134. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

67. THE MOLECULAR CONSTITUTIONS OF CATENARIN AND ERYTHROGLAUCIN, METABOLIC PRODUCTS RE- SPECTIVELY OF *HELMINTHOSPORIUM CATENARIUM* DRECHSLER AND OF SPECIES IN THE *ASPERGILLUS* *GLAUCUS* SERIES

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CHARLES *et al.* [1933] isolated from the mycelium of *Helminthosporium gramineum* Rabenhorst two hitherto undescribed hydroxyanthraquinones which they named helminthosporin (4:5:8-trihydroxy-2-methylanthraquinone) and hydroxy-isohelminthosporin, $C_{15}H_{10}O_6$. Since the latter substance was found by Raistrick *et al.* [1934] to be the main colouring matter of *Helminthosporium catenarium* Drechsler its name was altered by these workers to catenarin. Catenarin was shown by them to contain four hydroxyl groups, to give 2-methylanthracene on zinc dust distillation and, for reasons for which the original work must be consulted, was believed to be a 1:5:8-trihydroxy(hydroxy-methyl)-anthraquinone with the hydroxymethyl group either in position 2, 6 or 7.

We have now shown that when catenarin is reduced with hydriodic acid and red phosphorus, emodin anthranol is formed in good yield and gives, on oxidation with chromic acid, *Frangula*-emodin, $C_{15}H_{10}O_5$, 4:5:7-trihydroxy-2-methylanthraquinone (I). Hence it follows that catenarin must be a hydroxy-emodin and must therefore be II, III, IV, V or VI. Of these II is at once excluded since this must be reserved for ω -hydroxyemodin, a metabolic product of a strain of *Penicillium cyclopium* Westling [Anslow *et al.* 1940] and ω -hydroxy-emodin and catenarin are quite different substances. The belief that catenarin contains a side chain $-CH_3$ group and not a $-CH_2OH$ group is confirmed by the fact that it gives 1 mol. of acetic acid in a Kuhn-Roth oxidation.

The following experimental facts previously established by Raistrick *et al.* [1933; 1934] lead to the exclusion of structures III, V and VI for catenarin and support the view that catenarin is IV.

(a) From the similarity of the absorption curves in conc. H_2SO_4 of, on the one hand, helminthosporin and cynodontin each of which has been conclusively shown to contain nuclear hydroxyl groups in the 1:4:5-positions, and, on the other hand, catenarin, it is logical to conclude that catenarin is also a derivative of 1:4:5-trihydroxyanthraquinone. Only IV and V satisfy this condition.

(b) Catenarin gives a fluorescent solution in acetic acid and this fluorescence disappears on reduction to emodin. Raistrick *et al.* [1934] have shown that this

$R = H$, *Frangula*-emodin.
 $R = CH_3$, physcion.

ω -Hydroxyemodin.

$R = H$, catenarin.
 $R = CH_3$, erythroglaucin.

fluorescence, among polyhydroxyanthraquinones, is characteristic of derivatives of quinizarin, 1:4-dihydroxyanthraquinone. Only IV and V satisfy this condition.

(c) Catenarin is not a strong mordant dye and hence cannot be a derivative of alizarin, 1:2-dihydroxyanthraquinone. Only IV satisfies this condition.

(d) Charles *et al.* [1933] showed that treatment of catenarin tetra-acetate with chromic acid in acetic anhydride-acetic acid solution failed to oxidize the side chain to carboxyl and we have confirmed this observation. Several workers have attempted to carry out a similar oxidation of the acetates of polyhydroxy-methylanthraquinones with the following results.

(I) *Successful oxidations.* (1) *Frangula*-emodin, 4:5:7-trihydroxy-2-methylanthraquinone, to emodic acid, 4:5:7-trihydroxyanthraquinone-2-carboxylic

acid [Fischer & Gross, 1911; Anslow *et al.* 1940]. (2) ω -Hydroxyemodin 4:5:7-trihydroxy-2(hydroxymethyl)-anthraquinone, to emodic acid [Anslow *et al.* 1940]. (3) Helminthosporin, 4:5:8-trihydroxy-2-methylanthraquinone to helminthosporic acid, 4:5:8-trihydroxyanthraquinone-2-carboxylic acid [Charles *et al.* 1933].

