705. *5,7,4′-Trihydroxy-3′,6-dimethoxyflavone, a Pigment from* Digitalis lanata *L.*

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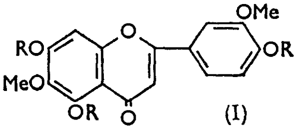
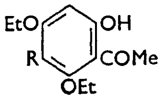
A flavonoid pigment extracted from *Digitalis lanata* L. residues is 5,7,4′-trihydroxy-3′,6-dimethoxyflavone.

The isolation of several flavones from various species of *Digitalis* has been reported. *Digitalis thapsii* L. furnishes 6,4′-dihydroxy-3,5,7,8-tetramethoxyflavone[1] (thapsin). *D. lutea* L. yields 5,7,3′,4′-tetrahydroxyflavone (luteolin).[2] Luteolin 7-glucoside and 5,3′-dihydroxy-3,6,7,8,4′,5′-hexamethoxyflavone (digicitrin) have been obtained[3] from *D. purpurea* L.

Through the courtesy of Messrs. Burroughs Wellcome and Co. we have been able to investigate a crystalline, yellow fraction isolated from the leaves of *D. lanata* L. during the preparation of the cardiac glycosides. The principal constituent of this pigment fraction has been identified as 5,7,4′-trihydroxy-3′,6-dimethoxyflavone (I; R = H). The occurrence of this flavone in Nature has not been previously described and the isolation of only one other flavonoid with this oxygenation pattern, namely, 5,6,7,3′,4′-pentamethoxyflavone from orange peel,[4] has been reported.



(I)



(II)

Methylation of the *D. lanata* pigment furnished the pentamethoxyflavone (I; R = Me) which was identified unequivocally by degradation and by comparison with an authentic specimen. The orientation of the substituents was readily determined by ethylation of the pigment to yield the ether (I; R = Et), alkaline degradation of which furnished 4-ethoxy-3-methoxybenzoic acid together with 4,6-diethoxy-2-hydroxy-5-methoxyacetophenone (II; R = OMe). This acetophenone was identified by comparison of its *p*-nitrobenzoate with a specimen prepared from 4,6-diethoxy-2-hydroxyacetophenone (II; R = H) by way of the stage (II; R = OH). The structure of the ether (II; R = OMe) was confirmed by its non-identity with 5,6-diethoxy-2-hydroxy-4-methoxyacetophenone.

Whilst the demethylation of 5,7,4′-trihydroxy-3′,6-dimethoxyflavone with aluminium chloride furnishes the corresponding pentahydroxy-derivative the use of hydriodic–acetic acid yields a mixture from which only a small quantity of 5,6,7,3′,4′-pentamethoxyflavone could be isolated after remethylation. It thus appears that, although 5,6,7-trihydroxy- and 5,6,7,4′-tetrahydroxy-flavone do not rearrange to the 5,7,8-derivatives under the influence of hydriodic acid,[5] 5,6,7,3′,4′-oxygenated flavones suffer a partial rearrangement to the isomeric 5,7,8-compound under these conditions. The high m. p. (315°) of the demethylation product from 6-hydroxy-5,7,3′,4′-tetramethoxyflavone with hydriodic acid[6] is in accord with the presence of a proportion of the 5,7,8-derivative.

The chemistry of the minor components of this *Digitalis* pigment will be described in a subsequent paper.

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[1] Karrer, *Helv. Chim. Acta*, 1934, **17**, 1560; Karrer and Venkataraman, *Nature*, 1935, **135**, 878.

[2] Fleischer and Fromm, *Ber.*, 1899, **32**, 1184; Diller and Kostanecki, *Ber.*, 1901, **34**, 1453; Kiliani and Meyer, *ibid.*, p. 3577.

[3] Nakamura, Ota, and Fukuchi, *J. Pharm. Soc. Japan*, 1936, **56**, 107; Meier and Fuerst, *Helv. Chim. Acta*, 1962, **45**, 232.

