

to isocercosporin when it is refluxed for a long time even at a low temperature. The extraction was continued until the color of the pigment had disappeared from the extract. The total yield of crystals was about 34 g.

This crude cercosporin (800 mg.) was dissolved in chloroform and chromatographed on 250 g. of dehydrated calcium hydrogen phosphate (tube  $37 \times 500$  mm.). (Superheating should be avoided in the dehydration of the calcium hydrogen phosphate.) The pigment adsorbed on the top of the column was developed with chloroform containing 0.5% of methanol. A small amount of a yellow substance and isocercosporin formed during extraction were discarded, and the developing solvent was changed to chloroform containing 1.5% of methanol. A dark red band of cercosporin was eluted, leaving a small amount of brown impurity at the top of the column. The eluted solution was concentrated *in vacuo* below  $50^\circ$  and kept overnight in an ice-box. Pure cercosporin separated as prisms (yield 720 mg.) which were recrystallized from chloroform and benzene.

**Isolation of Pigment from Infected Soy Beans.**—The epidermis (450 mg.) obtained from 30 grains of infected soy beans, was soaked in 8 ml. of glacial acetic acid at room temperature for 3 days; the pigment dissolved completely in the acetic acid. Ether (50 ml.) was then added to the solution which was washed several times with water to remove the acetic acid. The ether layer was dried and evaporated. When subjected to paper chromatography, the resulting pigment showed the same  $R_f$  value (0.61) as the cercosporin obtained from cultured mycelia.

**General Properties of Cercosporin.**—The samples for elementary analysis and measurement of molecular weight were recrystallized from benzene-chloroform, or alcohol, and dried at  $130^\circ$  (0.001 mm.) for 32 hours. Cercosporin contains no nitrogen, chlorine, sulfur or phosphorus and melts at  $241^\circ$  without sublimation or decomposition.  $[\alpha]_{D}^{20}$   $+470^\circ$  (*c* 0.5, chloroform);  $\lambda_{max}^{MeOH}$  223, 260, 271, 275 and 470  $\mu$ , ( $E_{1\%}^{1\text{cm}}$  940, 638, 652, 650 and 498);  $\lambda_{max}$  (in 1 *N* NaOH) 253, 299, 478, 610, 620 and 645  $\mu$ , ( $E_{1\%}^{1\text{cm}}$  535, 802, 562, 263, 257 and 375). Major bands in the infrared absorption spectrum (KBr window): 3400m, 2940m, 1619vs, 1585vs, 1554vs, 1455m, 1428m, 1395w, 1348w, 1315m, 1268s, 1223m, 1170s, 1145m, 1113m, 1075m, 1055m, 1017m, 978w, 938w, 921w, and 860m  $\text{cm}^{-1}$ ; bands in the 1700–1550  $\text{cm}^{-1}$  region (in chloroform): 1614vs, 1583s and 1551  $\text{cm}^{-1}$  (in tetrahydrofuran): 1615, 1585 and 1651  $\text{cm}^{-1}$ .

*Anal.* Calcd. for  $C_{28}H_{22}O_8(OCH_3)_2$  (548.52): C, 65.69; H, 5.15;  $OCH_3$ , 11.32. Found: C, 65.50; H, 4.83;  $OCH_3$ , 11.61 (from chloroform-benzene). C, 65.67; H, 4.85;  $OCH_3$ , 11.94 (from alcohol); C- $CH_3$  (by Kuhn-Roth), 4.70.

The solubilities of cercosporin in aqueous solutions are as follows: it is insoluble in sodium hydrogen carbonate, partially soluble in sodium carbonate, readily soluble in ammonia and very soluble in dilute alkali giving a clear green solution. It is readily soluble in pyridine, dioxane, chloroform, alcohol and acetone, soluble in ether and benzene,

and insoluble in petroleum ether, its solution in concentrated sulfuric acid has a stable purple-blue color and shows no fluorescence; when water is added, a black-purple precipitate is formed. Cercosporin gives a red color with ferric chloride and a green color with magnesium acetate<sup>6</sup> in alcohol solution.