(II) *Unsuccessful oxidations.* (1) Morindone, 1:5:6-trihydroxy-2-methylanthraquinone [Simonsen, 1918]. (2) Cynodontin, 1:4:5:8-tetrahydroxy-2-methylanthraquinone [Raistrick *et al.* 1933]. The accepted explanation for these differences is that the presence of a nuclear hydroxyl group adjacent to a methyl or hydroxymethyl group prevents the oxidation taking place, e.g. morindone and cynodontin, while oxidation proceeds smoothly in compounds not containing a so-placed nuclear hydroxyl group, e.g. emodin, ω -hydroxyemodin and helminthosporin. Only III and IV contain a nuclear hydroxyl group adjacent to the methyl group.

(e) 1:4:5:7-Tetrahydroxy-6-methylanthraquinone has been synthesized by Charlesworth & Robinson [1934] by an unequivocal method. This substance is not identical with catenarin but it gives colour reactions which are so similar to those given by catenarin as to justify the belief that the four hydroxyl groups are in the same positions in the two compounds. Thus to quote Charlesworth & Robinson "The following are the points of divergence noted. The colour in H_2SO_4 is slightly bluer than that of catenarin. Alkaline solutions have identical colours, but those of catenarin are more rapidly oxidized in air. The orange alcoholic solution of the synthetic compound exhibits a very feeble fluorescence, that of catenarin being much more pronounced. The synthetic compound is slightly less soluble in aqueous alcohol." There are only four possible 1:4:5:7-tetrahydroxy-methylanthraquinones and of these only the one having the methyl group in position 2 (IV) could possibly give *Frangula*-emodin on removal of one hydroxyl group by reduction.

Hence it is seen that, of the four possible structures for catenarin, i.e. III, IV, V and VI, only IV satisfies all the conditions laid down in (a), (b), (c), (d) and (e) above. The final proof that IV is in fact the correct structural formula for catenarin was obtained as follows. Catenarin tetramethyl ether (VII) was oxidized with chromic acid in acetic acid-acetic anhydride solution and 10% of 4-methyl-3:6-dimethoxyphthalic anhydride (VIII) and 2% of 3:5-dimethoxyphthalic anhydride (IX) were isolated from the products of oxidation.

The origin of VIII and IX from VII, by cleavage at the dotted lines, is clear and further it is obvious that, of the four possible structures III, IV, V and VI, only the tetramethyl ether of IV could possibly give rise to VIII and IX.

Finally, although it is theoretically possible to couple together the nuclei in VIII and IX to give the tetramethyl ether of a polyhydroxyanthraquinone of structure X, catenarin cannot have this structure since X could not give *Frangula*-emodin (I, $R = H$) on reduction.

Catenarin is therefore to be regarded as *1:4:5:7-tetrahydroxy-2-methylanthraquinone* (IV). The hydroxyl group in catenarin which is removed on reduction with HI to emodin is thus the one in position 1.

4-Methyl-3:6-dimethoxyphthalic anhydride, previously unknown, was synthesized, for comparison with the degradation product from catenarin tetramethyl ether, by a method similar to that used by Thiele & Meisenheimer [1900] and Thiele & Gunther [1906] for the synthesis of 3:6-dimethoxyphthalic anhydride from benzoquinone. Toluquinone was treated with nascent HCN in ethanol to give *2:5-dihydroxy-3:4-dicyanotoluene* which on methylation gave *2:5-dimethoxy-3:4-dicyanotoluene*. This substance, on hydrolysis with H_2SO_4 , yielded directly *4-methyl-3:6-dimethoxyphthalic anhydride* (VIII).

