

19. *The Chemistry of Extractives from Hardwoods. Part V.* The Isolation of 3 : 7 : 4'-Trimethylquercetin (Ayanin) from the Heartwood of Distemonanthus Benthamianus.*

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From the heartwood of the tree *Distemonanthus Benthamianus* a mixture of yellow colouring matters has been extracted. One of the constituents (ayanin) has been shown by degradation and synthesis to be 5 : 3'-dihydroxy-3 : 7 : 4'-trimethoxyflavone, a hitherto unknown derivative of quercetin.

THE West African tree, *Distemonanthus Benthamianus*, is the source of a commercial timber known variously as ayan, movingui, or Nigerian satinwood. The heartwood is pale to bright yellow with a lustrous surface, and being moderately durable and termite-resistant it finds many applications in furniture-making and joinery. It is reported to have acid-resisting properties, and also to stain in contact with sodium carbonate, but these observations do not appear to have been followed by any chemical examination of the timber. This communication presents the preliminary results of an investigation of the material extracted by means of organic solvents, which show that the heartwood contains a mixture of yellow colouring matters believed from their general properties to belong to the flavone series. Only one of the component compounds has so far been

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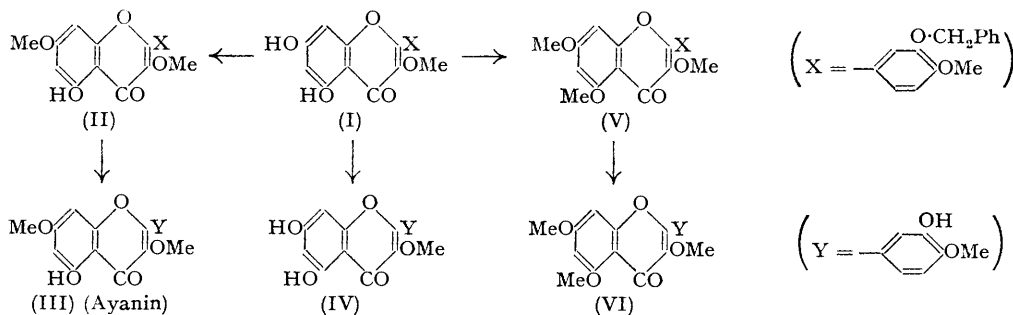
isolated in quantity sufficient to allow of its complete recognition; work on the accompanying pigments is continuing.

The identified constituent—termed ayanin in reference to the name commonly applied to the wood—is a hitherto unknown trimethyl ether of quercetin, namely, 5:3'-dihydroxy-3:7:4'-trimethoxyflavone. It was obtained from the heartwood by the usual sequence of extractions with boiling light petroleum, ether, and chloroform. The new colouring matter was present in all three solvent extracts, the combined yields being approximately 0.1% of the timber.

The molecular formula, $C_{18}H_{16}O_7$, for ayanin includes three methoxyl groups (Zeisel). The phenolic character of the pigment was evident from its solubility in aqueous sodium carbonate and hydroxide, but not in sodium hydrogen carbonate, to give dark orange solutions, and the presence of two hydroxyl groups was shown by the preparation of a diacetate. Indications of the nuclear structure were afforded by crimson colour reactions with magnesium and hydrochloric acid and with sodium amalgam followed by acid, denoting a flavonol nucleus with a blocked, *e.g.*, methylated, 3-hydroxyl group (Asahina and Inubuse, *Ber.*, 1928, **61**, 1646; 1929, **62**, 1256; Briggs and Locker, *J.*, 1949, 2158). The ultra-violet light absorption of ayanin solutions resembles typical flavonol spectra except for the absence of the maximum at 3100 Å characteristic of the free hydroxyl in the 3-position (Skarzynski, *Biochem. Z.*, 1939, **301**, 150), thus supporting the conclusions drawn from the colour reactions.

Evidence of the flavonol structure and, incidentally, of the orientation of the five substituent oxygen atoms was also found in the hydrolysis of ayanin with boiling hydriodic acid to a pentahydroxyflavone which gave an acetate apparently identical with pentaacetylquercetin. The relation to quercetin was confirmed by methylation experiments: either a tetramethoxy-compound, or under more vigorous conditions, a pentamethoxyflavone resulted, and the identity of these products was conclusively established by comparison with the corresponding derivatives prepared from an authentic specimen of quercetin. Moreover, from its reluctance to undergo complete methylation, which is, in general, a property of all 5-hydroxyflavones, etc., it was apparent that ayanin contained a free phenolic group at the 5-position.

