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Cercosporin. A Pigment of *Cercosporina Kikuchii Matsumoto et Tomoyasu.* III. The Nature of the Aromatic Ring of Cercosporin

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Zinc dust distillation and zinc dust fusion of cercosporin yielded a yellow hydrocarbon which showed an ultraviolet absorption spectrum characteristic of benzo[*ghi*]perylene. Oxidation of cercosporin with nitric acid gave mellitic acid in good yield. On Thiele's acetylation or alkaline hydrogen peroxide oxidation of tetramethylcercosporin, one of the two methoxyl groups which were originally present in cercosporin was demethylated and pentamethylnorcercosporin was produced. Permanganate oxidation of this compound yielded a lactone which on zinc dust distillation afforded benzo[*e*]pyrene. The nature of the cercosporin aromatic ring system and the location of the side chain and methoxyl group are discussed in the light of these observations. Photo-induced and aluminum chloride demethylation of cercosporin and its derivatives are also described.

Cercosporin, a pigment of *Cercosporina Kikuchii M. et T.* was studied spectroscopically and described as a polycyclic hydroxyquinone having an extended quinone system.[2] The present paper presents information concerning the nature of the cercosporin aromatic ring system and its substituents.

Zinc dust distillation of cercosporin and noranhydrocercosporin yielded a yellow hydrocarbon having an ultraviolet absorption spectrum that closely resembled that of benzo[*ghi*]perylene (Fig. 1). However, zinc dust distillation is a drastic

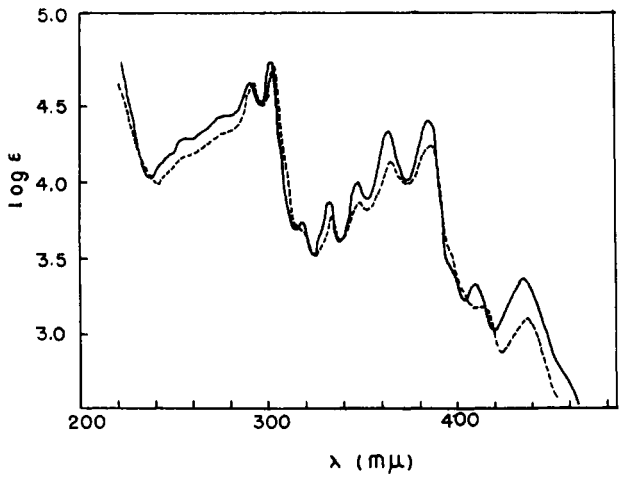

Fig. 1. Absorption spectra of benzo[*ghi*]perylene (———) and the product from zinc dust distillation of noranhydrocercosporin (– – – –)

procedure and frequently causes undesirable reactions such as ring fission or ring closure.[3] Therefore, zinc dust fusion which is a milder and more

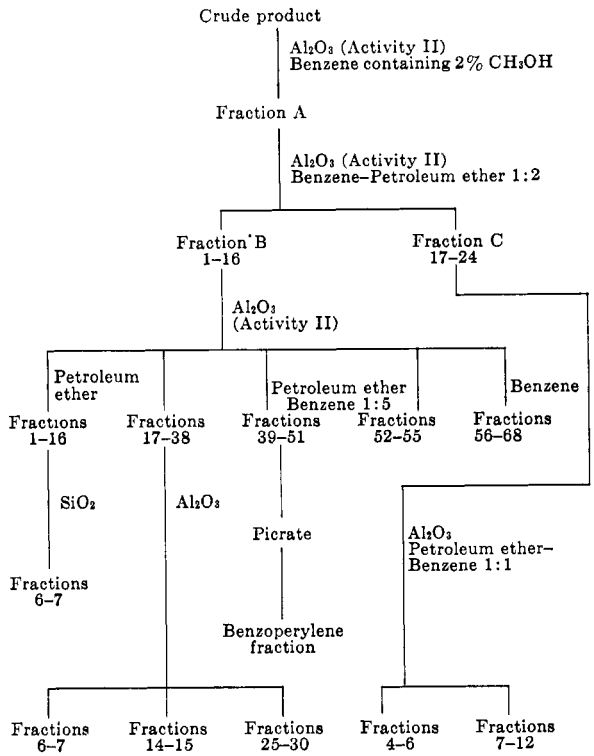

Fig. 2. Fractionation procedure for product of zinc dust fusion

suitable method than zinc dust distillation,[4] was employed for the conversion of the polycyclic quinone pigment cercosporin to its parent hydrocarbon. The product was fractionated by the process illustrated schematically in Fig. 2. The ultraviolet absorption spectrum of each fraction was determined but the only hydrocarbon detected was benzo[*ghi*]perylene. The production of mellitic acid in good yield by nitric acid oxidation of the pigment provided additional evidence that cercosporin contains a condensed ring system.