Paper chromatography was carried out on one-dimensional paper strips ( $2 \times 15$  cm.) which were developed (ascending flow) with the upper layer of a benzene-glacial acetic acid-water mixture (2:1:1). Cercosporin shows a spot with an  $R_f$  value of 0.61.

**Isomerization of Cercosporin.**—Cercosporin (200 mg.), dissolved in 40 ml. of toluene, was refluxed for 15 minutes. The reaction was followed by paper chromatography. Soon after the refluxing was begun, a new spot due to the formation of isocercosporin ( $R_f$  0.74) appeared in addition to the spot due to cercosporin. The isomerization reached equilibrium within 15 minutes with equal amount of the two isomers present. On cooling purple-black crystals separated, each of which contained the same amount of cercosporin and isocercosporin. These "mixed crystals" were also obtained when cercosporin was fused at its melting point ( $241^\circ$ ). To separate isocercosporin from this mixture, it was dissolved in chloroform and chromatographed on calcium hydrogen phosphate with chloroform containing 0.5% of methanol as the developing solvent. The eluted isocercosporin was concentrated *in vacuo*. When small amount of petroleum ether was added, isocercosporin separated as dark red prisms which were recrystallized from chloroform and benzene containing a small amount of petroleum ether, and dried at  $100^\circ$  (0.001 mm.) for 32 hours.  $\lambda_{max}^{MeOH}$  223, 260, 267, 329, 470 and 475  $\mu$ , ( $E_{1\%}^{1\text{cm}}$  800, 670, 70, 544 and 560),  $\lambda_{max}$  (in 0.1 *N* NaOH) 256, 299, 472, 610, 635 and 645  $\mu$ , ( $E_{1\%}^{1\text{cm}}$  472, 755, 475, 202, 245 and 316); Major bands in the infrared absorption spectrum (KBr window): 3400m, 2940m, 1619vs, 1584s, 1554s, 1462m, 1434m, 1397w, 1349w, 1319w, 1279vs, 1227m, 1174s, 1154m, 1119w, 1076m, 1054m, 1024s, 981w, 937w, 920m and 863m  $\text{cm}^{-1}$ .

*Anal.* Calcd. for  $C_{28}H_{22}O_8(OCH_3)_2$ : C, 65.69; H, 5.15;  $OCH_3$ , 11.32. Found: C, 65.64; H, 5.25;  $OCH_3$ , 11.79.

Isocercosporin dissolves more readily in chloroform, benzene and alcohol than cercosporin; its solubility in carbonate and alkali solutions is about the same; it gives the same color reactions with ferric chloride and magnesium acetate.

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(6) Color reaction for hydroxyquinones; S. Shibata, *J. Pharm. Soc. Japan.*, **61**, 320 (1941).

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## Cercosporin. A Pigment of *Cercosporina Kikuchii* Matsumoto et Tomoyasu. II. Physical and Chemical Properties of Cercosporin and its Derivatives

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Several acetyl and methyl derivatives of cercosporin were prepared; the results indicate that cercosporin contains two quinoid carbonyl, two phenolic hydroxyl and two alcoholic hydroxyl groups. The infrared, ultraviolet and the visible spectra of these derivatives suggest that cercosporin is a polyhydroxy derivative of a polycyclic quinone having an extended quinone system in which two phenolic hydroxyl groups are present in positions *peri* to the quinone carbonyls. By treatment with concentrated sulfuric acid, cercosporin is converted into noranhydrocercosporin,  $C_{28}H_{20}O_8$ , which contains no methoxy or alcoholic hydroxyl groups. The physical and chemical properties of noranhydrocercosporin and its derivatives are described.

In a previous paper<sup>1</sup> we reported the isolation of a new crystalline red pigment, cercosporin, from

(1) S. Kuyama and T. Tamura, *THIS JOURNAL*, **79**, 5725 (1957).

the cultured mycelia of *Cercosporina Kikuchii* M. et T.;  $C_{30}H_{28}O_{10}$  was proposed as the molecular formula, and the presence of two methoxy and several

acidic hydroxy groups was established. In this paper, the preparation of several derivatives of cercosporin and their physical and chemical properties are reported.

