 25 mg. of white needles (III), m.p. 69–70°. The solubilities and color reactions of this substance were similar to those of II.

Anal. Calcd. for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53; neut. equiv., 236. Found: C, 71.21, 71.03; H, 8.47, 8.59; neut. equiv., 238 (potentiometric titration against a hydrogen electrode).

Hydrolysis of Sorbicillin.—A mixture of 1.0 g. of pigment (I) and 100 ml. of 2*N* sodium hydroxide solution was refluxed for two hours. At the end of this period the solution was cooled, acidified and extracted with ether. The ether layer was dried, evaporated to a small volume and run through a small column of acid washed alumina. The column filtrate produced on evaporation 250 mg. of white solid, which when recrystallized once from methanol and water and twice from a mixture of glacial acetic acid and petroleum ether yielded 30 mg. of white flakes (IV), m.p. 187–188°. This substance showed the same solubility properties and color reactions as II and III.

Anal. Calcd. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25; mol. wt., 250; neut. equiv., 250. Found: C, 67.45, 67.15; H, 7.34, 7.46; mol. wt., 246 (in camphor); neut. equiv., 243 (potentiometric titration against a hydrogen electrode).

The filtrates from the crystallizations of IV were combined, evaporated to dryness and sublimed at 110° under high vacuum to produce 125 mg. of white solid, m.p. 150–165°. Two recrystallizations of the substance from xylene yielded 40 mg. of white prisms (V), m.p. 177–178°. The color reactions and solubilities of this compound were similar to those of II, III and IV.

Anal. Calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71; neut. equiv., 180; mol. wt., 180. Found: C, 66.67, 66.53;

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[9] This substance was prepared by the procedure of Gattermann, *Ann.*, **357**, 371 (1907).

[10] Gould and Raistrick, *Biochem. J.*, **28**, 1640 (1934).

[11] The analyses were carried out by Melton Burney.

H, 6.99, 6.52; neut. equiv., 188 (potentiometric titration against a hydrogen electrode); mol. wt., 160 (in camphor).

The diacetate was prepared by heating 3 mg. of V in 1 ml. of acetic anhydride and a trace of pyridine, flooding the mixture with water, and recrystallizing the precipitate from methanol and water, m.p. 96–97°.

Synthesis of 2,4-Dihydroxy-3,5-dimethylacetophenone (V).—Formylation of resorcinol with zinc cyanide and dry hydrogen chloride gas in ether solution gave a 55% yield of 2,4-dihydroxybenzaldehyde, m.p. 134–135° (lit.[12] 135–136°). Methylation of this phenol with dimethyl sulfate and alkali in a solution of methanol in the usual manner produced a yield of 75% of 2,4-dimethoxybenzaldehyde, m.p. 67–68° (lit.[9] 71°).

The reduction of this substance with hydrazine was carried out as follows. A mixture of 30 g. of the dimethyl ether, 30 g. of potassium hydroxide, 24 ml. of hydrazine hydrate and 230 ml. of triethylene glycol was boiled under reflux for one hour, and the temperature was then raised to 185° for four hours (the apparatus was arranged for distillation at the end of the reflux period). The mixture was cooled, the distillate combined with the material in the distillation flask, an equal volume of water added and the whole extracted with ether. The ether layer was washed first with water, then with dilute hydrochloric acid, dried, evaporated to dryness and distilled; yield 82%, b.p. 104–105° at 12 mm. This material was demethylated by heating under reflux with 48% hydrobromic acid for four hours to give an 82% yield (based on the dimethyl ether) of 2,4-dihydroxytoluene, m.p. 104–105° (lit.[13] 104–105°).

Acetylation of 2,4-dihydroxytoluene with zinc chloride and acetic acid according to the procedure of Yanagita[5] produced a yield of 46% of 2,4-dihydroxy-5-methylacetophenone, m.p. 170–171° (lit.[5] 170°).

The following procedure gave the maximum yield of 2,4-dihydroxy-3,5-dimethylacetophenone. Solid potassium hydroxide (2.24 g.) was dissolved in 13 ml. of dry methanol, the solution cooled and mixed with 1.55 g. of 2,4-dihydroxy-5-methylacetophenone and 7.1 g. of methyl iodide. The flask containing the mixture was tightly stoppered and allowed to stand in an ice-chest overnight. The solution was then poured into water, the mixture acidified, and the product that crystallized was recrystallized twice from ether to give 300 mg. (white needles) of 2,4-dihydroxy-3,5-dimethylacetophenone, m.p. 181–182° (lit.[4] 183°). This substance shows a slight variation in melting point depending on the solvent used for crystallization. Boiling xylene produces prisms, m.p. 178°, and methanol and water yields needles, m.p. 182–183°. A mixed melting point of the synthetic substance crystallized from xylene with V obtained by the degradation of sorbicillin gave 177–178°. The diacetate of the synthetic substance was prepared, m.p. 96–97 (lit.[4] 95–96°). A mixed melting point of the synthetic diacetate and that prepared from V showed no depression.