Ashley *et al.* [1939] isolated from the mycelium of a large number of species in the *Aspergillus glaucus* series two polyhydroxyanthraquinones, i.e. physcion, *4:5-dihydroxy-7-methoxy-2-methylanthraquinone* (I, $R=\text{CH}_3$) and a hitherto undescribed mould metabolic product, erythroglaucon. Erythroglaucon, $\text{C}_{16}\text{H}_{12}\text{O}_6$, was shown to be a monomethyl ether of a tetrahydroxy-methylanthraquinone, the fact that it contains a $-\text{CH}_3$ and not a $-\text{CH}_2\text{OH}$ side-chain following from a Kuhn-Roth estimation. We have now shown that *catenarin tetramethyl ether* is identical with erythroglaucon trimethyl ether. Hence it follows that erythroglaucon must be a monomethyl ether of catenarin and since catenarin is soluble in dilute aqueous Na_2CO_3 and in dilute aqueous ammonia while erythroglaucon is quite insoluble in these reagents it appeared probable that it is the hydroxyl group in position 7 in catenarin which appears as a methyl ether grouping in erythroglaucon. This conclusion, which is based on the well known and generally accepted belief that among the polyhydroxyanthraquinones only those possessing a hydroxyl group in a β position are soluble in dilute aqueous Na_2CO_3 , has been confirmed by the conversion of catenarin into erythroglaucon. The possibility that catenarin contains a β -hydroxyl group was discussed by Raistrick *et al.* [1934], but was rejected, despite the solubility of catenarin in aqueous Na_2CO_3 , since it was shown that catenarin is unaffected by heating for 8 hr. at 100° with methyl iodide and sodium methoxide in methanol. In this experiment a large excess of sodium was used, the proportions of reagents being catenarin (0.4 g.), methyl iodide (1 g. $\equiv$ 5.0 mol.) and sodium (1 g. $\equiv$ 31.0 mol. dissolved in methanol, 10 ml.). We have now found that if the proportions of reagents are altered to catenarin ($\equiv$ 1 mol.), methyl iodide ($\equiv$ 4 mol.) and sodium ($\equiv$ 4 mol.) erythroglaucon is formed in good yield. The same method was used for the conversion of *Frangula-emodin* (I, $R=\text{H}$) into its 7-methyl ether, physcion (I, $R=\text{CH}_3$) by Jowett & Potter [1903] and by Tutin & Clewer [1910]. There can thus be no reasonable doubt that erythroglaucon is the 7-methyl ether of catenarin and that it is in fact *1:4:5-trihydroxy-7-methoxy-2-methylanthraquinone*. Hence erythroglaucon (IV, $R=\text{CH}_3$) bears exactly the same relationship to catenarin (IV, $R=\text{H}$) as does physcion (I, $R=\text{CH}_3$) to *Frangula-emodin* (I, $R=\text{H}$).

EXPERIMENTAL

Two highly purified specimens of catenarin were used during this work.

We prepared the first specimen from a commercial sample of catenarin, m.p. 230–232°, which was isolated from the mycelium of *Helminthosporium gramineum* Rabenhorst L.S.H.T.M. Cat. No. Ag 106 [Charles *et al.* 1933] and was kindly given to us by Mr M. Barrowcliff, research manager of British Dyestuffs Corporation Ltd. The original sample was purified by acetylation with acetic anhydride and conc. H_2SO_4 , hydrolysis of the purified acetate with aqueous 2*N* NaOH in an atmosphere of nitrogen and crystallization from ethanol of the regenerated catenarin. The purified specimen formed large, glistening, deep red plates which melted sharply at 246° (Raistrick *et al.* [1934] give 246°). (Found, on material dried at 110°: C, 62.95; H, 3.55%. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_6$: C, 62.91; H, 3.52%.) On oxidation with chromic acid (Kuhn-Roth method) evidence of one side-chain methyl group was obtained. (Found: 102.4, 99.5% of 1 mol. CH_3COOH .)

The second specimen was prepared for us by our colleague Dr J. Breen by a much simpler method. The crude mixture of catenarin and helminthosporin (22.4 g.) obtained by ether and chloroform extraction of the dried mycelium (110 g.) of *Helminthosporium catenarium* Drechsler, L.S.H.T.M. Cat. No. Ag 127 [Raistrick *et al.* 1934] was vigorously stirred for 30 min. with cold 2% aqueous Na_2CO_3 (5½ litres). The deep purple solution was filtered from undissolved helminthosporin (3.8 g.), acidified with HCl and heated to flocculate the gelatinous precipitate of catenarin. This was filtered, washed and dried and gave pure catenarin (17.5 g.) after one crystallization from ethanol.

