

## CONSTITUTION OF OROXYLIN-A AND SYNTHESIS OF ITS DIETHYL-ETHER

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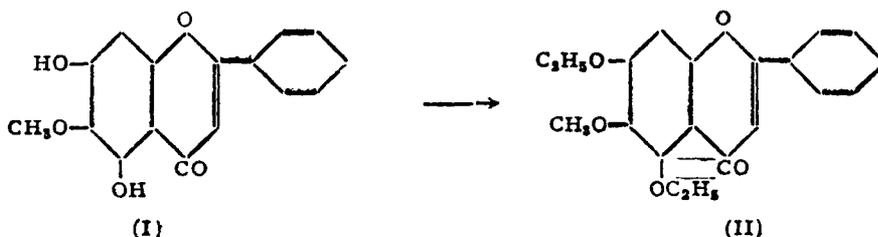
THE name oroxylin-A was given by Shah, Mehta and Wheeler<sup>1</sup> to a substance that was isolated from the root bark of *Oroxylum indicum* having a melting point of 231–32°. It was considered to be a monomethyl ether of baicalein and based on its properties and colour reactions it was given the constitution of baicalein-6-methyl ether (I). In the root bark it was associated with baicalein which however could be removed easily because of its instability in alkali.

The stem bark of the same plant was studied in detail by Bose and Bhattacharya.<sup>2</sup> They showed that the colouring matter called oroxylin by earlier workers consisted of a mixture of three substances. They could isolate in a pure condition chrysin and baicalein and definitely show the presence of the 6-methyl ether of baicalein though they could not separate it from the mixture. From certain considerations which were not mentioned they inferred that this methyl ether should have a melting point lower than 230°. After removing baicalein they subjected the remaining mixture to detailed fractionation and found that a very small fraction melting at 220–23° was the nearest approach to the 6-methyl ether of baicalein (Found: methoxyl, 9.78; C<sub>16</sub>H<sub>12</sub>O<sub>5</sub>, requires 10.92%). The preparation of the 7-methyl ether of baicalein was described by Sastri and Seshadri.<sup>3</sup> Its properties were different from those of oroxylin-A, thus eliminating this possibility.

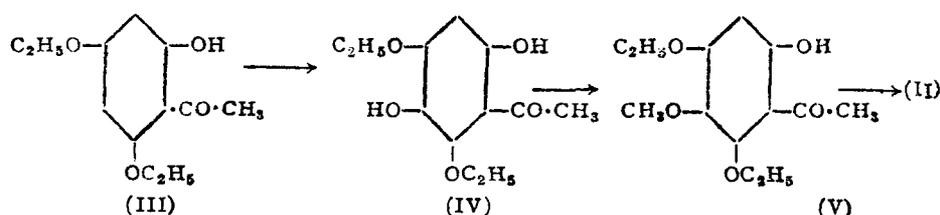
Since Shah, *et al.*,<sup>1</sup> were unsuccessful in their attempt to synthesise oroxylin-A, getting wogonin instead, we proposed to provide confirmation of its constitution by the synthesis of its diethyl ether according to the scheme adopted earlier in the case of patuletin which is the 6-methyl ether of quercetagenin.<sup>4</sup> An authentic sample of oroxylin-A was required for this purpose and as it was not available, it was prepared from the root bark of *Oroxylum indicum* obtained from Travancore. Using the method of the earlier workers it was easy to obtain the substance melting at 231–32° and free from baicalein. Though its methylation yielded baicalein trimethyl