The acetylation of cercosporin gave a tetraacetate, while reductive acetylation yielded hexaacetyldihydrocercosporin (cercosporin leucoacetate) which showed an intense green fluorescence in solution. Methylation with methyl iodide and silver oxide gave a red tetramethyl ether, while methylation with dimethyl sulfate and anhydrous potassium carbonate in acetone gave a red dimethyl ether, which was insoluble in aqueous alkali and gave no color reaction with ferric chloride and magnesium acetate. Further methylation of the dimethyl ether with methyl iodide and silver oxide yielded the tetramethyl ether. These results suggest that two of the four hydroxyl groups are phenolic and two are alcoholic. This conclusion is supported by the infrared spectrum of cercosporin tetraacetate which shows carbonyl bands of phenolic acetate and of alcoholic acetate at 1776 and 1740  $\text{cm}^{-1}$ , respectively.

As previously mentioned,<sup>1</sup> treatment of cercosporin with concentrated sulfuric acid yields purple-black crystals from which it is difficult to remove the solvent of recrystallization. This substance was formulated as  $\text{C}_{28}\text{H}_{20}\text{O}_8$  and called noranhydrocercosporin. Noranhydrocercosporin contains no methoxy groups (Zeisel). Acetylation gave a diacetate; while reductive acetylation or hydrogenation with Adams catalyst in the presence of acetic anhydride and anhydrous sodium acetate gave a dihydrotetraacetate. Reductive methylation gave a dihydrotetramethyl ether. The infrared spectrum of diacetylnoranhydrocercosporin shows only the phenolic acetate carbonyl band at 1766  $\text{cm}^{-1}$  and the aliphatic acetate carbonyl band is absent. These findings indicate that the two methoxy and two alcoholic hydroxyl groups of cercosporin are involved in the reaction, cercosporin  $\rightarrow$  noranhydrocercosporin. This conclusion was confirmed by the finding that dimethylnoranhydrocercosporin, prepared by the methylation of noranhydrocercosporin with dimethyl sulfate, was also obtained by treating dimethylcercosporin with concentrated sulfuric acid. Therefore, the action of concentrated sulfuric acid causes the loss of two alcoholic hydroxyl and two methoxy groups from cercosporin. The presence of alcoholic hydroxyl groups may be necessary for this reaction, since tetramethylcercosporin does not undergo this reaction. Noranhydrocercosporin was also obtained from isocercosporin, but no isomerization was observed in the noranhydrocercosporin series. Isomers of both cercosporin and dimethylcercosporin but not of tetramethylcercosporin were detected. This indicates that the isomerization and the formation of noranhydrocercosporin by sulfuric acid are closely related to the alcoholic hydroxyl groups present in cercosporin.

Noranhydrocercosporin is quite different from cercosporin, especially in the color of the crystals and in solubility. The visible spectrum of noranhydrocercosporin shows a remarkable bathochro-

mic shift, and there is an appreciable difference in ultraviolet and visible spectra between hexaacetyldihydrocercosporin and tetraacetyldihydrocercosporin; these facts suggest that the reaction with sulfuric acid causes a change in the chromophoric system probably in the position closely related to the nucleus. The infrared spectrum of noranhydrocercosporin is quite different from that of cercosporin (Fig. 1).

