The Synthesis of Euparin and Dehydrotremetone

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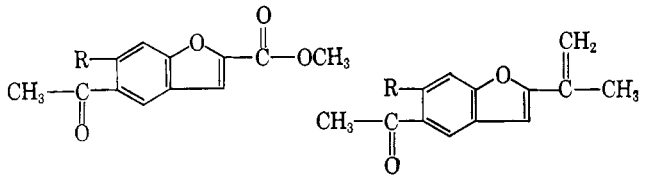
Euparin (III) has been synthesized from methyl 5-acetyl-6-hydroxycoumarilate (I) *via* the O-acetate, the ethylene ketal, and the readily dehydrated tertiary alcohol obtained by reaction of methylmagnesium iodide with the latter. Acetylation of methyl coumarilate gave methyl 5-acetylcoumarilate (IV) as shown by conversion to 5-ethylcoumarilic acid and independent synthesis of this latter compound. Dehydrotremetone (VII) was obtained by addition of methylmagnesium iodide to the ketal of IV followed by dehydration and removal of the ketal group.

The structure of euparin (III), a yellow substance from *Eupatorium purpureum*, was determined by Kamthong and Robertson,[1] who isolated the material from the roots of the plant. It subsequently has been isolated from *E. cannabinum*[2] and from *E. japonica*.[3]

In an investigation of the point of attack in the boron trifluoride-catalyzed acetylation of isomeric methyl methoxycoumarilates,[4] it was found that methyl 6-methoxycoumarilate was acetylated at 80° to yield methyl 5- and 7-acetyl-6-hydroxycoumarilates which could be conveniently separated by fractional crystallization. Milder conditions gave methyl 5- and 7-acetyl-6-methoxycoumarilates which were not easily separable.



I, R = HO
II, R = CH_3CO

III, R = HO
VII, R = H

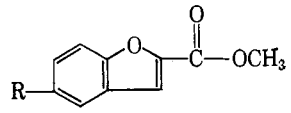
IV, R = H

The availability of I, therefore, suggested the preparation of euparin (III). The O-acetate II formed a ketal with ethylene glycol followed apparently by loss of the acetyl group, since attempts to form the ketal from I were not successful. The ketal with excess methylmagnesium iodide gave a tertiary alcohol which easily lost water to form the isopropylene group. Chromatography on acid washed alumina cleaved the ketal to produce euparin (III) directly. The ease of dehydration of the tertiary alcohol is in contrast to the more difficult dehydration of 2-(5′-acetyl-2′,3′-dihydro-2′-benzofuryl)-2-propanol.[5]

The synthetic euparin melted at 121–122° and formed a semicarbazone, a dinitrophenylhydrazone, and a maleic anhydride adduct all in agreement with the published data.[1,2] The green color with ferric chloride and the color pattern with concentrated sulfuric acid were those reported.[1]

Early work on the acetylation of esterified coumarilates revealed that ethyl coumarilate, in the only reported instance of acylation in the benzenoid ring of a benzofuran, had been acetylated to yield ethyl 5-acetylcoumarilate.[6] We found that methyl coumarilate was not attacked by acetic acid–acetic anhydride–boron trifluoride[7] and at higher temperatures, tars were produced. With aluminum chloride–acetyl chloride, methyl 5-acetylcoumarilate (IV) was obtained. The attack at the 5-position agreed with other reports[8] on the reaction of electrophilic reagents with methyl coumarilate. The assigned structure was confirmed by conversion of the oxime of IV to methyl 5-acetylaminocoumarilate, hydrolysis of the N-acetyl group to the 5-amino compound followed by diazotization, and treatment with cuprous chloride to yield the known methyl 5-chlorocoumarilate.[9] The catalytic reduction of IV to V gave further support to the structure of IV in that comparison was made with a sample of V whose structure was established by synthesis. For this purpose, *p*-ethylphenol was converted, although in poor yield, to 5-ethyl-2-hydroxybenzaldehyde which gave V when allowed to react with ethyl bromomalonate.[10]

The ethylene ketal VI of IV gave a tertiary alcohol after reaction with excess methylmagnesium iodide. This was both cleaved and dehydrated with *p*-toluenesulfonic acid to dehydrotremetone (VII). The synthetic material gave an infrared curve identical to dehydrotremetone obtained from rayless goldenrod, *Aplopappus heterophyllus*,[11] and to dehydrotremetone from white snakeroot, *Eupatorium urticaefoleum*.[12] Mixtures with samples from each of these sources melted without depression. The mixture of the oxime of our dehydrotremetone with the oxime of that from *E. urticaefolium* melted undepressed.