When correction was made for the bathochromic shift due to substituents, the ultraviolet and visible

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(2) S. Kuyama and T. Tamura, *J. Am. Chem. Soc.*, **79**, 5726 (1957).

(3) G. Kraemer, A. Spilker, and P. Eberhardt, *Ber. deut. chem. Ges.*, **23**, 3272 (1890); A. Spilker, *Ber. deut. chem. Ges.*, **26**, 1545 (1893).

(4) E. Clar, *Ber. deut. chem. Ges.*, **72**, 1645 (1939).

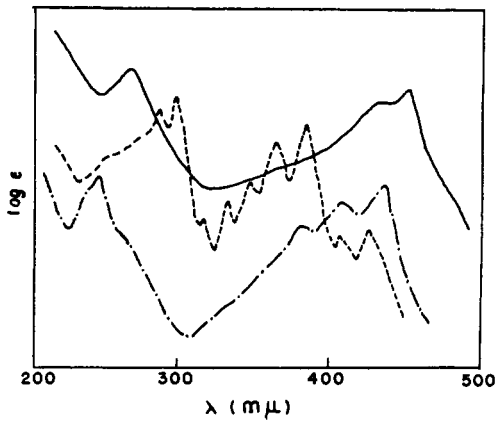

Fig. 3. Absorption spectra of hexaacetyldihydrocercosporin (———), benzo[*ghi*]perylene (– – – –), and perylene (- · - · - · -)

spectra of hexaacetyldihydrocercosporin were found to be similar to those of perylene but unlike those of benzo[*ghi*]perylene (Fig. 3). From the present findings and infrared data presented previously,[2] it is possible to conclude that cercosporin is a derivative of 4,9-dihydroxyperylene-3,10-quinone with one or more carbon side chains attached to the nucleus.

Because of cyclization during zinc dust distillation, it was not possible to determine the exact number and location of the side chain(s). There are many possible explanations for the formation of benzo[*ghi*]perylene by zinc dust distillation of cercosporin. The more probable of these will be tested and considered in a future report of this series.

Demethylation of cercosporin to yield a crystallizable product was not accomplished with hydrobromic or hydroiodic acid but the pigment was demethylated readily with anhydrous aluminum chloride in boiling benzene. The crystalline product had no methoxyl group; it was soluble in dilute sodium hydroxide and produced a deep blue color. Methylation of the product by means of dimethyl sulfate and potassium carbonate in acetone yielded dimethylcercosporin which was found to be identical with the product obtained by methylation of cercosporin. Apparently the two methoxyl groups were demethylated by anhydrous aluminum chloride in boiling benzene without rearrangement or other undesirable side reaction.

Acetylation of the demethylated product (norcercosporin) with acetic anhydride and pyridine yielded a hexaacetate which demonstrated stronger absorption at the phenolic acetate band of 1774 cm.$^{-1}$ than at the aliphatic acetate band of 1743 cm.$^{-1}$ Cercosporintetraacetate absorbed at 1776 and 1740 cm.$^{-1}$ but in this case the intensity of the aliphatic was greater than that of the phenolic band. These observations indicate that the two hydroxyl groups formed as a result of demethylation are either phenolic or enolic. However, the latter possibility seems the less likely since the two methoxyl groups were found to be extremely resistant to treatment with hydrochloric acid. The influence of demethylation of the bathochromic shift in the ultraviolet and visible spectra of cercosporin in neutral solution provided further evidence that the two methoxyl groups are attached to the perylene nucleus of the pigment.

If cercosporin contained a perylenequinone system and the quinoid rings were not substituted in at least two positions, the pigment would undergo several nucleophilic reactions characteristic of quinones, *i.e.*, amination with amines, addition of potassium cyanide or hydrogen chloride, and Thiele's acetylation. Perylene-3,10-quinone has an unsubstituted nucleus and when aminated forms the 1-substituted monoamine compound. However, oxidative hydrolysis of the product of Thiele's acetylation of perylene-3,10-quinone does not proceed as expected. The compound formed is 4,9-dihydroxyperylene-3,10-quinone rather than monohydroxyperylene-3,10-quinone.[5]