The distribution of the remaining substituents finally became apparent as the result of the degradation of ayanin by ozonolysis. Alkaline hydrolysis of the product, presumably an aroylated *o*-hydroxybenzoylformic ester, led to the isolation of *isovanillic* acid. This acid was also formed by the hydrolysis of ayanin with aqueous-alcoholic potassium hydroxide. The definite location of two further substituents in this way, combined with the information already gained regarding the nature of the groups at the 3- and the 5-position, completely determined the constitution of ayanin which was thereby identified as 5:3'-dihydroxy-3:7:4'-trimethoxyflavone (III).



The conclusions thus drawn from analytical data have been confirmed by a synthesis based on the general method of Allan and Robinson (*J.*, 1924, 2192). The necessary flavonol intermediate, 3'-benzyloxy-5:7-dihydroxy-3:4'-dimethoxyflavone (I), was prepared in excellent yield from ω -methoxyphloracetophenone and a mixture of the anhydride and sodium salt of benzylisovanillic acid, the fusion being carried out under

reduced pressure. Treatment in acetone solution with potassium carbonate and a limited amount of methyl sulphate caused methylation only of the 7-hydroxy group, giving 3'-benzyloxy-5-hydroxy-3 : 7 : 4'-trimethoxyflavone (II). Hydrolysis of the benzyl group with mixed hydrochloric and acetic acids completed the synthesis, the resulting dihydroxy-trimethoxyflavone (III) being identical with ayanin, as were also the respective diacetates. The availability of the intermediate (I) was utilised in the synthesis of two further new methyl ethers of quercetin. Of these, 5 : 7 : 3'-trihydroxy-3 : 4'-dimethoxyflavone (IV) was prepared simply by acid hydrolysis of the benzyl ether (I). To obtain 3'-hydroxy-3 : 5 : 7 : 4'-tetramethoxyflavone (VI), the parent compound (I) was first alkylated with excess of methyl sulphate thus forming the benzyl tetramethyl ether (V) from which the benzyl group was then removed by mild hydrolysis.

EXPERIMENTAL

Ayanin.—The ayan heartwood used in these experiments was supplied by the Forest Products Research Laboratory, Princes Risborough, through the kindness of Dr. W. P. K. Findlay. The powdered wood (2 kg.) was first extracted with boiling light petroleum (b. p. 40—60°) and, of the material thus obtained (2.1 g.), the small alkali-soluble portion (0.05 g.) was subsequently identified as ayanin. Boiling ether then removed a brownish-yellow friable solid (28.2 g.) which was heated with methanol (200 c.c.) under reflux for 1 hour. When the insoluble matter (0.4 g.) (A) had been removed, the solution was concentrated to 50 c.c. and left at room temperature for 1—2 weeks. The crystalline deposit (3 g.) which had then formed consisted of crude ayanin. When it had been collected, a further product (5 g.) (B) was obtained by concentrating the filtrate to 35 c.c. and setting it aside for several days.

The chloroform extract also consisted of a brittle light brown substance (23.1 g.) which was likewise digested with boiling methanol (100 c.c.). The residue (1 g.) (C) was collected and, after the filtrate had been concentrated to 50 c.c. and set aside for some time, a further quantity (3.7 g.) of impure ayanin was obtained. By repeated crystallisation of the combined crude fractions, pure *ayanin* (2 g.) was obtained as orange-yellow plates, m. p. 172—173° [Found, in the material dried at 110° in a vacuum : C, 62.6; H, 4.7; OMe, 27.5. $C_{15}H_7O_4(OMe)_3$ requires C, 62.8; H, 4.6; OMe, 27.0%]. Light absorption in methanol : maxima at 2540 (log $\epsilon = 3.93$), 2710 (log $\epsilon = 3.93$), and 3380 Å (log $\epsilon = 3.88$). Ayanin is sparingly soluble in ether, readily soluble in acetone, chloroform, and the simple alcohols. It dissolved in concentrated hydrochloric acid to a bright yellow solution from which it was precipitated by water. In alcoholic solution it gave a brownish-green colour with ferric chloride. Acetylation of ayanin with acetic anhydride-pyridine or acetic anhydride-sodium acetate afforded the *diacetyl* derivative which separated from methanol in colourless leaflets, m. p. 176—177° (Found, in a specimen dried at 100° in a vacuum : C, 60.8; H, 4.6; OMe, 21.7. $C_{22}H_{20}O_9, \frac{1}{2}H_2O$ requires C, 60.4; H, 4.8; OMe, 21.3%). The investigation of products A, B, and C is in progress.