V, R = C_2H_5
VI, R = CH_3—C(O)(O)—

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(12) (a) J. I. DeGraw and W. A. Bonner, *Tetrahedron*, **18**, 1295 (1962); (b) W. A. Bonner, J. I. DeGraw, D. M. Bowen, and V. R. Shah, *Tetrahedron Letters*, **12**, 417 (1961); (c) J. I. DeGraw and W. A. Bonner, *J. Org. Chem.*, **27**, 3917 (1962).

The acetylation of V with acetyl chloride–aluminum chloride gave a monoacetyl compound to which is assigned the structure methyl 7-acetyl-5-ethylcoumarilate without proof. This is analogous to the structure suggested for the product of chloromethylation of methyl 5-methylcoumarilate.[13]

Experimental[14]

Methyl 5-Acetyl-6-hydroxycoumarilate Ethylene Acetal.—The O-acetate II of methyl 5-acetyl-6-hydroxycoumarilate[4] (I) formed colorless flakes from methanol, m.p. 132.8–133.6°. The ferric chloride test was negative; λ_{max}^{EtOH} 244, 271, 336 mμ (ε 3800, 17,600, 17,800); λ^{CCl_4} 5.65, 8.07 (acetate), 5.95 μ.

Anal. Calcd. for $C_{14}H_{12}O_6$: C, 60.87; H, 4.38. Found: C, 60.64; H, 4.65.

A gram of this acetate II after 4 hr. in refluxing benzene containing 1 ml. of ethylene glycol and 250 mg. of *p*-toluenesulfonic acid gave 0.8 g. of the ketal which gave a green ferric chloride test. Crystallization from methanol gave material, m.p. 166.0–167.2°; λ_{max}^{EtOH} 229, 262 mμ (ε 10,800, 18,600); λ^{CCl_4} 5.75, 5.80 μ (ester) (no bands at 5.95 and 8.07 μ).

Anal. Calcd. for $C_{14}H_{14}O_6$: C, 60.43; H, 5.07. Found: C, 59.98; H, 4.86.

Attempted ketalization without prior formation of the O-acetate gave only starting material.

5-Acetyl-6-hydroxy-2-isopropenylbenzofuran (Euparin) (III).—A solution of 2.0 g. of the ester ketal in 200 ml. of benzene was added slowly over a 0.5-hr. period to methylmagnesium iodide prepared from 1.6 g. of magnesium and 10 g. of methyl iodide in 20 ml. of anhydrous ether. After stirring for 2 hr. and allowing to stand overnight, the reaction mixture was decomposed by the cautious addition of saturated ammonium chloride solution. The residue on removal of the solvent, after the usual work-up, was eluted from acid-washed alumina with benzene producing 600 mg. of a bright yellow solid, m.p. 116–117°, raised to m.p. 121–122° by crystallization from methanol (lit.[1] m.p. 118.5–121°, lit.[2b] 121–122°); λ_{max}^{EtOH} 263, 358 mμ (ε 34,400, 5900); λ^{CCl_4} 6.10, 7.80, 8.00, 9.18 w, 11.45, 11.60 μ. The compound gave a green ferric chloride test and an orange color with concentrated sulfuric acid which changed through red and brown to green as reported.[1,2a] The compound gave a deep yellow color in aqueous sodium hydroxide, removed by acidification as reported.[2a]

Anal. Calcd. for $C_{13}H_{12}O_3$: C, 72.21; H, 5.60. Found: C, 72.08; H, 5.93.

The 2,4-dinitrophenylhydrazone, recrystallized from ethyl acetate, melted at 254–255° (lit.[1] m.p. 252°) and the semicarbazone from methanol melted at 244–245° (lit.[1] m.p. 245°).