Reduction of catenarin. Formation of Frangula-emodin

A mixture of catenarin (1 g.), glacial acetic acid (20 ml.), HI (4 ml. sp.gr. 1.7) and red phosphorus (1 g.) was boiled under a reflux condenser for 5 hr. The mixture was cooled, poured into water (200 ml.) and decolorized by the addition of a slight excess of aqueous NaHSO_3 . The product which separated (1.38 g.) was collected and extracted with boiling glacial acetic acid (150 ml.). The reduction product separated as pale yellow micro-crystalline rectangular plates (0.68 g.) which decomposed with intense blackening from 250° upwards and had all the other properties of emodin anthranol. This product (0.68 g.) was dissolved in boiling glacial acetic acid (150 ml.), the solution cooled to 60°, and a solution of CrO_3 (0.68 g.) in water (10 ml.) and acetic acid (10 ml.) was quickly added. The dark brown reaction mixture was held at 60° for 30 min. and then evaporated *in vacuo* to 25 ml. The cinnamon-coloured broad needles which separated were collected, washed well with acetic acid and dried (0.35 g.; m.p. 253–254°). On crystallization from toluene (norite) it was obtained as fine, dull orange needles (0.23 g.; m.p. 257°). A mixture with authentic *Frangula-emodin* (m.p. 254–255°) melted at 255–256°. (Found: C, 66.60; H, 3.60%. Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_5$: C, 66.64; H, 3.73%.) Specimens of the product from catenarin and of *Frangula-emodin* gave identical reactions with the following reagents: aqueous 2% NaHCO_3 , insol.; 2% Na_2CO_3 , red solution; 2*N* NaOH, red solution with a bluish shade; conc. H_2SO_4 , red solution with a bluish shade; glacial acetic acid, pure yellow, non-fluorescent solution. These reactions are quite different from those given by catenarin which, *inter alia*, gives an orange solution with a marked greenish fluorescence in acetic acid.

The identity of the product from catenarin with *Frangula-emodin* was confirmed by conversion into the trimethyl ether. To a boiling solution of the

product from catenarin (0.15 g.) in acetone (10 ml.) and dimethyl sulphate (3 ml.), 2*N* NaOH (17 ml.) was added in portions of 2–3 ml. After the addition of 12 ml. of NaOH the methylated product separated as a voluminous mass. The methyl ether was collected (0.18 g.) and crystallized first from ethyl acetate and then from acetone. Canary yellow rods, m.p. 225°, not depressed on admixture with authentic emodin trimethyl ether (m.p. 225°), prepared from *Frangula*-emodin by the same method. (Fischer *et al.* [1911], also give 225°.) (Found: C, 69.00, 69.16; H, 5.24, 5.18; CH₃O, 29.5, 29.7%. Calc. for C₁₈H₁₆O₅: C, 69.20; H, 5.17; 3CH₃O, 29.8%.) The two products dissolve in conc. H₂SO₄ to give identical permanganate-coloured solutions which are quite different from, and much bluer in tone than, that given by emodin itself.

Oxidation of tetra-acetyl catenarin

The experimental conditions used were the same as those followed successfully by Anslow *et al.* [1940] for the oxidation of triacetyl emodin and tetra-acetyl ω -hydroxyemodin, a yield of 70–80% of triacetyl emodic acid being obtained in each of these cases. Tetra-acetyl catenarin (1 g.) was dissolved in a mixture of glacial acetic acid (20 ml.) and acetic anhydride (20 ml.) at 50–60° and, while maintaining this temperature, a solution of chromic anhydride (2 g.) in water (1.6 ml.) and glacial acetic acid (20 ml.) was added drop by drop during 30 min. The temperature was then raised and held at 65–70° for 3 hr. The reaction mixture, which slowly became deep green in colour, was cooled, and poured into water (600 ml. at 50°), when there was immediate separation of pale yellow fine needles, which were insoluble in 2% aqueous NaHCO₃. Weight 0.39 g., m.p. 232°, mixed m.p. with authentic tetra-acetyl catenarin, 234–235°. It was confirmed that this material was unchanged tetra-acetyl catenarin by regenerating the parent hydroxyanthraquinone which, after crystallizing from ethanol, melted at 243–244° and did not depress the m.p. of pure catenarin, m.p. 246°. The green filtrate from the recovered tetra-acetyl catenarin was evaporated *in vacuo* to about 100 ml. and was exhaustively extracted with ether in a continuous extractor. On removal of the solvent a small amount of gummy material remained (0.09 g.) which was obviously not an anthraquinone carboxylic acid. Hence, although considerable oxidation had obviously taken place this did not result in the formation of the carboxylic acid corresponding to catenarin, as occurs with emodin, ω -hydroxyemodin and helminthosporin, but in extensive breakdown of the molecule as a whole.