Demethylation of Ayanin.—When the solution obtained by heating ayanin (0.1 g.) with hydriodic acid (6 c.c.; *d* 1.7) and phenol (1.5 g.) at 160° for 4 hours was poured into water, a precipitate of quercetin was obtained. This was collected and acetylated with acetic anhydride-sodium acetate to give penta-acetylquercetin which crystallised from ethanol as leaflets, m. p. and mixed m. p. 193—194°.

5-Hydroxy-3 : 7 : 3' : 4'-tetramethoxyflavone (O-Monomethylayanin).—A solution of ayanin (0.2 g.) in methanol (4 c.c.) was treated with excess of ethereal diazomethane. After 2 days, the solution was evaporated and the gummy residue triturated with aqueous sodium hydroxide until solid. The product (0.15 g.) crystallised from methanol in long flat lemon-coloured leaflets, m. p. 158—159°, alone or mixed with 3 : 7 : 3' : 4'-tetramethylquercetin. The respective acetyl derivatives, m. p. 168—169°, were also shown to be identical.

3 : 5 : 7 : 3' : 4-Pentamethoxyflavone (OO-Dimethylayanin).—Ayanin (0.2 g.) was heated under reflux with methyl sulphate (1 c.c.) and potassium carbonate (2 g.) in dry acetone (30 c.c.) for 8 hours. The filtered acetone solution was evaporated and the yellow gummy residue stirred with aqueous sodium hydroxide. The solid residue, repeatedly crystallised from methanol, gave colourless plates having m. p. 152.5°, which was not depressed by pentamethylquercetin (Found, in a specimen dried at 100° in a vacuum : C, 64.7; H, 5.4; OMe, 40.6. Calc. for $C_{20}H_{20}O_7$: C, 64.5; H, 5.4; OMe, 41.4%).

Degradation of Ayanin.—(i) Diacetylavanin (0.53 g.) was dissolved in ethyl acetate (45 c.c.), and the solution treated at 0° with excess of ozonised oxygen. When the solvent had been removed at low temperature in a vacuum, the gummy residue was treated with water (100 c.c.)

on a steam-bath. The insoluble product was collected and hydrolysed with warm 2*N*-sodium hydroxide (5 c.c.) in an atmosphere of hydrogen. Acidification precipitated *isovanillic acid* (0.03 g.) which after crystallisation from methanol (charcoal) formed colourless plates, m. p. alone or mixed with an authentic specimen 252°. Acetylation gave *acetylisovanillic acid* in needles, m. p. and mixed m. p. 214—215° (Found: C, 57.1; H, 4.8. Calc. for C₁₆H₁₀O₅: C, 57.1; H, 5.2%). By methylating the acid with diazomethane and distilling the product an oil was obtained which subsequently crystallised from light petroleum in plates, identical with a specimen of methyl veratrate, m. p. 58°.

(ii) When *ayanin* (0.5 g.) was heated under reflux for 8 hours with potassium hydroxide (2.8 g.) in water (25 c.c.) and alcohol (10 c.c.) in an atmosphere of hydrogen, yellow needles (0.3 g.) of the flavone potassium salt were obtained on cooling. After the salt had been collected, the filtrate was saturated with carbon dioxide and extracted with ether, and then strongly acidified and again extracted with ether. Both ethereal extracts yielded uncrystallisable oils of which the first, *i.e.*, the phenolic fraction, gave on vacuum-sublimation a small quantity of *isovanillic acid*, identified by its mixed m. p.