ether fairly readily, its ethylation product melted low and indefinitely, and had to be repeatedly recrystallised a number of times in order to raise the melting point to that of a synthetic sample of 6-methoxy-5:7-diethoxy-flavone. A similar difficulty was noted in regard to the acetylation product which did not melt sharp (m.p. 131–35°). Methoxyl estimation gave low values. It appeared therefore that the original substance was still a mixture though rather sharp melting. Very similar results were obtained when stem bark samples procured from Travancore as well as from Calcutta were extracted adopting the same procedure. Attempts were then made to separate the mixture by the chromatographic method; they were unsuccessful. Eventually fractional crystallisation of the acetates from ethyl alcohol was found to be satisfactory. The least soluble fraction melted high and yielded pure chrysin acetate on further crystallisation. The middle fraction yielded on further crystallisation a sharp melting acetate (139–40°) which had the correct composition of the diacetate of oroxylin-A, this name being retained for the 6-methyl ether of baicalein. On deacetylation using acid or alkali it yielded a sharp melting uniform sample having the correct composition required for the 6-methyl ether of baicalein. But the melting point was 219–20°, considerably lower than that found for the mixture. Its correctness was confirmed in the following manner. From the methoxyl value of our sample melting at 231–2°, it could be estimated that it was roughly a mixture of 70 parts of oroxylin-A and 30 of chrysin. A mixture of this composition was made from pure entities and also another in the proportion of 60:40. The former melted at 233–4° and the latter at 236–7°. Though in these mixtures the major component was oroxylin-A and chrysin may be considered to be an impurity, the melting points were definitely higher than that of pure oroxylin-A.

The purified sample of oroxylin-A melting at 219–20° yielded a sharp melting acetate (139–40°) and a sharp melting diethyl ether readily. The latter was found to be identical with a synthetic sample of 6-methoxy-5:7-diethoxy flavone (II), thus providing definite confirmation of the proposed constitution of oroxylin-A.



The synthesis started from phloroacetophenone which was first converted into the 4:6-diethyl ether (III) by partial ethylation in acetone solution using two moles of ethyl iodide and excess of anhydrous potassium carbonate (compare Row and Seshadri<sup>4</sup>). This compound was earlier prepared by Kostanecki<sup>5</sup> by the partial de-ethylation of phloroacetophenone-triethyl ether with anhydrous aluminium chloride. When oxidised with potassium persulphate in alkaline medium it yielded 2:5-dihydroxy-4:6-diethoxy acetophenone (IV) which was also characterised by the preparation of its dibenzoate. The constitution of this dihydroxy compound arrived at by close analogies was further supported by its properties. It was then subjected to partial methylation to form 2-hydroxy-5-methoxy-4:6-diethoxy-acetophenone.



Later two methods were adopted. The above ketone was condensed with benzoic anhydride and sodium benzoate according to the method of Allan and Robinson. In order to decompose the 3-benzoyl derivative that was formed, boiling with aqueous sodium carbonate was employed. The product gave appreciable colour with alcoholic ferric chloride indicating that some de-ethylation had occurred in the 5-position during the above condensation. It was therefore finally ethylated to yield (II). As the second alternative the ketone (V) was converted into the flavone (II) according to the procedure of Baker and Venkataraman. The same compound melting at 115-16° was obtained by both ways and as already mentioned it was identical with O-diethyl-oroxylin-A.

From the results recorded above it could be concluded that the root as well as stem barks of *Oroxylum indicum* contain baicalein and chrysin along with oroxylin-A. Its correct melting point is 219-20° and its constitution as 6-O-methyl-baicalein is confirmed by the unambiguous synthesis of its diethyl ether.

#### EXPERIMENTAL

##### *Extraction of the Root Bark of Oroxylum indicum*

The powdered root bark (1.5 kg.) was extracted in the cold with acetone for 48 hours. The extract was tapped off and the extraction repeated twice

again. The combined extracts were distilled to recover the solvent. The concentrate on allowing to stand for two days deposited a heavy yellow crystalline solid. It was filtered and washed with small amounts of cold acetone. Yield, 33.0 g. It sintered at 210° and melted at 222–4°.

The crude product (10 g.) was crystallised twice from alcohol when it was obtained as a bright yellow crystalline solid. It was powdered well in a mortar and ground up thoroughly with 10% aqueous sodium hydroxide (50 c.c.). The greenish blue mixture was transferred to a conical flask and treated with a further volume (200 c.c.) of aqueous alkali. After shaking thoroughly it was left overnight. The heavy greenish blue precipitate was filtered off and a clear yellowish brown filtrate obtained. It was acidified with hydrochloric acid and the pale yellow solid precipitate recovered by filtration. It was crystallised twice from alcohol when the product was obtained as yellow shining narrow rectangular plates melting at 231–2°. Yield, 6.0 g. (Found: CH<sub>3</sub>O, 7.2; C<sub>16</sub>H<sub>12</sub>O<sub>5</sub> requires CH<sub>3</sub>O, 10.9%).