Thiele's acetylation and several attempts under various conditions to aminate cercosporin were not successful. No consistent product was obtained. However, Thiele's acetylation of tetramethylcercosporin followed by hydrolysis, yielded a product that had a strong hydroxyl band at 3400 cm.$^{-1}$ and which in aqueous sodium hydroxide produced a clear blue solution. The same compound was obtained also by treating an alkaline solution of tetramethylcercosporin with hydrogen peroxide and by passing air through a methanolic sodium hydroxide solution of tetramethylcercosporin. Acetylation of this compound yielded a product that absorbed at the acetate carbonyl band of 1760 cm.$^{-1}$, indicating that a phenolic hydroxyl group had formed on the nucleus. This reaction appeared similar to the hydrogen peroxide or atmospheric oxidation of perylene-3,10-quinone to 1-hydroxyperylene-3,10-quinone.[5] However, the compound obtained by oxidative hydrolysis of the product of Thiele's acetylation of tetramethylcercosporin had a molecular formula of $C_{33}H_{34}O_{10}$ rather than $C_{34}H_{34}O_{11}$ as was expected. Since methylation, by means of methyl iodide and silver oxide or dimethyl sulfate and potassium carbonate in acetone yielded the original compound (tetramethylcercosporin), it was concluded that under the conditions of Thiele's acetylation or treatment with hydrogen peroxide in alkali one of the methoxyl groups of tetramethylcercosporin was demethylated and pentamethylnorcercosporin was formed.

The methoxyl group liberated by Thiele's acetylation of tetramethylcercosporin was not alcoholic or derived from the *peri* position but was that present in the original cercosporin molecule. This conclusion was based on the following in-

(5) B. R. Brown and A. R. Todd, *J. Chem. Soc.*, 1280 (1954).

frared data. In 4,9-dihydroxyperylene-3,10-quinone the *peri* hydroxyl group is strongly chelated, causing the carbonyl band to shift to a lower frequency and the hydroxyl band to disappear completely.[6] In the case of 2,11-dihydroxyperylene-3,10-quinone, which has five- rather than six-membered chelation, the carbonyl band is shifted to a still lower frequency but the spectrum retains a hydroxyl band at 3300 cm.$^{-1}$ 1-Hydroxyperylene-3,10-quinone, which lacks chelation demonstrates a double carbonyl band (1647 and 1621 cm.$^{-1}$) and an intense hydroxyl band at 3322 cm.$^{-1}$ [5].

Pentamethylnorcercosporin had a strong band at 3400 cm.$^{-1}$ so the hydroxyl group was not *peri*. The presence of a carbonyl band at 1613 cm.$^{-1}$ with an accompanying shoulder at approximately 1640 cm.$^{-1}$ suggests that the hydroxyl group of pentamethylnorcercosporin is located on the quinone side of the pigment nucleus. Furthermore, pentamethylnorcercosporin was soluble in aqueous alkali and its acetate derivative demonstrated absorption at 1762 cm.$^{-1}$ which is characteristic of phenolic and not alcoholic acetates.

To obtain direct evidence for the location of the methoxyl groups several attempts were made to oxidize cercosporin and its acylated and methylated derivative. However, the appropriate derivative of phenanthrenepolycarboxylic acid was not detected. It appeared that the nucleus either remained unchanged or was destroyed completely. Oxidation of pentamethylnorcercosporin by potassium permanganate in alkali yielded a yellow compound which formed a yellow solution in aqueous alkali and had a strong green fluorescence. This reaction resembled the formation of 4-oxo-4*H*-benz[*de*]-anthracene-7,8-dicarboxylic acid anhydride by permanganate oxidation of perylene-3,10-quinone, which has as an intermediate 1-hydroxyperylene-3,10-quinone.[5] The infrared spectrum of the oxidation product of pentamethylnorcercosporin had a strong band at 1780 cm.$^{-1}$ (potassium bromide) but had no hydroxyl group band. In chloroform solution it absorbed at 1795 and 1780 cm.$^{-1}$; the latter band was more intense than the former. The addition of piperidine to this solution caused the two carbonyl bands to disappear and a new band typical of the carboxylate ion was formed at 1625 cm.$^{-1}$ This suggests that the quinone ring of pentamethylnorcercosporin to which the hydroxyl group is attached is cleaved by oxidation and forms a lactone, believed to be an unsaturated γ-lactone.[7]

Provided that the two carbon atoms which give rise to benzo[*ghi*]perylene by zinc dust distillation were located on the quinones zinc dust distillation of the lactone obtained by oxidation of pentamethylnorcercosporin would yield benzo[*e*]pyrene. If, on the other hand, the two carbon atoms were not located on the quinones, the product of zinc dust distillation would then be either 3*H*- or 6*H*-benzo[*cd*]pyrene. On zinc dust distillation of the lactone a hydrocarbon was obtained having an ultraviolet absorption spectrum very similar to benzo[*e*]pyrene (Fig. 4). This and the infrared

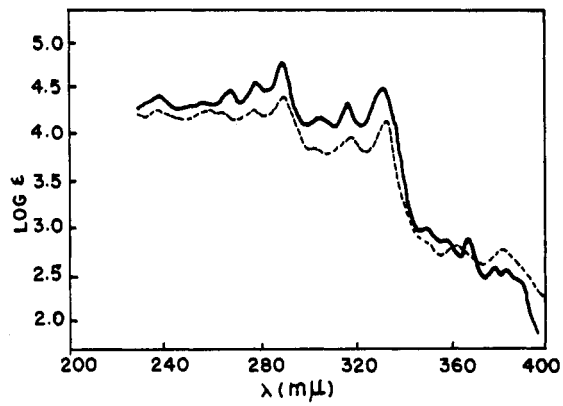

Fig. 4. Absorption spectra of benzo[*e*]pyrene (———) and the product from zinc dust distillation of the lactone (- - - -)

data obtained with pentamethylnorcercosporin provide complementary evidence that at least one methoxyl group and the carbon substituents of tetramethylcercosporin are located on the quinone ring system.