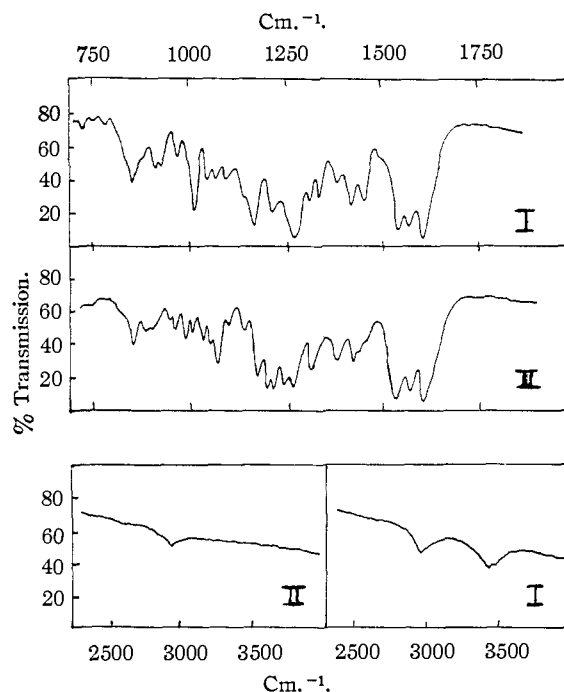


Fig. 1.—Infrared spectra (in KBr disk): I, cercosporin; II, noranhydrocercosporin.

The infrared spectrum of cercosporin shows a quinone carbonyl band at 1619  $\text{cm}^{-1}$  which shifts to 1638  $\text{cm}^{-1}$  in the tetraacetate and disappears on reductive acetylation. It is usually observed that when a hydroxyl group is present in the position *peri* to a quinoid carbonyl group, the quinone carbonyl band is shifted to a much lower frequency and the hydroxyl band near 3300  $\text{cm}^{-1}$  is also shifted to a lower frequency; the latter band becomes weak and occasionally disappears. Since an appreciable shift of the quinone carbonyl band of cercosporin to higher frequency is caused by acetylation and since noranhydrocercosporin shows no hydroxyl band (Fig. 1), it seems reasonable to assume that the two hydroxyl groups in cercosporin are situated in positions *peri* to the quinone carbonyl so as to form a six-membered chelate ring. It should be noted that the quinone carbonyl band of tetraacetylcercosporin remains at a considerably lower frequency than those of anthraquinone, naphthoquinone or benzoquinone.

The infrared spectra of polycyclic quinones have been investigated by D. Hadzi,<sup>2</sup> and A. W. Johnson and co-workers,<sup>3</sup> who concluded that whenever a quinone system was present in one nucleus it

(2) D. Hadzi and N. Sheppard, *THIS JOURNAL*, **73**, 5460 (1951).

(3) J. P. E. Human, A. W. Johnson, S. F. MacDonald and A. R. Todd, *J. Chem. Soc.*, 2633 (1951).

showed a maximum at 1660–1680  $\text{cm}^{-1}$ ; whereas the maximum is observed at 1635–1655  $\text{cm}^{-1}$  when the quinone system is extended through more than one ring. On the basis of these data and of the fact that cercosporin shows a bright yellow color with intensive green fluorescence even in a reduced state, it seems reasonable to conclude that cercosporin is a polyhydroxy derivative of a polycyclic quinone and has an extended quinone system. These conclusions were confirmed by the results of zinc dust distillation and by the finding that oxidation with nitric acid gives mellitic acid in good yield; they will be reported later.

Two pigments, which like cercosporin have an extended quinone system, have been found in nature: they are hypericin,<sup>4</sup> which has been characterized as a polyhydroxymesodianthren derivative, and erythroaphin,<sup>3,5,6</sup> which is believed to be a dihydroxyperylene derivative. Since these pigments are photodynamic, cercosporin should be examined for photodynamic properties.

### Experimental

Except for tetraacetylcercosporin which was dried at 61° (0.001 mm.), the samples for analysis were dried at 130° (0.001 mm.) for 32–48 hours.

The infrared spectra were measured by a Hilger H 800 double beam spectrometer with a rock salt prism.

Paper chromatography was carried out with the following three solvent systems: solvent A, upper layer of a benzene-glacial acetic acid-water mixture (2:1:1); solvent B, upper layer of a petroleum ether (b.p. 70–80°)–benzene-glacial acetic acid-water mixture (4:6:5:5); solvent C, upper layer of a petroleum ether–ether–glacial acetic acid-methanol–water mixture (10:2.5:5:5:1).