Methyl 5-Acetylcoumarilate (IV).—A solution of 7.04 g. of methyl coumarilate, m.p. 54–55°,[15] in 180 ml. of carbon disulfide and 40 ml. of acetyl chloride was treated with 28 g. of anhydrous aluminum chloride over a 30-min. period during which time the temperature was held below 5°. The mixture was then refluxed with stirring for 24 hr., cooled, the carbon disulfide decanted, and the residual complex decomposed with ice and hydrochloric acid. Crystallization from benzene gave 4.3 g. (50%), m.p. 159–162°. Further purification and sublimation *in vacuo* gave material, m.p. 161.5–163.2°; λ_{max}^{EtOH} 246, 300 mμ (ε 47,000, 12,700); λ^{CHCl_3} 5.80, 5.97 μ.

Anal. Calcd. for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 65.83; H, 4.83.

Attempted acetylation in acetic acid–acetic anhydride with boron trifluoride at 80°[7] gave only starting material.

The oxime of IV formed colorless needles from methanol, m.p. 181.0–181.8°.

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.79; H, 4.76. Found: C, 61.91; H, 4.81.

Methyl 5-Aminocoumarilate.—The oxime IV (150 mg.) in 55 ml. of benzene with 150 mg. of phosphorus pentachloride was refluxed on the steam bath for 45 min. The solvent was removed and water was added to produce 135 mg. (90%) of compound m.p. 155–159°. From benzene–petroleum ether (b.p. 90–120°) colorless needles, m.p. 161.2–162.4°, were obtained.

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.79; H, 4.76. Found: C, 61.88; H, 4.73.

One gram of the acetylamino compound in 10% hydrogen chloride in methanol refluxed for 2 hr. on the steam bath gave 810 mg. of the hydrochloride of the amine, m.p. 274° dec. The amine obtained by careful addition of 5% sodium hydroxide weighed 750 mg. (91%), m.p. 110–114°. From methanol–water (1:5) colorless needles, m.p. 111.0–111.8°, were obtained.

Anal. Calcd. for $C_{10}H_9NO_3$: C, 62.82; H, 4.75. Found: C, 62.55; H, 4.89.

Methyl 5-Chlorocoumarilate.—A suspension of 383 mg. of the previously prepared amino compound in 10 ml. of concentrated hydrochloric acid was diazotized in the usual way and the solution was poured slowly into a boiling solution of cuprous chloride (from 625 mg. of copper sulfate) in 20 ml. of 6 *N* hydrochloric acid. When the evolution of nitrogen had ceased, 50 ml. of water was added, the solution was cooled, and the product obtained was recrystallized from petroleum ether (b.p. 60–90°). The colorless crystals weighed 25 mg., m.p. 94–96° (lit.[9] m.p. 96–97°).

5-Ethylcoumarilic Acid.—Hydrogenation of 1 g. of methyl 5-acetylcoumarilate over 75 mg. of platinum oxide in 40 ml. of acetic acid at a pressure slightly above atmospheric consumed two moles of hydrogen. After filtration and evaporation of the filtrate, an oil was obtained which was saponified. The acid obtained (0.91 g.) melted at 190–195° and formed colorless needles from aqueous methanol, m.p. 198.2–199.8°. These melted at 198.0–199.5° when mixed with the 5-ethylcoumarilic acid prepared subsequently; λ_{max}^{EtOH} 267, 299 mμ (ε 17,700, 4780).

Anal. Calcd. for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.81; H, 5.51.

5-Ethylcoumarilic Acid from 5-Ethyl-2-hydroxybenzaldehyde.—A solution of 40 g. of *p*-ethylphenol in sodium hydroxide treated with chloroform[16] gave 13 g. (26%) of the aldehyde, b.p. 112–115° (19 mm.); lit.[16] b.p. 115–116° (16 mm.).

The reaction product from 3.1 g. of this aldehyde, 5.0 g. of ethyl bromomalonate, and 2.5 g. of potassium carbonate in 10 ml. of methyl ethyl ketone[10] was saponified at once to give 3.0 g. (79%) of 5-ethylcoumarilic acid, m.p. 195–198°. Crystallization from benzene gave colorless needles, m.p. 198.0–199.5°; λ^{CHCl_3} 2.36, 5.89 μ, identical with the infrared spectra of 5-ethylcoumarilic acid prepared previously.