It is of interest that although a solution of cercosporin in methanol and other organic solvents is stable on exposure to sunlight, derivatives in which the two phenolic hydroxyl groups of the pigment are methylated are extraordinarily photosensitive. When a methanolic solution of tetramethylcercosporin was exposed to direct sunlight the orange solution turned immediately to purple-red and showed a strong ferric chloride reaction. From this solution a deep red compound was obtained which had a molecular formula that corresponded to tetramethylnorcercosporin ($C_{32}H_{32}O_{10}$). This compound was obtained also by exposure to sunlight of methanolic solution of pentamethylnorcercosporin. In aqueous sodium hydroxide it produced a clear blue color solution. Acetylation of tetramethylnorcercosporin yielded the diacetate, which had a phenolic acetate band at 1768 cm.$^{-1}$ Methylation of tetramethylnorcercosporin by means of dimethyl sulfate and potassium carbonate in acetone or dimethyl sulfate and aqueous sodium hydroxide produced the parent compound, tetramethylcercosporin. This proves that the two methoxyl groups were demethylated by the action of sunlight. All attempts to obtain a dilactonic compound by the oxidation of tetramethylnorcercosporin in a manner similar to that employed for oxidation of pentamethylnorcercosporin were unsuccesful. However, the ease of methylation and the similarity of the ultraviolet spectra of tetra

(6) A. W. Johnson, J. R. Quayle, T. S. Robinson, N. Sheppard, and A. R. Todd, *J. Chem. Soc.*, 2633 (1951).

(7) R. N. Jones, C. L. Angell, T. Ito, and R. J. D. Smith, *Can. J. Chem.*, **57**, 2007 (1959).

and pentamethylnorcercosporin in neutral, alkaline, and concentrated sulfuric acid solution indicate that the group demethylated by sunlight was not *peri* or alcoholic but was one of the methoxyl groups present in the original cercosporin molecule.

The pigments, hypericin[8] and erythroaphin,[9] have a condensed aromatic ring and extended quinone system. The former was isolated from a higher plant and the latter from an insect. Fungi contain many bianthraquinones[10] and related compounds,[11] but the present investigation appears to be the first in which a fungus pigment was demonstrated to have a polyaromatic ring and extended quinone system.

EXPERIMENTAL

Zinc dust distillation of noranhydrocercosporin. An intimate mixture of 800 mg. of noranhydrocercosporin and 56 g. of acid activated zinc dust was divided into 75 equal portions. Each portion was placed in a small glass tube with one end drawn out to a small diameter and sandwiched between two layers of zinc dust. The larger end of the tube was sealed. The zinc dust layer at the tube exit was heated slowly until the sample layer was red and then the sample layer was heated directly. The yellow distillate which solidified on cooling was dissolved in benzene and evaporated to dryness. The yellow-brown residue (6.7 mg.) was dissolved in petroleum ether and chromatographed on 15 g. of alumina (Activity II) and developed with a mixture of petroleum ether and benzene (5:1). The faint yellow colored fraction which had an intense blue fluorescence under an ultraviolet light was collected, concentrated by evaporation, and recrystallized from methanol as greenish yellow prisms (1.4 mg.), m.p. 210–215°. λ_{max} (in methanol containing 1% chloroform) 253, 278, 290, 302, 315, 330, 346, 364, 384, 410, and 435 mμ ($E^{1\%}_{1\,cm}$ 610, 750, 1640, 1840, 200, 200, 274, 555, 665, 53, and 53). λ_{max} (in concd. sulfuric acid) 294, 302, 317, 386, 407, 427, 455, 515, 662, and 760 mμ ($E^{1\%}_{1\,cm}$ 485, 475, 400, 180, 264, 180, 211, 337, 169, and 105).