2,4-Dihydroxy-3,6-dimethylacetophenone (B).—The synthesis of 2,4-dihydroxy-6-methylacetophenone was carried out by the acetylation of orcinol according to the procedure of Hoesch.[8] A yield of 75% was obtained, m.p. 158–159° (lit.[8] 159°).

Nuclear methylation of 2,4-dihydroxy-6-methylacetophenone (by the procedure described above for the preparation of 2,4-dihydroxy-4,6-dimethylacetophenone) produced a yield of 27% of 2,4-dihydroxy-3,6-dimethylacetophenone, m.p. 149–150° (white plates from methanol and water). A solution of this substance in a 50% methanol-water mixture gave a purple-black color with a trace of ferric chloride. Its solubility characteristics were similar to V.

Anal. Calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.52; H, 6.88.

When the nuclear methylation of 2,4-dihydro-6-methylacetophenone was carried out by the procedure of Robinson and Shah,[7] the compound 2-hydroxy-3,6-dimethyl-4-methoxyacetophenone was isolated, yield 15%, m.p. 90–91° (white needles from glacial acetic acid).

Anal. Calcd. for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.82; H, 7.23.

In an attempt to demethylate the methoxyl group of the above compound, 100 mg. was refluxed with 48% hydrobromic acid for four hours. The solution was cooled, diluted with water and extracted with ether. The ether solution was dried, treated with charcoal, filtered, evaporated to a low volume and cooled. The product that separated was recrystallized from methanol and water to give prisms of 2,5-dimethylresorcinol, m.p. 161–162° (lit.[14] 163°). Preparation of the diacetate by the usual procedure gave needles (from methanol and water) m.p. 69° (lit.[14] 69°).

2,4-Dihydroxy-5,6-dimethylacetophenone (C).—Formylation of orcinol with zinc cyanide and dry hydrogen chloride gas in ether solution by a procedure similar to that reported by Gattermann[9] produced a yield of 71% of 2,4-dihydroxy-6-methylbenzaldehyde, m.p. 181–182° (lit.[9] 181–182°). Methylation of this substance with dimethyl sulfate and alkali in the usual manner gave a 94% yield of 2,4-dimethoxy-6-methylbenzaldehyde, m.p. 64–65° (lit.[9] 65°). This compound was reduced with hydrazine and potassium hydroxide by the procedure described above for the conversion of 2,4-dimethoxybenzaldehyde to 2,4-dimethoxytoluene. A yield of 76% of 3,5-dimethoxy-*o*-xylene was obtained, b.p. 118–119° (at 11 mm.), and this compound was demethylated with 48% hydrobromic acid to give a 94% yield of 2,4-dihydroxy-*o*-xylene, m.p. 134–135° (lit.[15] 136–137°).

The preparation of 2,4-dihydroxy-5,6-dimethylacetophenone was carried out by the following procedure. Dry hydrogen chloride gas was passed into a stirred mixture of 3 g. of 3,5-dihydroxy-*o*-xylene, 1.7 g. of acetonitrile, 1.3 g. of zinc chloride and 25 ml. of dry ether. After the mixture had been saturated with gas, it was allowed to stand for two hours, the ether was decanted, 20 cc. of water was added, the mixture was boiled for ten minutes and cooled. Recrystallization of the product from methanol and water gave 2.3 g. of white needles, m.p. 155–156°. Further recrystallization of the substance did not alter its melting point. A solution of this phenol in a 50% methanol–water mixture turned black when a trace of ferric chloride was added.

Anal. Calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.43; H, 6.70.

Acknowledgment.—The author takes pleasure in expressing appreciation to Dr. R. B. Woodward of Harvard University for suggestions regarding this investigation, and to Drs. Max Tishler and R. T. Major of Merck & Co., Inc., for their encouragement and for a supply of sorbicillin.

Summary

The structure of the mold pigment sorbicillin has been elucidated through its degradation and the synthesis of its degradation products. The compound was shown to be 1-[2,4-dihydroxy-3,5-dimethylphenyl]-hexadiene-2,4-one-1. Procedures for the synthesis of 2,4-dihydroxy-3,5-dimethylacetophenone, 2,4-dihydroxy-3,6-dimethylacetophenone and 2,4-dihydroxy-5,6-dimethylacetophenone have been described.

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(12) Shoesmith and Haldane, *J. Chem. Soc.*, **123**, 2704 (1923).

(13) Neville and Winther, *Ber.*, **15**, 2981 (1882).

(14) Kostanecki, *ibid.*, **19**, 2321 (1886).

(15) Herzig, Wenyel and Hornstein, *Monatsh.*, **27**, 795 (1906).