

ANTHOXANTHIN PIGMENTS OF THE LEAVES OF *DIGITALIS LANATA* EHRH. AND THE CONSTITUTION OF A NEW PIGMENT DINATIN

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THE occurrence of luteolin in the leaves of *Digitalis purpurea* was reported long time back.¹ The 7-glucoside of luteolin has also been found to occur in the same plant.² Digitolutein, a new yellow pigment of *D. lutea*³ and *D. purpurea*,⁴ has been shown to be the 3-methyl alizarin 1-methyl ether.^{5,6} In the course of work on the preparation of digitoxin from the leaves of a Spanish *Digitalis* of uncertain botanical identity but considered to be very probably *D. thapsi*, Karrer⁷ reported the isolation of a flavone pigment thapsin which was subsequently found⁸ to be identical with calycopterin isolated from the leaves of *Calycopteris floribunda*.⁹

In the course of our work on the commercial *D. lanata* leaves grown in Kashmir for cardioactive principles we found that the drug contains an appreciable quantity of flavone pigments. The present paper deals with the chemical examination of the pigments isolated from this material.

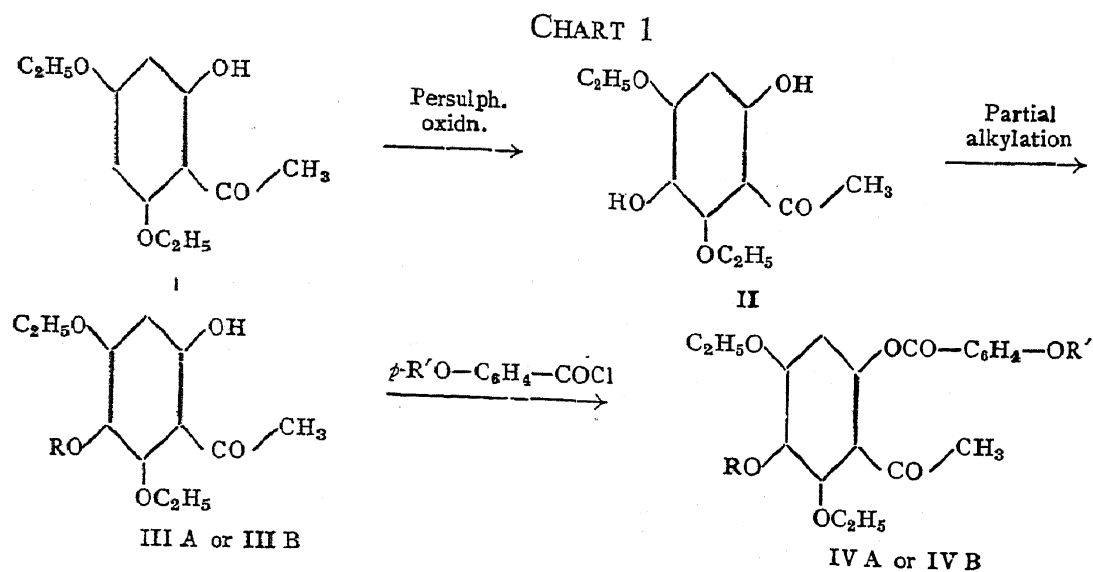
The powdered leaves were extracted with alcohol in the cold. From the concentrate after removal of lipid material with petroleum ether and benzene the pigments were extracted with ether. Two fractions 1 and 2 were obtained, both of which were yellow in colour and answered colour reactions for anthoxanthins.