Zinc dust fusion of cercosporin. An intimate mixture consisting of cercosporin (1.0 g.), zinc dust (20 g.), sodium chloride (10 g.), and zinc chloride (50 g.) was divided equally into twenty test tubes. Each tube was heated on a metal bath at 220° for 5 min. and then at 300–310° for 10 min. and stirred with a glass rod. After cooling, the contents of each tube were heated with 10% hydrochloric acid on a boiling water bath for a few minutes, then filtered, dried, and thoroughly extracted with benzene. The benzene solution was washed with 5% sodium hydroxide to remove acidic products. The dried solution was concentrated, added to a column containing 20 g. of alumina (Activity II), and developed with 2% methanol in benzene as solvent. The yellow solution which resulted was evaporated, redissolved in a mixture of benzene–petroleum ether (1:2), and chromatographed on 60 g. of alumina (Activity II). Separation was accomplished as shown in Fig. 2. Ten milliliters of each fraction was collected and fractions 1–6 were obtained by development with the same solvent. Fractions 17–24 were developed with benzene. Fractions 1–16 were rechromatographed on 50 g. of alumina (Activity II) and separated into five groups. The first group (fractions 1–16) were rechromatographed on 10 g. of silica gel and developed with petroleum ether. Fractions 8–9 yielded a yellow viscous oil after removal of the solvent.

The second group (fractions 17–38) was evaporated, dissolved in petroleum ether, and chromatographed on 15 g. of alumina with petroleum ether as the solvent. Five milliliters of each fraction was collected and divided into three groups. Fraction 14–15 yielded greenish yellow crystals (2.9 mg.) on removal of the solvent.

The third group (fractions 39–51) was evaporated and sublimed under reduced pressure. The sublimates were converted to picrates (11.9 mg.) and regenerated by passage through an alumina column. The eluate was evaporated and the residue (3.8 mg.) recrystallized from alcohol as greenish yellow prisms; each fraction showed an ultraviolet absorption spectrum characteristic of benzo(*ghi*)perylene.

Oxidation of cercosporin with nitric acid. Cercosporin (150 mg.) was oxidized with 1.5 ml. of nitric acid (sp. gr. 1.38) in a sealed tube for 3 hr. at 170°. On cooling, white crystals of mellitic acid formed. This was methylated using ethereal diazomethan solution and recrystallized from dilute methanol and benzene–petroleum ether mixture as colorless needles, m.p. 187° (103.6 mg.).

Oxidation of noranhydrocercosporin with nitric acid. Noranhydrocercosporin (150 mg.) was treated as described for cercosporin. The yield of mellitic acid hexamethyl ester was 93.2 mg.

Except for hexaacetylnorcercosporin which was dried at 61° (0.001 mm.), the following samples for analysis were dried at 130–140° (0.001 mm.) for 32–48 hr.

Norcercosporin. To a solution of cercosporin (500 mg.) in 200 ml. of boiling benzene 2 g. of fine powdered anhydrous aluminum chloride was added and the mixture boiled for 1 hr. During storage overnight a purple precipitate was formed. This was separated and dissolved in water. The purple-red solution was made alkaline, using sodium hydroxide, then reacidified with concd. hydrochloric acid, saturated with sodium chloride, and chilled in an ice box overnight. The red precipitate formed was collected by filtration, dried, added to a column containing 65 g. of dehydrated calcium hydrogen phosphate, and then developed with chloroform. The chloroform eluate was discarded and elution was continued using chloroform containing 5% ethanol. This eluate was concentrated *in vacuo* and benzene was then added. The precipitate obtained was recrystallized from alcohol and benzene as purple black crystals (80 mg.), m.p. 253°.

Anal. Calcd. for $C_{28}H_{24}O_{10}$: C, 64.61; H, 4.65. Found: C, 64.70; H, 4.87.

$\lambda^{CH_3OH}_{max}$ 228, 276, and 510 mμ (log ε 4.72, 4.58, and 4.37). λ_{max} (in 1*N* sodium hydroxide containing 20% methanol) 235, 287, and 590 mμ (log ε 4.62, 4.70, and 4.35). $\lambda^{conc.\ H_2SO_4}_{max}$ 220, 240, 260, infl. 310, 405, 520, 550 infl., and 640 infl. (log ε 4.64, 4.64, 4.56, 4.06, 3.80, 4.34, 4.92, and 3.87). Infrared: (potassium bromide) 3560_s, 3400_s, 1625_s, 1600_{vs} cm.$^{-1}$ (OH, C=O).

It was readily soluble in methanol but slightly soluble in chloroform and benzene. It dissolved in sodium carbonate forming a purple color and in sodium hydroxide the color was blue. A methanol solution produced a purple-red color with ferric chloride and a blue color with magnesium acetate.