Methanolic hydrogen chloride gave methyl 5-ethylcoumarilate (V) (89%), b.p. 161–165° (15 mm.). The ester was a colorless solid melting at room temperature; λ_{max}^{EtOH} 274 mμ (ε 18,950); λ^{CCl_4} 5.73, 5.78 μ.

Anal. Calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.93. Found: C, 70.53; H, 5.91.

Methyl 7-Acetyl-5-ethylcoumarilate.—Acetylation of 1.9 g. of methyl 5-ethylcoumarilate in carbon disulfide with aluminum chloride as described in the previous preparation gave 0.92 g. of product (43%), m.p. 140–144°. Crystallization from benzene gave colorless needles, m.p. 145.3–146.3°.

Anal. Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 67.86; H, 5.86.

Methyl 5-Acetylcoumarilate Ethylene Acetal (VI).—A solution of 4.5 g. of IV, 1.34 g. of ethylene glycol, and 20 mg. of *p*-toluenesulfonic acid in 30 ml. of benzene was refluxed for 24 hr. with continuous separation of water. The product (5.2 g., 96%) melted at 101–110° and formed colorless needles after repeated crystallization from petroleum ether (b.p. 60–90°), m.p. 117.5–119.0°; λ_{max}^{EtOH} 273 mμ (ε 22,200); λ^{CCl_4} 5.75, 5.80 (no 5.97) μ.

Anal. Calcd. for $C_{14}H_{14}O_5$: C, 64.11; H, 5.38. Found: C, 64.44; H, 5.40.

Ethylene Ketal of 2-(5′-Acetyl-2′-benzofuryl)-2-propanol.—To a solution of methylmagnesium iodide prepared from 1.94 g. of magnesium and 11.36 g. of methyl iodide in 40 ml. of ether was added dropwise over a 30-min. period a solution of 2.10 g. of VI in 10 ml. of benzene and 20 ml. of ether. After stirring for 2 hr. at room temperature and decomposition of the complex with aqueous ammonium chloride, 1.5 g. of the carbinol, m.p. 90–94°, was obtained. This formed colorless needles from aqueous methanol; m.p. 94.0–95.0°; λ_{max}^{EtOH} 244, 284 mμ (ε 17100, 4080); λ^{CCl_4} 2.79, 2.90 μ.

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.58; H, 7.01.

Dehydrotremetone (VII).—A solution of 500 mg. of the previously described carbinol with 50 mg. of *p*-toluenesulfonic acid

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(14) Melting points of analytically pure compounds are corrected.

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in 25 ml. of benzene was refluxed for 10 min. on the steam bath. The bicarbonate-washed benzene solution on evaporation gave, after crystallization from aqueous methanol, 325 mg. (85%) of dehydrotremetone (VII), m.p. 83–85°. This, crystallized four times from the same solvent, gave material, m.p. 84.0–85.5° (lit.[12a] m.p. 87.5–88.5°); λ_{max}^{EtOH} 253, 280.5, 292.5 mμ (ε 39,100, 19,150, 14,900) [lit.[12a] λ_{max}^{EtOH} 252, 280, 292 mμ (ε 39,000, 19,000, 15,000)]. Mixed with a sample of VII isolated from *Aplopappus heterophyllus*,[11] m.p. 80–82° cor.; the mixture melted unchanged. The infrared spectra of the two materials were identical throughout, the chief bands being at 5.98, 7.00, 7.41, 7.74, 8.68, and 11.14 μ, all in carbon tetrachloride.

A mixture of this synthetic VII with a sample, m.p. 83.5–85.5° cor., from *Eupatorium urticaefolium*,[12a] melted at 83.5–85.0° cor. The infrared spectra of this natural dehydrotremetone[12a] in carbon tetrachloride was identical with the two curves obtained previously.

The oxime of dehydrotremetone, from aqueous methanol, melted at 131.5–133.5° (lit.[12a] m.p. 131–132°). A mixture of our oxime with the oxime of VII derived from *E. urticaefolium*[12a] melted at 132–133°.