**Tetraacetylcercosporin.**—To cercosporin (400 mg.), dissolved in 8 ml. of pyridine, was added 4 ml. of acetic anhydride with cooling. After standing in an ice-box overnight, the reaction mixture was poured into 300 ml. of cold water. The resulting orange precipitate was filtered, washed with water and dried; yield 490 mg. It was dissolved in benzene and chromatographed on  $\text{CaHPO}_4$  (cf. preceding paper) with benzene as solvent. The procedure was carried out in the dark, since the compound is oxidized rapidly in the presence of light. A small amount of impurity was discarded, and the solvent was changed to benzene containing 0.3% of methanol. The main orange zone of tetraacetylcercosporin was developed. The orange solution was evaporated to dryness under reduced pressure at low temperature. The residue was dissolved in 10 ml. of glacial acetic acid and poured into water with vigorous stirring. Tetraacetylcercosporin, which separated as an orange amorphous powder, was filtered, thoroughly washed with water and dried; m.p. 90–95°;  $R_f$  0.32 with solvent C;  $[\alpha]_{\text{D}}^{20, \text{CHCl}_3} +265^\circ$  ( $c$  2.0, chloroform). Major bands in the infrared absorption spectrum (KBr window): 2900m, 1776s, 1740s, 1638vs, 1592s, 1567s, 1470m, 1455m, 1380s, 1320w, 1280m, 1245s, 1200s, 1142s, 1155m, 1010m, 979w, 961w, 920w, and 900w  $\text{cm}^{-1}$ ; absorption bands in the 1700–1550  $\text{cm}^{-1}$  region (in chloroform): 1635, 1590 and 1562  $\text{cm}^{-1}$ ; (in tetrahydrofuran): 1643, 1593 and 1568  $\text{cm}^{-1}$ .

*Anal.* Calcd. for  $\text{C}_{30}\text{H}_{24}\text{O}_8(\text{OCOCH}_3)_4$ : C, 63.68; H, 5.06;  $\text{CH}_3\text{CO}$ , 24.02. Found: C, 63.97; H, 5.16;  $\text{CH}_3\text{CO}$ , 23.7.

It was readily soluble in all solvents except petroleum ether and water.

(4) H. Brockmann, F. Pohl, K. Maier and M. N. Hashad, *Naturwiss.*, **27**, 550 (1939); *Ann.*, **553**, 1 (1942); N. Pace and G. Mackinney, *This Journal*, **63**, 2570 (1941); H. Brockmann and H. Eggers, *Ang. Chem.*, **67**, 706 (1955).

(5) H. Duewell, J. P. E. Human, A. W. Johnson, S. F. MacDonald and A. R. Todd, *Nature*, **162**, 759 (1948).

(6) J. P. E. Human, A. W. Johnson, S. F. MacDonald and A. R. Todd, *J. Chem. Soc.*, **477**, 485 (1950); **4925**, **4928** (1952); **107**, **1280**, **1285**, **2378** (1954); **954**, **959** (1955).

**Hexaacetyldihydrocercosporin.** (*Cercosporin Leucoacetate*).—To cercosporin (500 mg.), dissolved in 10 ml. of pyridine, was added 7 ml. of acetic anhydride with cooling, followed by 0.5 g. of zinc powder. The red solution was immediately reduced to yellow solution which had an intense green fluorescence. After standing overnight in an ice-box, it was poured into 400 ml. of cold water. The bright yellow precipitate was filtered, washed with water and dried; yield 600 mg. The crude product (200 mg.) dissolved in benzene, was chromatographed on 80 g. of  $\text{CaHPO}_4$  with benzene as the solvent. After a small amount of impurities had been eluted, the main fraction of hexaacetyldihydrocercosporin was developed. The solution was evaporated under reduced pressure at a low temperature, ether and petroleum ether were added. Pure hexaacetyldihydrocercosporin separated as bright yellow prisms which were recrystallized from benzene–ether–petroleum ether mixture; m.p. 203°;  $R_f$  0.16 with solvent C;  $\lambda_{\text{max}}^{\text{MeOH}}$  220, 277, 326, 330, 340, 362, 368, 372, 380, 426 and 456  $\text{m}\mu$ , ( $E_{1\%}^{1\text{cm}}$  830, 415, 41.5, 43, 40, 55, 57, 52.5, 56, 189 and 223). Major bands in the infrared spectrum (KBr window): 2900w, 1767s, 1730s, 1580m, 1465w, 1435w, 1360s, 1250s, 1198s, 1158m, 1130m, 1048s, 1005w, 960w and 903  $\text{cm}^{-1}$ .