Hexaacetylnorcercosporin. To norcercosporin (50 mg.), dissolved in 2 ml. of pyridine was added 2 ml. of acetic anhydride at room temperature. After storage in an ice box overnight, the reaction mixture was poured into water. The orange precipitate formed was filtered, dried, dissolved in benzene, and chromatographed in a column containing 15 g. of calcium hydrogen phosphate, using benzene plus 0.05% of methanol as solvent. Hexaacetylnorcercosporin was developed as the main orange band; the eluted solution was evaporated to dryness under reduced pressure. The residue was dissolved in a small amount of glacial acetic acid and added to water with vigorous stirring. Hexaacetylnorcercosporin, which separated as an orange amorphous

(8) H. Brockmann, von Falkenhausen, Neeff, Dorlars, and Budde, *Chem. Ber.*, **84**, 865 (1951).

(9) B. R. Brown, A. W. Johnson, S. F. MacDonald, J. R. Quale, and A. R. Todd, *J. Chem. Soc.*, 4928 (1952).

(10) B. H. Howard and H. Raistrick, *Biochem. J.*, **56**, 56 (1954).

(11) A. E. Oxford and H. Raistrick, *Biochem. J.*, **34**, 790 (1940).

powder was filtered, throughly washed with water, and dried, m.p. 113–116°. All attempts to crystallize this compound were unsuccessful.

Anal. Calcd. for $C_{28}H_{18}O_{10}(CH_3CO)_6$: 62.17; H, 4.70; CH_3CO, 33.4. Found: C, 62.31; H, 4.83; CH_3CO, 34.1.

Infrared: potassium bromide 1774, 1733, and 1645 cm.$^{-1}$ (C=O).

Octaacetyldihydronorcercosporin. To norcercosporin (50 mg.), dissolved in 2 ml. of pyridine, 2 ml. of acetic anhydride, and 0.3 g. of zinc powder were added. After standing 5 hr. at room temperature the mixture was added to 100 ml. of water, and the bright yellow precipitate which formed was filtered, washed with water, and dried. It was dissolved in benzene and chromatographed on 15 g. of calcium hydrogen phosphate with benzene as the solvent. The main fraction of octaacetyldihydronorcercosporin was collected and evaporated under reduced pressure. The residue was dissolved in a small amount of benzene and then ether and petroleum ether were added. A pure octaacetyl compound separated as bright yellow prisms (50 mg.), which were recrystallized from a benzene–ether–petroleum ether mixture; m.p. 236°.

Anal. Calcd. for $C_{28}H_{18}O_{10}(CH_3CO)_8$: C, 61.53; H, 4.93; CH_3CO, 40.1. Found: C, 61.88; H, 5.26; CH_3CO, 39.8.

$\lambda_{max}^{CH_3OH}$ 276, 430, and 452 mμ (log ε 4.62, 4.27, and 4.33).

Methylation of norcercosporin. Norcercosporin (30 mg.) was dissolved in 30 ml. of acetone and treated as described for the preparation of dimethylcercosporin. The crude product was purified by chromatography on acid-washed alumina and recrystallized from methanol, m.p. 252°. This product was demonstrated to be identical with dimethylcercosporin by mixed melting point and paper chromatography (solvent system, *cf.* preceding paper).

Pentamethylnorcercosporin. A. *By Thiele's acetylation.* To a solution of tetramethylcercosporin (500 mg. in 7 ml. of acetic anhydride), one drop of concd. sulfuric acid was added. After 3 hr. the solution was poured into 400 ml. of water and the precipitate that formed was harvested by filtration. This was dissolved in 30 ml. of methanol containing 3 ml. of 10% sodium hydroxide solution and heated over a water bath for 3 min. The solution immediately turned blue. An additional 100 ml. of water was added and the solution then extracted with chloroform to remove the residual tetramethylcercosporin. The blue alkaline layer was acidified with hydrochloric acid, reextracted with chloroform, and the deep red chloroform extract evaporated to dryness. The residue was dissolved in benzene and chromatographed on 50 g. of calcium hydrogen phosphate, using benzene. The major orange red zone was collected, concentrated by evaporation, and a small amount of methanol was added. The pentamethylnorcercosporin which separated, was recrystallized from methanol as red prisms, m.p. 153°.

Anal. Calcd. for $C_{33}H_{34}O_{10}$: C, 67.11; H, 5.80; OCH_3, 26.27. Found: C, 66.84; H, 5.64; OCH_3, 26.27.

$\lambda_{max}^{CH_3OH}$ 225, 274, 330 infl., and 485 mμ (log ε 4.71, 4.60, 3.70, and 4.43). λ_{max} (in *N* sodium hydroxide containing 20% methanol) 271, 430, 500, and 640 mμ ($E^{1\%}$ 615, 190, 180, and 290). λ_{max} (in concd. sulfuric acid) 238, 265 infl., 310, 410, 520, and 580 mμ ($E^{1\%}$ 780, 595, 250, 105, 374, and 365). Infrared: (potassium bromide), 3400, 1613 cm.$^{-1}$ (OH, C=O).