[4] Born, *Chem. and Ind.*, 1960, 264.

[5] Sastri and Seshadri, *Proc. Indian Acad. Sci.*, 1946, **24**, *A*, 245.

[6] Murti and Seshadri, *Proc. Indian Acad. Sci.*, 1948, **27**, *A*, 219.

EXPERIMENTAL

5,7,4′-*Trihydroxy*-3′,6-*dimethoxyflavone*.—Purification from acetone of the crude pigments remaining after removal of the cardiac glycosides from *D. lanata* L. furnished the *flavone* in yellow needles, m. p. 227—228° [Found: C, 61·8; H, 4·4; OMe, 18·4%. *M* (Rast), 363. $C_{15}H_8O_5(OMe)_2$ requires C, 61·8; H, 4·3; OMe, 18·8%; *M*, 330], giving an intense green-brown ferric reaction in alcohol and a positive Gibbs test

Prepared quantitatively by the pyridine–acetic anhydride method the *triacetate* separated from acetone in needles, m. p. 220° [Found: C, 59·8; H, 4·4; OMe, 13·7. $C_{21}H_{14}O_8(OMe)_2$ requires C, 60·5; H, 4·4; OMe, 13·6%]. Prepared similarly, the *tribenzoate* separated from aqueous acetone in needles, m. p. 174° [Found: C, 71·1; H, 4·3; OMe, 9·9. $C_{36}H_{20}O_8(OMe)_2$ requires C, 71·0; H, 4·1; OMe, 9·7%].

Demethylation of the pigment (1 g.) with aluminium chloride (0·8 g.) in benzene (10 ml.) during 4 hr. at 100° furnished 5,6,7,3′,4′-*pentahydroxyflavone* in plates (0·6 g.), m. p. 284° (from ethyl acetate) (Found: C, 59·3; H, 3·5. $C_{15}H_{10}O_7$ requires C, 59·6; H, 3·3%). The penta-acetate separated from aqueous acetone in needles, m. p. 212° (Found: C, 58·2; H, 4·2. Calc. for $C_{25}H_{20}O_{12}$: C, 58·6; H, 3·9%). Oliverio, Marini-Bettolo, and Bergellini[7] report m. p. 207—209°. Methylation of this pentahydroxyflavone furnished 5,6,7,3′,4′-pentamethoxyflavone which separated from acetone in pale yellow prisms, m. p. 179°, identical with an authentic specimen [Found: C, 64·3; H, 5·6; OMe, 40·0. Calc. for $C_{15}H_5O_2(OMe)_5$: C, 64·5; H, 5·4; OMe, 41·7%]. Oliverio *et al.*[7] report m. p. 178°. The same product (0·9 g.) was obtained by methylation of 5,7,4′-trihydroxy-3′,6-dimethoxyflavone (1 g.) in boiling acetone (100 ml.) containing potassium carbonate (10 g.) during 3 hr.