3'-Benzyloxy-5 : 7-dihydroxy-3 : 4'-dimethoxyflavone (I).—Benzylisovanillic anhydride (4.3 g., 2.5 mols.) (Lovecy, Robinson, and Sugawara, *J.*, 1930, 817), potassium benzyloxyisovanillate (1.8 g., 1.6 mols.) and ω -methoxyphloracetophenone (0.75 g., 1 mol.) were thoroughly mixed and heated at 170—180°/30 mm. for 2 hours. The cooled product was powdered and dissolved in ethanol (36 c.c.) and water (3 c.c.) containing sodium hydroxide (3.7 g.), and the solution boiled for 10 minutes. The solvents were then removed under reduced pressure, and water (35 c.c.) and excess of acid added. The resulting emulsion was exhaustively extracted with ether, the ethereal solution washed with sodium hydrogen carbonate solution and evaporated, thus giving *3'-benzyloxy-5 : 7-dihydroxy-3 : 4'-dimethoxyflavone* (I) as a pale yellow solid (1.33 g., 89%) which crystallised from benzene in yellow plates, m. p. 211—212° (Found: C, 68.6; H, 5.2. C₂₄H₂₀O₇ requires C, 68.6; H, 4.8%).

3'-Benzyloxy-5-hydroxy-3 : 7 : 4'-trimethoxyflavone (II).—The *3'*-benzylflavone (I) (0.35 g.) was methylated in dry acetone (18 c.c.) with methyl sulphate (0.08 c.c., 1.1 mols.) and anhydrous potassium carbonate (2 g.) under reflux for 6½ hours. The suspension was filtered, and the filtrate and acetone washings evaporated, leaving a brown solid (0.35 g.) which by recrystallisation from methanol gave *3'-benzylayanin* as orange needles, m. p. 138° (Found: C, 68.5; H, 5.2; OMe, 23.8. C₂₅H₂₂O₇ requires C, 69.1; H, 5.1; OMe, 21.4%).

5 : 3'-Dihydroxy-3 : 7 : 4'-trimethoxyflavone (*Ayanin*).—The *3'*-benzylayanin (II) (0.15 g.) and a mixture of acetic acid (7 c.c.) and concentrated hydrochloric acid (3.7 c.c.) were heated on a steam-bath for 1½ hours. When the solution had been brought to neutrality with sodium hydroxide the *dihydroxytrimethoxyflavone* (III) (0.1 g., 89%) separated and by recrystallisation from methanol was obtained in orange-yellow plates, m. p. alone or mixed with the natural product, 173° (Found, in a specimen dried at 140° in a vacuum: C, 62.3; H, 4.3; OMe, 29.4%). Acetylation gave a diacetate, m. p. 115°, identical with diacetylayanin.

5 : 7 : 3'-Trihydroxy-3 : 4'-dimethoxyflavone (IV).—The benzyl ether (I) (0.1 g.) was heated in acetic acid (4.8 c.c.) and concentrated hydrochloric acid (2.6 c.c.) on a steam-bath for 1½ hours. Neutralisation of the solution and crystallisation of the precipitate (0.07 g., 90%) from methanol (charcoal) gave the *trihydroxydimethoxyflavone* (IV) as orange-brown plates, m. p., with slight previous softening, 232—233° (Found: C, 61.1; H, 4.4. C₁₇H₁₄O₇ requires C, 61.8; H, 4.3%).

3'-Benzyloxy-3 : 5 : 7 : 4'-tetramethoxyflavone (V).—The flavone (I) (0.3 g.) was heated under reflux for 24 hours with methyl iodide (5 c.c.) and acetone (50 c.c.) containing potassium carbonate (2 g.). After filtration, the solution was evaporated and the colourless product (0.32 g.) crystallised from ethanol, the *benzyloxytetramethoxyflavone* separating in plates, m. p. 143—144° (Found, in a specimen dried at 100° in a vacuum: C, 69.5; H, 5.5; OMe, 29.2. C₂₆H₂₄O₇ requires C, 69.6; H, 5.4; OMe, 27.7%).

3'-Hydroxy-3 : 5 : 7 : 4'-tetramethoxyflavone (VI).—Hydrolysis of the flavone (V) with a mixture of hydrochloric and acetic acids in the manner used for compounds (III) and (IV) gave *3'-hydroxy-3 : 5 : 7 : 4'-tetramethoxyflavone* which, when crystallised from methanol (charcoal), formed yellow elongated hexagonal plates, m. p. 220—222° (Found: C, 63.2; H, 5.3; OMe, 38.0. C₁₉H₁₈O₇ requires C, 63.7; H, 5.0; OMe, 34.6%).

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