It was very soluble in methanol, chloroform, and benzene, less soluble in ether, and insoluble in petroleum ether and aqueous sodium hydrogen carbonate. The product formed a clear blue solution in aqueous caustic alkali. The methanol solution gave a blue color with ferric chloride.

B. *By hydrogen peroxide.* To tetramethylcercosporin (1.0 g. in 70 ml. of methanol) 20 ml. of aqueous 10% sodium hydroxide and 10 ml. of hydrogen peroxide (30%) were added, and the solution was warmed in a water bath at 60°. It effervesced strongly and turned green immediately. After storage at room temperature for 20 min., 200 ml. of water was added, and the solution was extracted with chloroform to remove residual tetramethylcercosporin. The deep blue water layer was acidified with hydrochloric acid, reextracted with chloroform, and the red chloroform extract was dried and freed of solvent by evaporation. The residue was dissolved in benzene and chromatographed on calcium hydrogen phosphate column using benzene. The major red band was collected, the benzene removed by evaporation under reduced pressure, and the product crystallized by adding a small amount of methanol. The compound was recrystallized from methanol as red prisms; m.p. 153°, and its identity with pentamethylnorcercosporin as obtained in Method A was established by their mixed melting point and infrared spectra.

Monoacetylpentamethylnorcercosporin. Pentamethylnorcercosporin (100 mg.) was treated as described for hexaacetylnorcercosporin. The crude product was dissolved in benzene and chromatographed on calcium hydrogen phosphate with benzene as the solvent. The main orange band of monoacetylpentamethylnorcercosporin was collected, concentrated by evaporation, and a small amount of methanol was added. The orange-red prisms formed were recrystallized from methanol, m.p. 201°.

Anal. Calcd. for $C_{35}H_{36}O_{11}$: C, 66.44; H, 5.73; CH_3CO, 6.80. Found: C, 66.68; H, 5.53; CH_3CO, 6.5.

Infrared: (potassium bromide) 1762, 1632 cm.$^{-1}$ (C=O).

Methylation of pentamethylnorcercosporin. A mixture of pentamethylnorcercosporin (395 mg.), methyl iodide (10 ml.), and silver oxide (2 g.) was refluxed for 30 min. The excess methyl iodide was removed and the residue then extracted with chloroform. The solvent extracted was evaporated to dryness and the residue was dissolved in benzene and purified by chromatography on calcium hydrogen phosphate using benzene. The major band was collected, concentrated by evaporation, and the crude product was recrystallized from methanol as orange-red prisms, m.p. 204°.

This compound was demonstrated to be identical with tetramethylcercosporin by mixed melting point and infrared analyses.

Potassium permanganate oxidation of pentamethylnorcercosporin. To a solution of pentamethylnorcercosporin (320 mg. in 100 ml. of 1% aqueous sodium hydroxide) at room temperature, 12 ml. of 1% potassium permanganate solution was added dropwise. When the potassium permanganate was consumed completely, the manganese dioxide formed was removed by filtration. The filtrate was acidified with hydrochloric acid and then extracted with chloroform. The chloroform extract was shaken with saturated sodium hydrogen carbonate and then 1% sodium hydroxide solution to recover residual pentamethylnorcercosporin. This was retreated with potassium permanganate until converted to the sodium hydrogen carbonate soluble product. The sodium hydrogen carbonate solution, which has a yellow color and demonstrates intense green fluorescence, was acidified with hydrochloric acid and the brown precipitate formed was collected by shaking with chloroform. After removal of the solvent, the residue was dissolved in benzene and chromatographed on a column of calcium hydrogen phosphate using first benzene, and then benzene containing 0.5% of methanol. The bright yellow eluate was collected and concentrated. The crude product formed when a small quantity of ether was added was recrystallized once from chloroform–benzene and several times from methanol. The yield was 40 mg. of yellow prisms, m.p. 286°.

$\lambda_{max}^{CH_3OH}$ 244, 256, 321, 396, and 457 infl. ($E^{1\%}$ 612, 559, 245, 456, and 116). $\lambda_{max}^{conc.\ H_2SO_4}$ 254, 275, infl. 429, 480 infl., and 510 mμ ($E^{1\%}$ 805, 571, 465, 284, 344). Infrared chloroform 1800, 1780, 1640, and 615 cm.$^{-1}$ (C=O), [chloroform containing piperidine 1625 cm.$^{-1}$ (C=O)].

Zinc dust distillation of the permanganate oxidation product. Thirty-four milligrams of the product obtained by the above procedure was thoroughly mixed with zinc dust and divided into eight portions, each of which was treated as described for zinc dust distillation of noranhydrocercosporin. The yellow distillate was collected in hexane and

concentrated. The crude product was purified by the silica gel chromato-strip method, using hexane in an ascending system. The major fraction, which fluoresced blue in ultraviolet light, was eluted from the strip with 25 ml. of methanol. The ultraviolet absorption spectrum of this solution was very similar to that of benzo[*e*]pyrene (Fig. 4).