Catenarin tetramethyl ether

Catenarin cannot be satisfactorily methylated with methyl sulphate and NaOH either in aqueous or in boiling acetone solution by the method which is very suitable for the methylation of emodin (see above). The following method was therefore used. Catenarin (1 g.) was dissolved in boiling acetone (50 ml.) and methyl sulphate (5 ml.) and anhydrous K₂CO₃ (5 g.) were added in 1 ml. and 1 g. portions at suitable intervals during 6 hr. boiling under reflux. The mineral salts were separated by filtration and were thoroughly extracted with acetone. Evaporation of the solvent gave 0.84 g. of *catenarin tetramethyl ether*, m.p. 189–190°, raised to 190–191°, by recrystallization from acetone from which it separated in golden-yellow leaflets. (Found: C, 66.49, 66.60; H, 5.28, 5.21; CH₃O, 36.1, 35.9%. C₁₈H₁₈O₆ requires C, 66.63; H, 5.30; 4CH₃O, 36.3%.) Catenarin tetramethyl ether does not depress the m.p. of erythroglauca trimethyl ether (Ashley *et al.* [1939], m.p. 187–188°). Both substances are insoluble in aqueous 2*N* NaOH and both give a deep pure blue solution in conc. H₂SO₄.

Oxidation of catenarin tetramethyl ether. Isolation of 4-methyl-3:6-dimethoxyphthalic anhydride and 3:5-dimethoxyphthalic anhydride

Catenarin tetramethyl ether (3 g.) was dissolved in a mixture of acetic anhydride (30 ml.) and glacial acetic acid (20 ml.). To this solution, held in a vigorously boiling water bath, there was added during 15 min. and with mechanical stirring, a solution of CrO_3 (7.2 g.) in water (4.8 ml.) and glacial acetic acid (30 ml.). The heating and stirring were continued for a further 15 min.

The dark green reaction mixture was cooled, evaporated *in vacuo* to a syrup (20–25 ml.) and held at 0° for 1 hr. The syrup, now containing crystals, was triturated with four successive 200 ml. amounts of dry ether. The combined ethereal extracts, separated from the green solid (A), were evaporated to low bulk and finally to 5 ml. *in vacuo*. On standing, crystals separated which were filtered, washed with acetic anhydride and then with ether and dried. Treatment of the combined mother liquors and washings (B) is described later. The crystals (0.23 g., m.p. $196\text{--}198^\circ$) were recrystallized from benzene in pale yellow needles, m.p. 202° , of 4-methyl-3:6-dimethoxyphthalic anhydride. This specimen, a synthetic specimen (see below) and a mixture of the two, all melted at $202^\circ \pm 0.5^\circ$. The oxidation product also showed the same violet-blue fluorescence in benzene and green fluorescence in conc. H_2SO_4 as does the synthetic product. (Found, on the oxidation product: C, 59.61, 59.58; H, 4.62, 4.51; CH_3O , 27.9, 27.6%. $\text{C}_{11}\text{H}_{10}\text{O}_5$ requires C, 59.43; H, 4.54; $2\text{CH}_3\text{O}$, 27.9%.) On oxidation with chromic acid (Kuhn-Roth method) evidence of one side-chain CH_3 group was obtained, i.e. 102.7, 99.2% of 1 mol. CH_3COOH . The dried green solid (A) was exhaustively extracted (Soxhlet) with ether to give 0.17 g. of a yellow solid from which, after sublimation in a high vacuum followed by crystallization from benzene, a further 0.07 g., m.p. $199\text{--}201^\circ$, of 4-methyl-3:6-dimethoxyphthalic anhydride was obtained. Total yield, 0.30 g.