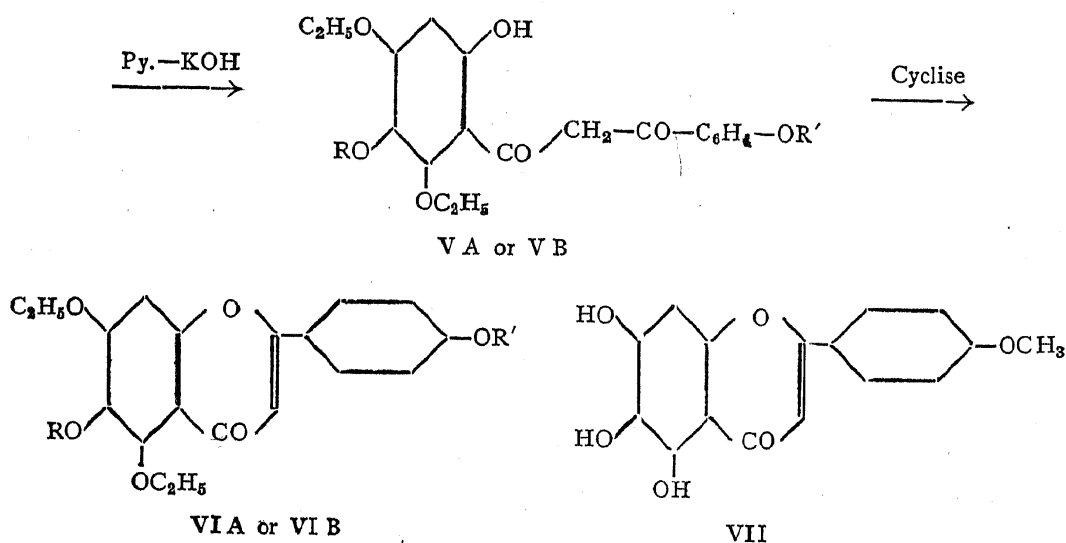
Fraction 1 could not be completely purified by mere fractional crystallization; however, fractional crystallization of the mixed acetate yielded two pure entities which were deacetylated to recover the original compounds. The properties and composition of the two acetates and their deacetylated products indicated that the substances in question were scutellarein (5:6:7:4'-tetrahydroxy flavone) and luteolin (5:6:3':4'-tetrahydroxy flavone). The identity of scutellarein which has no recognizable m.p. was confirmed by the preparation of its tetramethyl ether (this ether was also needed to settle the constitution of dinatin, see later).

Purification of fraction 2 yielded a compound, m.p. 274–76°. It answered anthoxanthin colour reactions, analysed for the formula $C_{16}H_{12}O_6$ with one methoxyl and formed a triacetate. Its complete methyl ether was found

to be identical with scutellarein tetramethyl ether and its demethylation product with scutellarein. Thus the pigment seems to be a monomethyl ether of scutellarein not described in the literature so far and is therefore designated as dinatin. The pigment was soluble in aqueous sodium carbonate solution indicating a free 7-hydroxy group. Of the three remaining positions for the methoxyl the 4' and 6 positions have a greater probability than the 5, since the 5-hydroxyl group in anthoxanthins is known to be relatively hindered. The first possibility (4'-position) for the methoxyl can easily be settled if on alkaline degradation anisic acid and/or *p*-methoxyacetophenone can be identified among the products. This approach however was ruled out as not enough material was available for a fission experiment. Hence recourse was had to the following synthetic approach. The compound dinatin was fully alkylated using ethyl sulphate and the complete mixed ether compared directly with synthetic specimens of 5:6:7-triethoxy-4'-methoxyflavone and 5:7:4'-triethoxy-6-methoxyflavone. The triethyl ether of dinatin was found to be identical with the former but not with the latter, indicating that dinatin is 4'-O-methyl scutellarein (VII). This structure for dinatin agrees with the observation that it gives a positive Bargellini¹⁰ test which is considered to be characteristic of flavones and flavonols having hydroxyl groups in the 5:6:7-positions.

The synthetic triethoxy-monomethoxy compounds mentioned above and which are not described in the literature were prepared from phloracetophenone diethyl ether along the lines described by Sastri and Seshadri,¹¹ except that the rearrangement of the substituted benzoyloxy compound to the diketone has been accomplished by the method of Gallagher *et al.*¹² The sequence of reactions is shown in Chart 1.





In formulæ III—VI

R = OC₂H₅ and R' = OCH₃ in the A series
 and R = OCH₃ and R' = OC₂H₅ in the B series

EXPERIMENTAL

Note.—The alkoxy content of mixed ethers described herein are calculated and expressed as ethoxyl.