Tetramethylnorcercosporin. A solution of tetramethylcercosporin (1.0 g. in 1 l. of methanol) was placed in a 2-l. flask and exposed to direct sunlight on a clear day with occasional shaking. After 6 hr. the solution, which had changed from orange to purple-red, was evaporated to dryness under reduced pressure. The residue was dissolved in benzene and chromatographed on 60 g. of calcium hydrogen phosphate, using benzene. Following a small amount of yellow colored impurity, the main fraction containing tetramethylnorcercosporin was eluted. This was concentrated by evaporation under reduced pressure and a small amount of benzene, ether, and petroleum ether added. Tetramethylnorcercosporin separated as dark red needles, which were recrystallized from methanol, mp. 241°. It was insoluble in aqueous sodium hydrogen carbonate, but in dilute aqueous-caustic alkali formed a clear blue solution. Alcoholic solutions gave a blue color with ferric chloride and purple color with magnesium acetate.

Anal. Calcd. for $C_{32}H_{32}O_{10}$: C, 66.66; H, 5.59; OCH_3, 21.60. Found: C, 66.41; H, 5.76; OCH_3, 21.30.

$\lambda_{max}^{CH_3OH}$ 226, 275, 330 infl. and 500 mμ (log ε 4.76, 4.63, 3.76, and 4.45). λ_{max} (in *N* sodium hydroxide containing 20% methanol), 240, 280 infl., 440, and 640 mμ ($E^{1\%}$ 850, 605, 155, 380). $\lambda_{max}^{coned.\ H_2SO_4}$ 238, 260 infl., 310, 400, 520, and 580 mμ ($E^{1\%}$ 830, 610, 250, 110, 380, and 390).

Diacetyltetramethylnorcercosporin. Tetramethylnorcercosporin (50 mg.) was treated with 2 ml. of pyridine and 2 ml. of acetic anhydride as described for the preparation of monoacetylpentamethylnorcercosporin. The crude product was purified by chromatography on calcium hydrogen phosphate using benzene. Diacetyltetramethylcercosporin was recrystallized from methanol as orange-red prisms, m.p. 225°.

Anal. Calcd. for $C_{36}H_{36}O_{12}$: C, 65.44; H, 5.49; CH_3CO, 13.0. Found: C, 65.50; H, 5.57; CH_3CO, 12.5.

Infrared: (potassium bromide) 1768, 1635 cm.$^{-1}$ (C=O).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MUSLIM UNIVERSITY]

Studies on the C-Methyl-γ-benzopyrone System. Orientation in the Isoflavone Series

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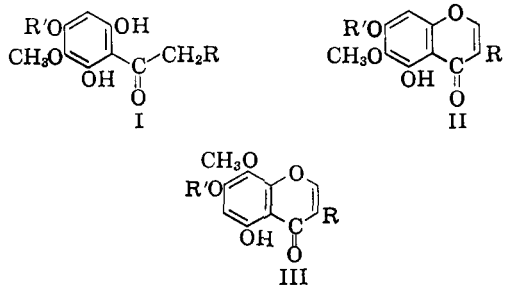
A tentative explanation of the different orientations of the isoflavones produced by ethoxalylation and by the ethyl formate-sodium synthesis has been advanced. The ethoxalylation of a C-methyl deoxybenzoin (2,4,6-trihydroxy-3-methyl-) (VII) has been studied. A mixture of two new isomeric isoflavones 5,7-dihydroxy-8-methyl-(IXa) and 5,7-dihydroxy-6-methyl-(IX-b), is obtained. The constitution of the two isoflavones has been proved by their synthesis through conventional methods. The difference in the behavior of the O-methyl deoxybenzoin of type I and C-methyl deoxybenzoin of type VII towards the same condensing agent, ethoxalyl chloride, has also been discussed.

It has been well established[1,2] that deoxybenzoins of type I(R = Ar) on treatment with ethyl formate and sodium yield the corresponding isoflavones of type II, which result from cyclization at the 6-hydroxyl group in I. However, when Baker, *et al.*[3] submitted the deoxybenzoin (I. R = *p*-HO-C_6H_4, R′ = H) to the ethoxalylation process, they obtained the product 5,7,4′-trihydroxy-8-methoxyisoflavone (III. R = *p*-HO−C_6H_4, R′ = H) resulting from cyclization involving the 2-hydroxyl group.



Baker *et al.* (*loc. cit.*) drew attention to this difference in the behavior of the two condensing agents and remarked that further cases of 5,7,8-orientation, and the reason causing the orientation in the ethoxalylation process to be different from the one in the ethyl formate–sodium method, would be

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