The combined mother liquors and washings (B) were evaporated to dryness *in vacuo* to yield a gum from which, by trituration with ether followed by crystallization from benzene, 0.12 g. of unchanged catenarin tetramethyl ether, m.p. $186\text{--}188^\circ$, was separated. The mother liquors from this substance were dried and sublimed in a high vacuum to give 0.18 g. of a yellow micro-crystalline sublimate with an indefinite m.p. and consisting essentially of a mixture of 3:5-dimethoxyphthalic anhydride and 4-methyl-3:6-dimethoxyphthalic anhydride. These two substances were separated by dissolving the mixture in hot 2N NaOH (3 ml.) and acidifying the cooled solution with HCl. 3:5-Dimethoxyphthalic acid separated first in thick compact prisms (0.09 g.) which are readily distinguished from 4-methyl-3:6-dimethoxyphthalic acid which separated later in fine thin needles. The 3:5-dimethoxyphthalic acid was heated for a few minutes at 170° when it melted and gave off water. The resulting 3:5-dimethoxyphthalic anhydride was purified by sublimation in a high vacuum. It was thus obtained in colourless micro-needles (0.06 g.). This specimen, an authentic specimen of 3:5-dimethoxyphthalic anhydride and a mixture of the two all melted at $147\text{--}148^\circ$ (lit. 147°). (Found: C, 57.73, 57.82; H, 3.87, 3.90; CH_3O , 29.9, 29.5%. $\text{C}_{10}\text{H}_8\text{O}_5$ requires C, 57.68; H, 3.88; $2\text{CH}_3\text{O}$, 29.8%.)

Synthesis of 4-methyl-3:6-dimethoxyphthalic anhydride

(a) *2:5-Dihydroxy-3:4-dicyanotoluene*. To a solution of toluquinone (10 g.) in ethanol (300 ml.), cooled in ice, conc. H_2SO_4 (12.5 ml.) in ethanol (25 ml.) was added. A solution of KCN (30 g.) in water (60 ml.) was cautiously added, with vigorous stirring, until the mixture became brown in colour and alkaline

in reaction. The mixture was then made slightly acid by the addition of alcoholic H_2SO_4 and after 1–2 hr. was evaporated *in vacuo* to remove the ethanol and excess HCN . The sticky residue, consisting of a mixture of 2:5-dihydroxy-3:4-dicyanotoluene, toluquinol and K_2SO_4 , was diluted with water (500 ml.) and the dark tarry matter which separated (0.27 g.) was filtered off and discarded. The filtrate was extracted with ether and the dark crystalline residue (11–12 g.) remaining on removal of the solvent and consisting of a mixture of 2:5-dihydroxy-3:4-dicyanotoluene and toluquinol was ground with three 100 ml. lots of cold chloroform which removed the toluquinol, the crude 2:5-dihydroxy-3:4-dicyanotoluene remaining as a buff-coloured powder (33.2 g. from 60 g. of toluquinone = 77.6 % theor.).

2:5-Dihydroxy-3:4-dicyanotoluene cannot be satisfactorily crystallized from any of the customary solvents but is readily characterized by its crystalline diacetate and dimethyl ether (see below). A specimen was purified for analysis by fractional sublimation in a high vacuum at 140–150° and was so obtained in pale yellow micro-crystals which have no definite m.p. but darken from 195° and become completely black at about 240°. (Found: C, 61.88; H, 3.42; N, 16.1 %. $\text{C}_9\text{H}_6\text{O}_2\text{N}_2$ requires C, 62.05; H, 3.48; N, 16.1 %.) The substance is readily soluble in ethanol, ethyl acetate, ether and acetone but is almost insoluble in chloroform, benzene and toluene. Unlike the corresponding benzene compound it is fairly readily soluble in cold water and aqueous solutions give the following reactions: (a) With FeCl_3 , a violet colour changing to brown on standing. (b) With dilute NaOH , the colourless solution, which shows a blue fluorescence, becomes yellow with an intense green fluorescence.