#### *Fractionation of Acetate*

The purified, dry product (6 g.) was refluxed with acetic anhydride (30 c.c.) and fused sodium acetate (5 g.) for an hour and a half. The excess of acetic anhydride was then decomposed by adding the cold mixture to ice-water (200 c.c.). The precipitated acetyl derivative was filtered, washed with water and crystallised from alcohol. The acetyl derivative was thus obtained as colourless needles melting between 131–135°.

#### *Fraction (i)—Chrysin Acetate*

The crude acetyl derivative was dissolved in just sufficient amount of boiling alcohol and filtered. On cooling, the filtrate deposited colourless crystals (1.5 g.) which formed fraction (i) and melted at 180–4°. It was crystallised twice from alcohol when the compound was obtained as colourless wedge-shaped plates melting at 192–3°. The mixed melting point with an authentic sample of chrysin acetate was not depressed. When deacetylated with alcoholic hydrochloric acid, it yielded pale yellow prismatic needles melting at 275–6° identical with an authentic sample of chrysin.

#### *Fraction (ii)—Acetate of Oroxylin-A*

The filtrate from fraction (i) was concentrated to half its original volume and was left aside. After a few hours, the crystalline solid that separated was filtered and washed with cold alcohol. This fraction (ii) melted at 137–38° and weighed 2.5 g. When recrystallised twice from alcohol, it came out in the form of colourless long rectangular plates and rods melting

at 139–40°. There was no rise in the melting point after a further crystallisation. (Found: C, 64.8; H, 4.5; CH<sub>3</sub>O, 8.7; C<sub>20</sub>H<sub>16</sub>O<sub>7</sub> requires C, 65.2; H, 4.3 and CH<sub>3</sub>O, 8.4%).

*Fraction (iii)*

The mother liquor from fraction (ii) was further concentrated. Only a semi-solid mass could be recovered from it. On pressing it over a filter-paper, some solid was obtained, which melted indefinitely between 95 and 105°. Further fractionation of this solid did not yield fruitful results.

*Oroxylin-A*

The hydrolysis of the acetate from Fraction (ii) was effected by two methods: (a) acid hydrolysis and (b) alkali hydrolysis.

*Acid hydrolysis.*—The acetate (0.5 g.) was dissolved in alcohol (50 c.c.) and an equal volume of concentrated hydrochloric acid added; the mixture was refluxed for two hours. The alcohol was removed under reduced pressure when a solid product separated out. It was crystallised twice from alcohol when yellow elongated rectangular prisms were obtained melting at 219–20°. Further crystallisation did not improve the melting point.

*Alkali hydrolysis.*—The acetate (0.5 g.) was left overnight with 10% aqueous sodium hydroxide with occasional stirring. The solid which at first was insoluble, slowly went into solution. The clear reddish yellow solution was acidified next morning. The pale yellow solid, obtained thereby, was crystallised from alcohol. After two crystallisations, yellow elongated rectangular prisms were obtained melting at 219–20° which could not be improved further by crystallisation. The mixed melting point with 7-O-methyl-baicalein which also melts at this temperature was considerably depressed. (Found: C, 68.0; H, 4.4; CH<sub>3</sub>O, 10.5; C<sub>16</sub>H<sub>12</sub>O<sub>5</sub> requires C, 67.6; H, 4.2 and CH<sub>3</sub>O, 10.9%). Pure oroxylin-A gave with alcoholic ferric chloride an olive brown colour and with alcoholic lead acetate a yellow precipitate which dissolved in excess of the reagent.

*Another method of isolating chrysin from the mixture*

The sample (1 g.) melting at 231–2° was dissolved in acetic anhydride (5 c.c.) and treated with hydriodic acid (d. 1.7; 10 c.c.). After refluxing for 1½ hours, the mixture was cooled, diluted with water and then saturated with sulphur dioxide. The bright yellow solid product was collected at the pump, washed with more sulphur dioxide-water and then transferred to aqueous sodium hydroxide (100 c.c., 10%). The mixture was shaken thoroughly and left overnight. It was then filtered through a fluted filter

and the clear reddish yellow filtrate acidified with hydrochloric acid. A pale yellow solid separated out which after a crystallisation from alcohol was obtained as pale yellow prismatic needles melting at 275–6°. A second crystallisation from the same solvent did not improve the melting point. It did not also depress the melting point of an authentic sample of chrysin; yield, 0.3 g.