The powdered leaves (3 kg.) were extracted with 90% alcohol (4×6 litres) in the cold. The combined extracts were concentrated under reduced pressure to 800 ml. and extracted in succession with petroleum ether (5×500 ml.), benzene (5×500 ml.) and ether (6×400 ml.). After these extractions the concentrate was almost devoid of anthoxanthin pigments (magnesium-hydrochloric acid reaction). All the extracts were washed with water and dried over sodium sulphate.

The petroleum ether extract (32 g.) and the benzene extract (41 g.) were dark-greenish-brown amorphous semi-solids and were not examined further.

Ether extract.—The bulk of the solvent was removed by distillation and the concentrate was set aside for three days when a yellow solid separated (fraction 1, 2·8 g.). It was filtered and washed with petroleum ether followed by ether. The filtrate was further concentrated and set aside for a number of days when some more yellow solid separated (fraction 2, 0·8 g.). The mother liquors of fraction 2 did not yield any more solid.

Fraction 1.—This gave a red colour with magnesium and hydrochloric acid. With lead acetate in alcohol solution it gave a yellow precipitate. It was soluble in aqueous sodium hydroxide and sodium carbonate solutions.

With alcoholic ferric chloride it gave a deep-brown colour. The solid melted indefinitely between 270° and 300°. Repeated fractional crystallizations from the common organic solvents did not yield wholly satisfactory results. The material (2 g.) was acetylated by heating under reflux with anhydrous sodium acetate (4 g.) and acetic anhydride (5 ml.) at 130–40° for 4 hours. The crude acetate (2.2 g.) after an initial crystallization from benzene-petroleum ether was fractionally crystallized from acetone. The less soluble fractions were combined and crystallized twice from alcohol-acetone when long silky needles were obtained, m.p. 238–40° (acetate A) (1.2 g.).

The more soluble fractions after repeated crystallizations from alcohol gave colourless needles, m.p. 215–17° (acetate B) (0.4 g.).

Characterization of acetates A and B

Acetate A, m.p. 238–40°.—On magnesium-hydrochloric acid reduction it gave an orange-red colour. With alcoholic ferric chloride no colour was obtained. Found: C, 60.4; H, 4.3; $-\text{COCH}_3$, 38.6%. $\text{C}_{23}\text{H}_{18}\text{O}_{10}$ (Scutellarein tetraacetate) requires: C, 60.8; H, 4.0; $-\text{COCH}_3$ (4), 37.9%.

Deacetylation of A to scutellarein.—A solution of the substance (300 mg.) in alcohol (20 ml.) and acetone (4 ml.) was refluxed with 1 N sulphuric acid (100 ml.) for 10 hours in a water-bath. The organic solvents were removed under reduced pressure and the aqueous residue cooled, when a yellow solid separated out, which was filtered, washed with water and dried (yield 180 mg.). It crystallized from methanol as yellow leaflets. It did not melt below 340° but decomposed above 350°. It gave a red colour on reduction with magnesium-hydrochloric acid, a yellow precipitate with lead acetate and a reddish-brown colour with alcoholic ferric chloride. It formed a yellow solution with aqueous sodium hydroxide and sodium carbonate solutions which immediately turned green. Found: C, 63.4; H, 3.8%. $\text{C}_{15}\text{H}_{10}\text{O}_6$ (scutellarein) requires: C, 63.0; H, 3.5%.

Methylation of scutellarein.—A solution of the substance (80 mg.) in anhydrous acetone (15 ml.) was refluxed for 30 hours with dimethyl sulphate (1 ml.) and anhydrous potassium carbonate (2 g.). The product was crystallized from acetone-petroleum ether and then from alcohol when colourless prisms were obtained, m.p. 156–58°. It gave no colour with ferric chloride. Found: C, 67.1; H, 4.9; $-\text{OCH}_3$, 34.8%. $\text{C}_{19}\text{H}_{18}\text{O}_6$ (scutellarein tetramethyl ether) requires: C, 66.7; H, 5.3; $-\text{OCH}_3$ (4), 36.2%.