*Anal.* Calcd. for  $\text{C}_{30}\text{H}_{24}\text{O}_8(\text{OCOCH}_3)_6$ : C, 62.84; H, 5.27;  $\text{CH}_3\text{CO}$ , 32.17. Found: C, 62.68; H, 5.24;  $\text{CH}_3\text{CO}$ , 32.2.

This compound gave no color with ferric chloride in alcohol. It was readily saponified in alcoholic alkali giving a green solution.

**Dimethylcercosporin.**—This procedure was carried out in the dark. Cercosporin (1 g.) was dissolved in 300 ml. of acetone containing 10 g. of finely powdered anhydrous potassium carbonate. To this solution, 6 ml. of dimethyl sulfate was added dropwise with stirring at 40–45°; after 2 hours 5 g. of  $\text{K}_2\text{CO}_3$  and 3 ml. of dimethyl sulfate were added and stirring was continued. Paper chromatography was used to follow the reaction. After 4 hours the color of the solution turned to orange. It was cooled and filtered rapidly and evaporated under reduced pressure. The residue was shaken with 20 ml. of 10% aqueous  $\text{K}_2\text{CO}_3$  and kept overnight in an ice-box. Crude dimethyl cercosporin separated as ruby red prisms. The crude compound (400 mg.) was dissolved in 50 ml. of chloroform and chromatographed on 40 g. of acid-washed alumina<sup>7</sup> with chloroform as the solvent. After orange-red colored impurities had been eluted, the solvent was changed to chloroform containing 0.5% of methanol, and development was continued. The orange-red solution was evaporated under reduced pressure and a small amount of methanol was added. Dimethylcercosporin which separated was recrystallized from dilute methanol as red prisms; m.p. 248°;  $R_f$  0.35 (tailing) with solvent A.

*Anal.* Calcd. for  $\text{C}_{28}\text{H}_{20}\text{O}_6(\text{OCH}_3)_2$ : C, 66.66; H, 5.59;  $\text{OCH}_3$ , 21.5. Found: C, 66.46; H, 5.81;  $\text{OCH}_3$ , 21.7.

It did not dissolve in aqueous caustic alkali and gave no color reaction with ferric chloride and magnesium acetate. It was readily soluble in alcohol, soluble in chloroform and slightly soluble in ether and cold benzene. The hot solution in benzene gelatinized on cooling. Dimethylcercosporin is partially isomerized by refluxing its toluene solution for a few minutes, or by fusing it at the melting point, to isodimethylcercosporin, which showed a spot at  $R_f$  0.46.

**Tetramethylcercosporin.**—Cercosporin (300 mg.) was refluxed with 35 ml. of methyl iodide and 1.5 g. of silver oxide. After 8 hours 300 mg. of silver oxide was added and refluxing was continued for 24 hours. The excess methyl iodide was recovered, and the residue extracted with chloroform and purified by chromatography on 20 g. of acid-washed alumina in the dark with chloroform as the developer. The eluted solution of tetramethylcercosporin was evaporated to dryness; the residue was recrystallized from dilute methanol as red prisms; m.p. 204.5°;  $R_f$  0.87 (solvent A),  $R_f$  0.57 (solvent C).

*Anal.* Calcd. for  $\text{C}_{28}\text{H}_{18}\text{O}_4(\text{OCH}_3)_4$ : C, 67.54; H, 6.00;  $\text{OCH}_3$ , 30.8. Found: C, 67.45; H, 6.22;  $\text{OCH}_3$ , 31.0.

It was insoluble in aqueous caustic alkali and gave no color reaction with ferric chloride or magnesium acetate.

(7) "Aluminium oxide for chromatographic absorption analysis according to Brockmann" (250 g.) was soaked in one l. of 1% hydrochloric acid, washed several times with water, filtered and dried at 100° for 4 hours.