Alkaline Degradation of the Fully Methylated Flavone.—A solution of the pentamethyl ether (1 g.) and potassium hydroxide (15 g.) in water (40 ml.) and alcohol (100 ml.) was refluxed for 6 hr. Isolated in the usual way, the acidic fraction furnished veratric acid (0·3 g.), prisms, m. p. 180° [from chloroform–light petroleum (b. p. 60—80°)], identical with an authentic specimen [Found: C, 59·3; H, 5·8; OMe, 35·2. Calc. for $C_7H_4O_2(OMe)_2$: C, 59·3; H, 5·5; OMe, 34·1%]. A solution of the phenolic fraction (0·25 g.) in chloroform–light petroleum (b. p. 60—80°) deposited 3,4,5-trimethoxyphenol, m. p. 147°, identical with an authentic specimen (Found: C, 58·3; H, 6·6; OMe, 50·0. Calc. for $C_9H_{12}O_4$: C, 58·7; H, 6·6; OMe, 50·5%). The oil remaining after evaporation of the mother-liquors exhibited an intense green ferric reaction in alcohol and with *p*-nitrobenzoyl chloride it gave the p-*nitrobenzoate* of 2-hydroxy-4,5,6-trimethoxyacetophenone, yellow needles, m. p. 123° (from methanol), identical with an authentic specimen [Found: C, 57·4; H, 4·7; OMe, 24·4; N, 3·9. $C_{15}H_8NO_5(OMe)_3$ requires C, 57·6; H, 4·6; OMe, 24·8; N, 3·7%]. The *semicarbazone* of 2-hydroxy-4,5,6-trimethoxyacetophenone separated from methanol–light petroleum (b. p. 60—80°) in plates, m. p. 212°, identical with an authentic specimen [Found: C, 51·6; H, 6·1; N, 14·8; OMe, 32·9. $C_9H_8N_3O_2(OMe)_3$ requires C, 50·9; H, 6·0; N, 14·8; OMe, 32·8%]. The 2,4-*dinitrophenylhydrazone* was purified by chromatography from benzene–chloroform (4 : 1) on alumina and formed red plates, m. p. 180°, identical with an authentic specimen (Found: C, 50·3; H, 4·4; N, 14·0. $C_{17}H_{18}N_4O_8$ requires C, 50·2; H, 4·4; N, 13·8%).

Methylation of the fraction containing 2-hydroxy-4,5,6-trimethoxyacetophenone furnished 2,4,5,6-tetramethoxyacetophenone (purified by chromatography), m. p. 53°, identical with an authentic specimen.

5,7,4′-*Triethoxy*-3′,6-*dimethoxyflavone*.—Treatment of the dimethoxyflavone (2 g.) with ethyl iodide (8 ml.) in boiling acetone (100 ml.) containing potassium carbonate (10 g.) during 18 hr. gave 5,7,4′-*triethoxy*-3′,6-*dimethoxyflavone* which separated from acetone in prisms (1·7 g.), m. p. 165—168° (Found: C, 66·7; H, 6·5. $C_{23}H_{26}O_7$ requires C, 66·7; H, 6·3%).

Hydrolysis of this flavone (1 g.) by the method previously described furnished 4-ethoxy-3-methoxybenzoic acid, plates (from acetone), m. p. 194°, identical with an authentic specimen (Found: C, 61·1; H, 6·3. Calc. for $C_{10}H_{12}O_4$: C, 61·2; H, 6·2%). The oily, phenolic fraction was identified as 4,6-diethoxy-2-hydroxy-5-methoxyacetophenone by conversion into the p-*nitrobenzoate* which separated from methanol in pale yellow needles, m. p. 74°, identical with an authentic specimen (Found: C, 59·9; H, 5·5; N, 3·2. $C_{20}H_{21}NO_8$ requires C, 59·6; H, 5·2; N, 3·5%). 4,6-Diethoxy-2,5-dihydroxyacetophenone[8] (0·8 g.) was converted during 7½ hr. in

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[7] Oliverio, Marini-Bettolo, and Borgellini, *Gazzetta*, 1948, **78**, 363.

[8] Row, Sastri, Seshadri, and Thiruvengadam, *Proc. Indian Acad. Sci.*, 1948, **28**, *A*, 189.

boiling acetone (70 ml.) containing potassium carbonate (2 g.) and methyl sulphate (0·5 g.) into the non-crystalline 4,6-diethoxy-2-hydroxy-5-methoxyacetophenone. This was characterised as the *p*-nitrobenzoate which separated from methanol in pale yellow needles, m. p. 74°, identical with the derivative prepared from the natural source (Found: C, 59·9; H, 5·2. Calc. for $C_{20}H_{21}NO_8$: C, 59·6; H, 5·2%).