Acetate B, m.p. 215–17°.—This substance gave an orange-red colour on reduction with magnesium-hydrochloric acid but gave no colour with ferric chloride. Found: C, 60.2; H, 4.5; $-\text{COCH}_3$, 38.6%. $\text{C}_{23}\text{H}_{18}\text{O}_{10}$

(luteolin tetraacetate) requires: C, 60.8; H, 4.0; $-\text{COCH}_3$ (4), 37.9%.

Deacetylation of B to luteolin.—The substance (0.3 g.) was dissolved in alcohol (25 ml.), treated with 1 N sulphuric acid (100 ml.) and the mixture refluxed for 10 hours in a water-bath. The yellow solid that separated out on working up the product was filtered, washed with water and dried (yield 160 mg.). It crystallized from alcohol as yellow needles, m.p. 334–38°. It gave an orange-red colour on reduction with magnesium-hydrochloric acid, a yellow precipitate with lead acetate and a greenish-brown colour with ferric chloride. Found: C, 62.8; H, 3.9%. $\text{C}_{15}\text{H}_{10}\text{O}_6$ (luteolin) requires C, 63.0; H, 3.5%.

Fraction 2.—Repeated fractional crystallizations from alcohol rejecting each time the less soluble portion gave pale-yellow needles, m.p. 274–76° (dinatin, yield 0.5 g.). It was soluble in sodium hydroxide and sodium carbonate solutions, gave an orange-red colour with magnesium-hydrochloric acid, yellow precipitate with lead acetate and dark-brown colour with ferric chloride. Bargellini test was positive. Found: C, 63.6; H, 4.5; $-\text{OCH}_3$, 10.4%. $\text{C}_{16}\text{H}_{12}\text{O}_6$ requires: C, 64.0; H, 4.0; $-\text{OCH}_3$ (1), 10.3%.

Dinatin triacetate.—Dinatin (100 mg.) was acetylated with anhydrous sodium acetate and acetic anhydride and the product crystallized twice from benzene-petroleum ether and once from ethyl acetate when it was obtained as prismatic needles, m.p. 168–70° (yield 70 mg.). It gave no colour with ferric chloride. Found: C, 62.5; H, 4.7; $-\text{OCH}_3$, 7.0; $-\text{COCH}_3$, 31.2%. $\text{C}_{22}\text{H}_{18}\text{O}_9$ (triacetate) requires: C, 62.0; H, 4.3; $-\text{OCH}_3$ (1), 7.3; $-\text{COCH}_3$ (3) 30.3%.

Methyl ether of dinatin.—The substance (100 mg.) was dissolved in dry acetone (15 ml.) and to the solution anhydrous potassium carbonate (2 g.) and dimethyl sulphate (1 ml.) were added and the mixture was refluxed for 30 hours. The product was crystallized from alcohol (charcoal) when colourless prisms, m.p. 158–60°, were obtained (40 mg.). The substance gave no colour with ferric chloride. Found: C, 67.0; H, 5.8; $-\text{OCH}_3$, 35.3%. $\text{C}_{19}\text{H}_{18}\text{O}_6$ (scutellarein tetramethyl ether) requires: C, 66.7; H, 5.3; $-\text{OCH}_3$ (4), 36.2%. Mixed m.p. with tetramethyl scutellarein (see above) was undepressed (156–58°).

Demethylation of dinatin.—The substance (100 mg.) was dissolved in glacial acetic acid (2 ml.) and treated with a saturated solution of hydrobromic acid in glacial acetic acid (4 ml.) and the mixture was heated in a boiling

water-bath for 2 hours. The product crystallized from alcohol as small yellow leaflets which did not melt below 340° . The substance answered all the colour reactions described above under scutellarein. Found: C, 62.6; H, 3.9; $-\text{OCH}_3$, nil. $\text{C}_{15}\text{H}_{10}\text{O}_6$ (scutellarein) requires: C, 63.0; H, 3.5%.