It was readily soluble in benzene, chloroform, alcohol; soluble in ether; and insoluble in petroleum ether. No isomers could be separated from the heated solution. It dissolved in concentrated sulfuric acid giving a purple color, but on the addition of water, tetramethylcercosporin was recovered unchanged.

**Noranhydrocercosporin.**—Cercosporin (1 g.) was dissolved in 30 ml. of concentrated sulfuric acid and filtered through a glass filter. The filtrate was then poured into 500 ml. of ice-water and kept overnight in an ice-box. The purple black precipitate was thoroughly washed with water, dried (yield 900 mg.) and recrystallized from chloroform-methanol as purple black leaflets; m.p. 316° dec.;  $R_f$  0.90 with solvent A,  $R_f$  0.59 (tailing) with solvent B;  $\lambda_{max}$  (in methanol containing 2% of chloroform): 274, 325, 522 and 526 m $\mu$ , ( $E_1^{1\%}$   $cm^{-1}$ : 971, 83, 562 and 540); infrared spectrum (KBr window): 2850w, 1625vs, 1588vs, 1550vs, 1433w, 1398w, 1340m, 1288s, 1258s, 1218s, 1201s, 1183m, 1150w, 1110w, 1076m, 1061w, 1040w, 1013w, 993w, 971w, 958w, 896 w and 863 w cm $^{-1}$ .

*Anal.* Calcd. for  $C_{28}H_{18}O_8$ : C, 69.42; H, 4.16. Found: C, 69.12; H, 4.18.

It was soluble in chloroform, nitrobenzene and acetic acid; less soluble in dioxane and benzene; almost insoluble in alcohol; and insoluble in aqueous sodium hydrogen carbonate and caustic alkali. The sodium salt separated as purple precipitate when the chloroform solution was shaken with aqueous sodium hydroxide. The dioxane solution gave a red color with ferric chloride and a purple color with magnesium acetate.

**Diacetylnoranhydrocercosporin.**—To noranhydrocercosporin (500 mg.), dissolved in 10 ml. of pyridine, was added 5 ml. of acetic anhydride with cooling. The red precipitate was filtered, dried (yield 430 mg.), dissolved in chloroform and chromatographed on acid-washed alumina with chloroform as the solvent. Diacetylcercosporin was developed as a red band and the eluted solution was concentrated under reduced pressure. When methanol was added, diacetylnoranhydrocercosporin separated as red prisms which were recrystallized from chloroform-methanol; m.p. 253° dec.; infrared spectrum (KBr window): 2850w, 1765vs, 1636vs, 1609s, 1584s, 1467w, 1441m, 1400m, 1377vs, 1296m, 1264s, 1199vs, 1140vs, 1074s, 1049s, 994m, 952m, 907m, 859w and 807w cm $^{-1}$ .

*Anal.* Calcd. for  $C_{28}H_{18}O_8(OCOCH_3)_2$ : C, 67.60; H, 4.26;  $CH_3CO$ , 15.1. Found: C, 67.66; H, 4.76;  $CH_3CO$ , 15.8.

It was soluble in chloroform and benzene, but only slightly soluble in alcohol. It gave no color with ferric chloride or magnesium acetate in alcohol.

**Tetraacetyldihydronoranhydrocercosporin (Noranhydrocercosporin Leucoacetate).**—(a) Noranhydrocercosporin (500 mg.) was treated as described for hexaacetyldihydrocercosporin; yield 600 mg. The crude product was dissolved in chloroform and chromatographed on 60 g. of acid-washed alumina with chloroform as the solvent. The eluted solution of tetraacetyldihydronoranhydrocercosporin (bright yellow solution with intense green fluorescence) was treated as above. The bright yellow prisms were recrystallized from benzene-methanol; m.p. 160–165°; infrared spectrum (KBr window): 2850w, 1768vs, 1633m, 1555w, 1442m, 1417m, 1373s, 1355s, 1192vs, 1147m, 1097w, 1042s, 970w and 894w cm $^{-1}$ .

*Anal.* Calcd. for  $C_{28}H_{18}O_4(OCOCH_3)_4$ : C, 66.05; H, 4.62;  $CH_3CO$ , 26.3. Found: C, 66.12; H, 5.19;  $CH_3CO$ , 25.6.