The Dehydrotremetone–Maleic Anhydride Adduct.—A solution of 500 mg. of VII and 500 mg. of maleic anhydride in 25 ml. of benzene was refluxed for 10 hr. On cooling, 150 mg. of the maleic anhydride adduct, m.p. 200–207° dec., was obtained and a second crop by concentration of the mother liquor weighed 180 mg., m.p. 195–205° dec. Extensive recrystallization from aqueous acetone gave material melting at 205–210° dec., not improved by further recrystallization.

Anal. Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.72; H, 4.23.

5-Acetyl-2-isopropylbenzofuran.—The hydrogenation of 200 mg. of dehydrotremetone (VII) in 8 ml. of ethyl acetate with 50 mg. of 5% platinum–charcoal at room temperature and a pressure slightly above atmospheric consumed 1 mole of hydrogen in 2.5 hr. and gave, after sublimation at 60–70° (6 mm.), 180 mg. of colorless needles, m.p. 46–48°. Resublimation gave material for analysis; m.p. 47.5–48.5°; λ^{CCl_4} 5.93 μ (no band at 11.14 μ); λ_{max}^{EtOH} 236.5 mμ (ε 21,100); λ_{infl}^{EtOH} 255 mμ (ε 5720).

Anal. Calcd. for $C_{13}H_{14}O_2$: C, 77.20; H, 6.98. Found: C, 77.58; H, 7.04.

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The Structure and Biosynthesis of Nidulin[1,2]

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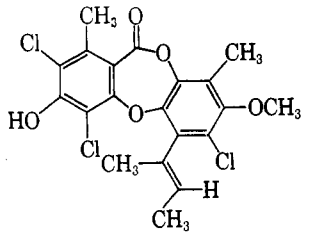
The structure of nidulin is confirmed by additional degradative evidence to be I. The biogenesis of this fungal metabolite is discussed and evidence is presented that it is derived from acetate and isoleucine.

In 1945, Kurung reported[5] that *Aspergillus ustus* produced a substance which was active *in vitro* in inhibiting the growth of *Mycobacterium tuberculosis* and *M. ranae*. Hogeboom and Craig[6] reported the isolation of two crystalline compounds from the same species of *Aspergillus*. Since these early reports much work on the metabolites of *A. ustus* (more properly now *A. nidulans*) has appeared,[7–12] and various partial structural proposals have been suggested.[8,10] Recently,[11,12] two complete structures for nidulin (I and II) have been advanced. Experiments described in the present paper revise the previous results which led to structure II and confirm structure I for nidulin.



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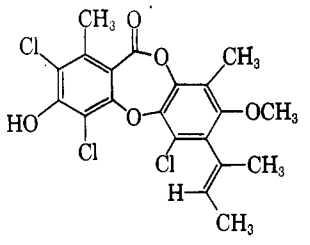
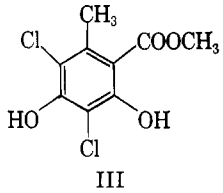


II

The structure of ring A of nidulin was established by the isolation of methyl 4,6-dichloro-*o*-sellinate (III)



III

as a degradation product of methyl nidulinate.[9] The presence of the depsidone nucleus was also deduced at an early date.[8] The nature of the five carbons attached to ring B was elucidated both by n.m.r. techniques[13] and by chemical degradations.[11]

The remaining question about the structure of nidulin concerns the distribution of groups about ring B. The assignment of structure I to nidulin[11] rested on the isolation of a dihydroxybenzoquinone derivative, $C_{11}H_{12}O_4$, obtained from nidulin by action of hydriodic acid on nidulin, followed by aerial oxidation of the resulting product in alkaline methanol. This substance was formulated as the 2,5-dihydroxybenzoquinone derivative, because its ultraviolet spectrum in neutral ethanol bears a close resemblance to that of 3-methyl-2,5-dihydroxybenzoquinone. The relative orientation of the two alkyl groups, the chlorine atom, and the oxygen substituent known to be disposed about ring B of

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(2) Presented at the 140th National Meeting of the American Chemical Society, Chicago, Ill., September, 1961.

(3) National Institute of Health Predoctoral Fellow.

(4) Alfred P. Sloan Fellow.

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