Triethyl ether of dinatin.—Dinatin (70 mg.) was dissolved in acetone (12 ml.) and to the solution anhydrous potassium carbonate (2 g.) and diethyl sulphate (0.8 ml.) were added and the mixture was refluxed for 48 hours. The product was twice crystallized from acetone-petroleum ether when colourless needles were obtained, m.p. $144-46^{\circ}$. Found: C, 68.4; H, 6.7; $-\text{OC}_2\text{H}_5$, 45.9%. $\text{C}_{22}\text{H}_{24}\text{O}_6$ requires: C, 68.7; H, 6.3; $-\text{OC}_2\text{H}_5$ (4), 46.9%.

Synthesis of 5:6:7-Triethoxy-4'-Methoxy Flavone

2:5-Dihydroxy-4:6-diethoxy acetophenone (II).—2-Hydroxy-4:6-diethoxy acetophenone¹³ (I), m.p. $84-85^{\circ}$ (2 g.), was treated with pyridine (20 ml.) and potassium hydroxide (5 g.) in water (30 ml.) and the solution cooled to a temperature of $15-20^{\circ}$ and kept continuously stirred. A solution of potassium persulphate (2.5 g.) in water (50 ml.) was then introduced dropwise during the course of 4 hours, the temperature being maintained at $15-20^{\circ}$ throughout the reaction. After allowing to stand for 24 hours the reaction mixture was rendered acid to congo-red by the addition of conc. hydrochloric acid. The unreacted original ketone which separated was filtered and washed with a small quantity of water. The combined aqueous filtrate and washings were treated with sodium sulphite (1g.), conc. hydrochloric acid (35 ml.) was then added and the solution heated at $85-90^{\circ}$ in a water-bath for 30 minutes. On cooling it rapidly deposited a brownish-yellow crystalline material which was filtered, washed with water and dried, m.p. $120-23^{\circ}$ (yield 0.9 g.). It crystallized from alcohol as yellow prisms, m.p. $125-26^{\circ}$ and gave the characteristic colour reactions of *p*-dihydroxy phenols.¹¹ Found: C, 60.5; H, 7.0; $-\text{OC}_2\text{H}_5$, 36.7%. $\text{C}_{12}\text{H}_{16}\text{O}_5$ requires: C, 60.0; H, 6.7%; $-\text{OC}_2\text{H}_5$ (2), 37.5%.

2-Hydroxy-4:5:6-triethoxy acetophenone (III A).—The 2:5-dihydroxy compound (650 mg.) was dissolved in acetone (10 ml.) and benzene (40 ml.) and treated with anhydrous potassium carbonate (1.5 g.) and diethyl sulphate (0.4 ml.; 1.1 mole) and the mixture refluxed in a water-bath for 12 hours. The inorganic salts were filtered and washed with hot benzene. The filtrate and washings were washed with 5% aq. sodium carbonate and water, dried over sodium sulphate and the solvent removed under vacuum. The reddish-brown oily residue was taken in ether and diluted with petroleum ether

when the last traces of unchanged dihydroxy compound got precipitated. The clear petroleum ether solution after the removal of solvent gave 2-hydroxy-4:5:6-triethoxy acetophenone as an orange-yellow oil (yield 350 mg.). It gave a deep violet-brown colour with ferric chloride.

2-Anisoyloxy-4:5:6-triethoxy acetophenone (IV A).—2-Hydroxy-4:5:6-triethoxy acetophenone (360 mg.) was anisoylated by heating with freshly distilled anisoyl chloride (0.5 ml.) and dry pyridine (10 ml.) for 2 hours on a boiling water-bath. The reaction mixture was poured into ice-cold water containing hydrochloric acid with stirring. The anisoyl derivative which separated as a semi-solid was taken in ether, the ether solution washed successively with dilute hydrochloric acid, aq. sodium carbonate and water, dried over sodium sulphate and the solvent distilled, when an orange-red semi-solid (0.5 g.) was obtained. Attempts to crystallise it proved unsuccessful. It was insoluble in sodium hydroxide and gave no colour with ferric chloride.