It is soluble in chloroform, benzene and dioxane, and slightly soluble in alcohol. It gave no color reaction with ferric chloride.

(b) A mixture of 200 mg. of noranhydrocercosporin, 100 mg. of anhydrous sodium acetate, 20 ml. of acetic anhydride

and 50 mg. of Adams' catalyst was shaken under an atmosphere of hydrogen. After 30 minutes, the color of the solution changed to bright yellow with an intense green fluorescence. It was then heated at 100° for 10 minutes and kept overnight. The catalyst was filtered and 50 ml. of methanol was added. After standing for a few hours, the solution was concentrated under reduced pressure. The crystals which separated upon the addition of methanol were recrystallized from benzene-methanol; m.p. 160–165°;  $R_f$  0.94 with solvent A,  $R_f$  0.73 with solvent B.

**Dimethylnoranhydrocercosporin.** (a) **Methylation of Noranhydrocercosporin.**—To a boiling mixture of 350 ml. of xylene and 10 g. of anhydrous potassium carbonate was added 6 ml. of dimethyl sulfate dropwise with stirring and boiling continued for 2 hours. The solution turned orange-red, and a red precipitate separated. After the reaction mixture had stood overnight in an ice-box, water was added to dissolve the potassium carbonate. The red precipitate was filtered, washed with alcohol, recrystallized from chloroform-methanol;  $R_f$  0.47 with solvent A. It did not melt at 330°.

*Anal.* Calcd. for  $C_{28}H_{18}O_8(OCH_3)_2$ : C, 70.30; H, 4.72;  $OCH_3$ , 12.1. Found: C, 70.18; H, 4.96;  $OCH_3$ , 11.31.

It is soluble in nitrobenzene and chloroform, but slightly soluble in most other solvents.

(b) **Dimethylnoranhydrocercosporin from Dimethylcercosporin.**—A solution of dimethylcercosporin (500 mg.) in 30 ml. of concentrated sulfuric acid was poured into 200 ml. of ice-water, and kept overnight. The red precipitate was washed with methanol, and recrystallized from chloroform-methanol;  $R_f$  0.47 with solvent A. It did not melt at 330°.

*Anal.* Calcd. for  $C_{28}H_{18}O_8(OCH_3)_2$ : C, 70.30; H, 4.72;  $OCH_3$ , 12.1. Found: C, 70.40; H, 4.94;  $OCH_3$ , 11.1.

No difference was found between this compound and dimethylnoranhydrocercosporin prepared as described in (a).

**Tetramethyldihydronoranhydrocercosporin.**—To a mixture of 500 mg. of noranhydrocercosporin, 40 ml. of 10% aqueous sodium hydroxide solution and 2 g. of sodium hyposulfite was added 10 ml. of dimethyl sulfate in 2-ml. portions with vigorous shaking; 20 ml. more of 10% aqueous sodium hydroxide was added to keep the solution alkaline. Shaking was continued until the separation of the yellow precipitate was completed. The dried precipitate, dissolved in chloroform, was chromatographed on acid-washed alumina in the dark with chloroform as the solvent. The eluted solution of tetramethyldihydronoranhydrocercosporin (bright yellow solution with intense green fluorescence) was concentrated and methanol was added. The resulting bright yellow prisms were recrystallized from benzene-methanol; m.p. 203°;  $R_f$  0.97 with solvent A, 0.34 with solvent C.

*Anal.* Calcd. for  $C_{28}H_{18}O_4(OCH_3)_4$ : C, 70.83; H, 5.57;  $OCH_3$ , 22.9. Found: C, 71.15; H, 5.22;  $OCH_3$ , 22.9.

It is readily soluble in chloroform and benzene, and soluble in alcohol. When ferric chloride was added, the alcoholic solution assumed a blue color which immediately changed to red. This red solution was extracted with a small amount of chloroform and the extract concentrated; the residue which was chromatographed with solvent A showed a spot at  $R_f$  0.47, which was identical with that of dimethylnoranhydrocercosporin.

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