*O-Diethyl-oroxylin-A*

The sample of oroxylin-A (0.5 g.) melting at 219–20° was ethylated by refluxing with diethyl sulphate (2 c.c.) and anhydrous potassium carbonate (5 g.) in anhydrous acetone (50 c.c.). After 20 hours, the solvent was distilled off and the residue treated with water. The precipitated solid was filtered, washed with water and crystallised once from aqueous alcohol. The ethyl ether was readily obtained as colourless rectangular plates melting at 115–16° which was not depressed by a synthetic sample. Yield, 0.4 g. (Found: C, 70.3; H, 5.8;  $C_{20}H_{20}O_5$  requires C, 70.6 and H, 5.9%).

*2-Hydroxy-4:6-diethoxy-acetophenone (III)*

Dry phloracetophenone (4.2 g.) was refluxed with ethyl iodide (4.3 c.c.) and anhydrous potassium carbonate (10 g.) in acetone solution (150 c.c.). After 12 hours, the solvent was distilled off and water added to the residue. The resulting solid was collected, washed with water and then taken up in ether. The ethereal solution was extracted thrice with 5% aqueous sodium hydroxide. The combined extracts were acidified with dilute hydrochloric acid when the ethyl ether was precipitated as a practically colourless crystalline solid. It was filtered, washed and crystallised from dilute alcohol. The ketone was now obtained as colourless thin, broad, rectangular plates melting at 86–87°. Its alcoholic solution developed an intense reddish brown colour with a drop of ferric chloride. Yield, 1 g.

*2:5-Dihydroxy-4:6-diethoxy-acetophenone (IV)*

To a continuously stirred solution of the above ketone (3.5 g.) in aqueous sodium hydroxide (60 c.c.; 10%) was added dropwise a solution of potassium persulphate (5 g.) in water (100 c.c.) during the course of 3 hours, the temperature being maintained at 15–20° throughout the addition. After 24 hours, the reddish brown solution was neutralised to litmus with hydrochloric acid and the unchanged ketone recovered by ether extraction. The liquid was then treated with sodium sulphite (5 g.) and concentrated hydrochloric acid (25 c.c.) and heated at 90° for half-an-hour. On cooling a brownish yellow crystalline solid was deposited. When crystallised from alcohol,

2:5-dihydroxy-4:6-diethoxy-acetophenone was obtained as yellow stout rhombic prisms and melted at 130–1°. It was easily soluble in hot alcohol and acetone but insoluble in petroleum-ether. In alcoholic solution, the substance gave a transient green colouration with a drop of ferric chloride and with excess of the reagent a deep reddish brown colour. It gave no precipitate with lead acetate in alcoholic solution. (Found: C, 59.9; H, 6.9;  $C_{12}H_{16}O_5$  requires C, 60.0; H, 6.7%). Yield, 0.8 g.

The dibenzoyl derivative was prepared by heating the dihydroxy ketone with benzoyl chloride and pyridine on the water-bath. The product was diluted with water, and the precipitated solid was filtered and crystallised from alcohol; m.p. 154–5°. (Found: C, 69.9; H, 5.5;  $C_{28}H_{24}O_7$  requires C, 69.6 and H, 5.4%).

*Partial methylation of the above ketone*

The 2:5-dihydroxy compound (IV) (1.6 g.) was dissolved in dry acetone (75 c.c.) and treated with acid-free dimethyl sulphate (0.7 c.c.) and anhydrous potassium carbonate (3 g.). After refluxing for 12 hours, the potassium salts were filtered and washed well with warm acetone. After distilling off the acetone from the filtrate, the residue was taken up in ether and extracted thrice with 5% aqueous alkali. The extracts were acidified with hydrochloric acid. The product was taken up in ether and the ether solution dried over sodium sulphate. On removing the solvent a reddish-brown oil was obtained which did not solidify even on keeping in the refrigerator for 3 days. It was then treated with light petroleum (40–60°) when the unchanged dihydroxy compound along with some resinous impurity was left behind. The petroleum ether solution was carefully decanted and the solvent distilled off when the partial methyl ether was obtained as a bright yellow oil. Yield, 0.8 g. It dissolved in aqueous sodium hydroxide to give a yellow solution and its alcoholic solution developed a violet-brown colour with a drop of ferric chloride. It did not solidify on standing or cooling and could not be further purified. It was used directly for the next stage of synthesis.