5,6-*Diethoxy*-2-*hydroxy*-4-*methoxyacetophenone*.—A solution of *O*-monoethylphloroglucinol [9] (8·8 g.) in ether (150 ml.) containing zinc chloride (5 g.) and acetonitrile (5 ml.)was saturated at 0° with hydrogen chloride. After 24 hr. the yellow ketimine hydrochloride was separated, washed with ether, dissolved in water (100 ml.) containing concentrated hydrochloric acid (5 ml.), and heated for 3 hr. The product was purified from aqueous alcohol, to give 6-*ethoxy*-2,4-*dihydroxyacetophenone* in pale yellow plates (7 g.), m. p. 178°, giving an intense plum-coloured ferric reaction in alcohol (Found: C, 61·3; H, 6·2; OEt, 22·4. $C_8H_7O_3{\cdot}OEt$ requires C, 61·2; H, 6·2; OEt, 23·0%). Methylation of this ketone (3·1 g.) in boiling acetone (150 ml.) containing potassium carbonate (10 g.) and methyl sulphate (2·3 g.) during 3 hr. gave 6-ethoxy-2-hydroxy-4-methoxyacetophenone (2·7 g.) in needles, m. p. 134°, from aqueous alcohol. Sonn [10] records m. p. 134°. The authenticity of this compound was proved by its identity with a specimen prepared from 2,6-dihydroxy-4-methoxyacetophenone,[11] diethyl sulphate, and potassium carbonate in boiling acetone during 3 hr.

A solution of potassium persulphate (6·7 g.) in water (100 ml.) was added during 4 hr. to a stirred solution of 6-ethoxy-2-hydroxy-4-methoxyacetophenone (5·3 g.) in water (700 ml.) containing sodium hydroxide (7 g.) at 15—20°. After 22 hr. the solution was acidified (Congo Red) with hydrochloric acid. Unchanged acetophenone (1·6 g.) was collected and the filtrate acidified with concentrated hydrochloric acid (60 ml.) and warmed on the steam-bath for ½ hr. Purification of the resultant precipitate from aqueous alcohol gave 6-*ethoxy*-2,5-*dihydroxy*-4-*methoxyacetophenone* in yellow needles (1·4 g.), m. p. 145°, giving a red-brown ferric reaction in alcohol [Found: C, 58·9; H, 6·2; OMe, 13·9; OEt, 20·2. $C_8H_6O_3(OMe){\cdot}OEt$ requires C, 58·4; H, 6·2; OMe, 13·7; OEt, 19·9%]. The *diacetate* separated from aqueous methanol in rods, m. p. 109° (Found: C, 58·3; H, 5·9. $C_{15}H_{18}O_7$ requires C, 58·1; H, 5·9%). Ethylation of the previous acetophenone (0·88 g.) with anhydrous potassium carbonate (2 g.), and diethyl sulphate (0·6 g.) in boiling acetone (60 ml.) during 7 hr. furnished 5,6-diethoxy-2-hydroxy-4-methoxyacetophenone as an oil which was characterised as the p-*nitrobenzoate*, yellow plates (from methanol), m. p. 128° [Found: C, 59·6; H, 5·1; N, 3·6; OMe, 7·6; OEt, 22·0. $C_{15}H_8NO_5(OEt)_2{\cdot}OMe$ requires C, 59·6; H, 5·3; N, 3·5; OMe, 7·7; OEt, 22·3%].

We thank Drs. T. Dewing and H. T. Openshaw for assistance in supplying the crude pigment. Financial assistance was provided by the Government Grants Committee of the Royal Society, The Department of Scientific and Industrial Research, and Imperial Chemical Industries Limited. One of us (K. Y. S.) is indebted to the Commissioners for the award of a Commonwealth Scholarship.

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[*Received, January 23rd*, 1963.]

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[9] Subramaniam and Robertson, *J.*, 1937, 289.
[10] Sonn, *Ber.*, 1928, **61**, 2300.
[11] Whalley, *J.*, 1951, 3229.