Diketone (V A).—The above anisoyl derivative (0.5 g.) was taken in dry pyridine (4 ml.) and freshly powdered potassium hydroxide (0.8 g.) was added to it. The mixture was shaken for 4 hours in a water-bath maintained at 45°, then treated with 20% ice-cold acetic acid (30 ml.) and left in the ice-chest overnight. The crude diketone was crystallized twice from methanol when bright yellow prismatic needles, m.p. 114–16°, were obtained (yield 0.3 g.). It gave a green colour with ferric chloride. Found: C, 64.9; H, 6.9; $-\text{OC}_2\text{H}_5$, 43.1%. $\text{C}_{22}\text{H}_{26}\text{O}_7$ requires: C, 65.7; H, 6.5; $-\text{OC}_2\text{H}_5$ (4), 44.8%.

5:6:7-Triethoxy-4'-methoxy flavone (VI A).—A mixture of the above diketone (0.2 g.), glacial acetic acid (3 ml.) and fused sodium acetate (0.5 g.) was gently boiled for 3 hours and diluted with water (20 ml.) when a colourless solid separated. It was filtered, washed with water, dried and crystallized from acetone-petroleum ether and ether-petroleum ether, when colourless needles were obtained, m.p. 146–48° (yield 70 mg.). It gave an orange-red colour with magnesium-hydrochloric acid but gave no colour with ferric chloride. Found: C, 68.4; H, 6.6; $-\text{OC}_2\text{H}_5$, 44.8%. $\text{C}_{22}\text{H}_{24}\text{O}_6$ requires: C, 68.7; H, 6.3; $-\text{OC}_2\text{H}_5$ (4), 46.9%. Mixed m.p. between this compound and triethyl dinatin was undepressed (145–47°).

Synthesis of 5:7:4'-Triethoxy-6-Methoxy Flavone.

2-Hydroxy-5-methoxy-4:6-diethoxy acetophenone (III B). 2:5-Dihydroxy-4:6-diethoxy acetophenone (II) (350 mg.) was partially methylated using 1.1 mole of dimethyl sulphate. The product was obtained as an

orange-yellow oil (270 mg.) which gave a violet-brown colour with ferric chloride.

2-(*p*-Ethoxy benzoyloxy)-5-methoxy-4:6-diethoxy acetophenone (IV B).—The above described product (270 mg.) was treated with pyridine (10 ml.) and freshly distilled *p*-ethoxy-benzoyl chloride (0.5 ml.) and the mixture heated under reflux for 3 hours in a water-bath. The product was worked up as described earlier under the anisoyloxy compound. The *p*-ethoxy-benzoyl derivative could be obtained only as semi-solid (yield 300 mg.) which did not dissolve in aq. sodium hydroxide and gave no colour with ferric chloride.

Diketone (V B).—The above acyl derivative (300 mg.) was dissolved in dry pyridine (3 ml.) and freshly powdered potassium hydroxide (0.6 g.) was added and the reaction carried out as already described. The crude diketone was crystallized from methanol when bright yellow prismatic needles, m.p. 97–98° (yield 200 mg.), were obtained. The substance gave a green colour with ferric chloride. Found: C, 65.2; H, 6.9; $-\text{OC}_2\text{H}_5$, 43.4%. $\text{C}_{22}\text{H}_{26}\text{O}_7$ requires: C, 65.7; H, 6.5; $-\text{OC}_2\text{H}_5$ (4), 44.8%.

5:7:4'-Triethoxy-6-methoxy flavone (VI B).—The above diketone (100 mg.) was cyclised by heating with glacial acetic acid and fused sodium acetate for 3 hours and the product crystallized from ether-petroleum ether when colourless needles, m.p. 138–40° (yield 40 mg.) were obtained. The substance gave an orange-red colour with magnesium-hydrochloric acid but gave no colour with ferric chloride. Found: C, 68.3; H, 6.7; $-\text{OC}_2\text{H}_5$, 45.2%. $\text{C}_{22}\text{H}_{24}\text{O}_6$ requires: C, 68.7; H, 6.3; $-\text{OC}_2\text{H}_5$ (4), 46.9%. Mixed m.ps. of this substance (VI B) with synthetic (VI A) described above and with triethyl dinatin separately were depressed (120–27° and 122–28° respectively).

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