(b) 2:5-Diacetoxy-3:4-dicyanotoluene. Crude 2:5-dihydroxy-3:4-dicyanotoluene (2 g.) was dissolved in a mixture of acetic anhydride (20 ml.) and conc. H_2SO_4 (1 ml.). The acetylation mixture, after standing for a short time, was poured into iced water and the precipitated acetate (2.3 g.) was crystallized from ethanol. 2:5-Diacetoxy-3:4-dicyanotoluene crystallizes in long colourless rods, m.p. 128°. (Found: C, 60.31; H, 4.00; N, 10.85 %. $\text{C}_{13}\text{H}_{10}\text{O}_4\text{N}_2$ requires C, 60.43; H, 3.90; N, 10.85 %.) Solutions of the substance in benzene and still more in ethanol show, in the light from a mercury vapour lamp, a blue fluorescence which is not visible in daylight.

(c) 2:5-Dimethoxy-3:4-dicyanotoluene. The crude mixture (11–12 g.) of 2:5-dihydroxy-3:4-dicyanotoluene and toluquinol from toluquinone (10 g.) (see section (a), above) was dissolved in acetone (150 ml.) and methyl sulphate (30 ml.). Aqueous 2*N* NaOH (150 ml.) was added in portions to the solution which was boiled for 30 min. 2:5-Dimethoxy-3:4-dicyanotoluene crystallized from the cooled mixture and was separated from the oily toluquinol dimethyl ether by filtration. It was washed with chilled 50 % aqueous acetone (100 ml.) followed by glacial acetic acid (50 ml.) and was so obtained as colourless needles (5.5 g.; m.p. 182°) which may be converted into the anhydride without further purification. It crystallizes with very little loss from glacial acetic acid in long colourless needles, m.p. 182°. (Found: C, 65.13; H, 4.90; N, 13.8; CH_3O , 30.4 %. $\text{C}_{11}\text{H}_{10}\text{O}_2\text{N}_2$ requires C, 65.31; H, 4.99; N, 13.9; $2\text{CH}_3\text{O}$, 30.7 %.) Solutions of the substance in ethyl acetate, glacial acetic acid, ethanol and benzene are colourless with no apparent fluorescence in daylight but all show a faint blue fluorescence in the light from a mercury vapour lamp.

(d) 4-Methyl-3:6-dimethoxyphthalic anhydride. 2:5-Dimethoxy-3:4-dicyanotoluene (2 g.) was dissolved in 20 ml. of a mixture of conc. H_2SO_4 (200 ml.) and water (20 ml.) and hydrolysed by heating in a boiling water bath for 20 min. The solution quickly became orange-yellow in colour with a strong green

fluorescence. It was cooled and poured into water (20 ml.) when there was a copious separation of pale yellow needles from the hot solution. A further 20 ml. of water were added, the mixture cooled and held at 0° for 2 hr. The crystals were separated by filtration, washed well with cold water and dried. Weight of almost pure anhydride from six such experiments, i.e. from 12 g. of dinitrile, 6.1 g. The yield of anhydride decreased on longer hydrolysis and was very small after 1.5 hr. (0.1 g. from 2 g.). The filtrate and washings, on evaporation on a boiling water bath, gave 3.2 g. of a mixture of crystalline substances from which a further 2.3 g. of the anhydride were isolated by extraction with aqueous 5% NaHCO₃ and fractional crystallization from benzene. *4-Methyl-3,6-dimethoxyphthalic anhydride* crystallizes well from glacial acetic acid or benzene in long pale yellow needles, m.p. 202°, which readily sublime in a high vacuum at 135°. (Found: C, 59.56; H, 4.49; N, nil; CH₃O, 27.9%. C₁₁H₁₀O₅ requires C, 59.43; H, 4.54; 2CH₃O, 27.9%.) The substance does not dissolve in cold 2*N* NaOH except on long standing, but quickly dissolves on heating. Dilute solutions in ethyl acetate, ethanol, glacial acetic acid or benzene have a strong violet-blue fluorescence. Solutions in cold conc. H₂SO₄ are yellow, with a strong green fluorescence.

*Methylation of catenarin with methyl iodide and sodium methoxide.
Formation of erythroglauцин*

A mixture of catenarin (1.144 g. $\equiv$ 1/250 mol.), methyl iodide (2.272 g. $\equiv$ 4/250 mol.) dissolved in methanol (10 ml.), and 10 ml. of a solution of Na (1.84 g.) in methanol (50 ml.), i.e. 0.368 g. Na $\equiv$ 4/250 mol., was heated in a sealed glass tube at 100° for 4 hr. The brownish purple reaction mixture was poured into water (200 ml.), acidified with HCl, filtered, washed and dried. The crude reaction product (1.09 g.) was crystallized from glacial acetic acid (100 ml. + norite) and gave 0.56 g. of almost pure *catenarin 7-methyl ether*, m.p. 197–200°. This material was recrystallized several times from glacial acetic acid to constant m.p. Pure catenarin 7-methyl ether was finally obtained in this way as large shining bright red plates, m.p. 205–206°. An authentic specimen of erythroglauцин, isolated from *Aspergillus ruber* (Spieckermann and Bremer) Thom and Church, L.S.H.T.M. Cat. No. A. 22, by Ashley *et al.* [1939] and recrystallized from acetic acid, melted at 205–206°, and a mixture with catenarin 7-methyl ether melted at the same temperature. (Found, on catenarin 7-methyl ether: C, 63.85, 63.93; H, 4.00, 4.08; CH₃O, 10.05, 10.1%. C₁₆H₁₂O₆ requires C, 63.98; H, 4.03; CH₃O, 10.3%.)

Catenarin 7-methyl ether and natural erythroglauцин, compared side by side, gave identical reactions with the following reagents. (a) Glacial acetic acid, an orange-red solution with a green fluorescence. (b) Conc. H₂SO₄, a solution which is pure blue in colour in very dilute solution but becomes a deep violet-blue by transmitted light in more concentrated solutions. (c) Aqueous *N* NaOH. The red crystals immediately become dark purple in colour and slowly dissolve to a permanganate-coloured solution which, on standing, slowly deposits a violet precipitate. (d) Aqueous 2% NaHCO₃, aqueous 2% Na₂CO₃ or aqueous 2*N* NH₄OH, quite insoluble. The behaviour towards aqueous Na₂CO₃ and aqueous NH₄OH is in marked contrast to that of catenarin which dissolves in 2% Na₂CO₃ giving a cerise solution and in 2*N* NH₄OH to a reddish violet solution.

Triacetylcatenarin-7-methyl ether. Catenarin-7-methyl ether (0.08 g., m.p. 205–206°) was dissolved in 4 ml. of a mixture of pyridine (10 ml.) and acetic anhydride (2 ml.) and held at 37° for 24 hr. The acetate (0.09 g.), which was

precipitated on pouring the acetylation mixture into water, was crystallized from ethanol. Yellow rods, m.p. 229–230°. (Found: C, 62.22, 62.01; H, 4.45, 4.12; CH_3O , 7.3, 7.1%. $\text{C}_{22}\text{H}_{18}\text{O}_9$ requires C, 61.95; H, 4.26; CH_3O , 7.3%.) Triacetylerythroglaucin, prepared similarly, melted at 230–231° and a mixture of the two substances at 230–231°.

SUMMARY

The molecular constitutions of catenarin and erythroglaucin have been determined.

Catenarin, $\text{C}_{15}\text{H}_{10}\text{O}_6$, which constitutes over 15% of the dry weight of the mycelium of *Helminthosporium catenarium* Drechsler, gives *Frangula*-emodin, 4:5:7-trihydroxy-2-methylanthraquinone, on reduction with HI and red phosphorus, followed by oxidation with chromic acid of the resulting anthranol. *Catenarin tetramethyl ether* on oxidation with chromic acid yields a mixture of 4-methyl-3:6-dimethoxyphthalic anhydride (the synthesis of which is described) and 3:5-dimethoxyphthalic anhydride. These, and other experimental facts previously established, determine the constitution of catenarin as 1:4:5:7-tetrahydroxy-2-methylanthraquinone.

Erythroglaucin, $\text{C}_{16}\text{H}_{12}\text{O}_6$, a metabolic product of species in the *Aspergillus glaucus* series, has been shown to be the 7-methyl ether of catenarin, i.e. 1:4:5-trihydroxy-7-methoxy-2-methylanthraquinone. It was prepared, in good yield, by methylating catenarin with methyl iodide and sodium methoxide in methanol solution.

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