*6-Methoxy-5:7-diethoxy flavone (II): (a) Allan-Robinson Method*

An intimate mixture of the above partial methyl ether (V) (2 g.), benzoic anhydride (12 g.) and sodium benzoate (4 g.) was heated between 170–80°, under reduced pressure for three hours. The cooled mass was then refluxed with alcoholic potash (10%; 75 c.c.) for 20 minutes and then after the removal, of the solvent by distillation under reduced pressure, dissolved in water and saturated with carbon dioxide. A dark brown solid separated out.

It was further boiled with aqueous alcoholic sodium carbonate (5%) for about two hours. After distillation of the alcohol and acidification the solid product was filtered and crystallised from alcohol using animal charcoal. It gave a green colouration with alcoholic ferric chloride indicating partial de-ethylation. So it was ethylated further using excess of ethyl sulphate and potassium carbonate in anhydrous acetone medium. After refluxing for 12 hours the solvent was distilled off and water added to the residue. A brownish white solid separated out. It was crystallised from aqueous alcohol when 6-methoxy-5:7-diethoxy flavone was obtained as colourless narrow rectangular plates melting at 115–6°. It gave no colour with ferric chloride in alcoholic solution and did not dissolve in aqueous alkali. (Found: C, 70.5; H, 5.8;  $C_{20}H_{20}O_5$  requires C, 70.6; H, 5.9%).

(b) *Baker and Venkataraman Method*

*Benzoylation.*—The partially methylated ketone (V) (1.5 g.) was benzoylated by heating with benzoyl chloride (1 c.c.) and pyridine (8 c.c.) on the water-bath for 30 minutes. The benzoyl derivative separated out as a pale brown oil when the mixture was diluted with ice-cold dilute hydrochloric acid. It was taken up in ether and washed with 3% aqueous sodium hydroxide in order to remove any unchanged ketone and benzoic acid and then with very dilute hydrochloric acid and water. After drying over calcium chloride, the ether was removed when the benzoyl derivative was obtained as a practically colourless semi-solid mass. Yield, 1.5 g. It was insoluble in aqueous alkali and gave no colour with alcoholic ferric chloride. Various attempts at crystallisation from alcohol, alcohol-benzene mixture, and petroleum ether-acetone mixture failed. It was, therefore, directly employed for conversion into the dibenzoyl methane.

*Transformation.*—The foregoing benzoyl derivative was heated with finely powdered sodamide (10 g.) in dry toluene medium on the water-bath for four hours with frequent shaking. The solid sodium derivative was then filtered, washed well with warm benzene and after drying added in small quantities to ice-water. After the excess of sodamide had completely decomposed, the bright yellow solution was saturated with carbon dioxide. The dibenzoyl methane was obtained as an orange yellow oil. It was taken up in ether and dried over sodium sulphate. When the solvent was removed again a bright yellow oil was obtained. It did not crystallise on standing or cooling. It easily dissolved in aqueous alkali and gave a deep reddish brown colour with a drop of ferric chloride. Yield, 0.9 g.

*Ring closure.*—The above dibenzoyl methane (0.7 g.) was treated with glacial acetic acid (8 c.c.) and anhydrous sodium acetate (2 g.) and the

mixture gently refluxed for 4 hours. After dilution with water, it was extracted with ether, the ether layer shaken with sodium hydroxide to remove acetic acid and any phenolic material. After washing with very dilute hydrochloric acid and water, the ether solution was dried over calcium chloride and the solvent removed. The flavone was obtained as a colourless oil which solidified on keeping in the refrigerator for 12 hours. It crystallised from petroleum-ether-acetone mixture as narrow rectangular plates and flat needles melting at 115–116°. It was insoluble in aqueous alkali and gave no colour with alcoholic ferric chloride. It agreed in every respect with the sample obtained by the Allan-Robinson method.

#### SUMMARY

The experiments described in this paper show that a pure sample of oroxylin-A melts at 219–20° (acetate, 139–40°), that the substance melting at 231–32° is a mixture of it with chrysin and that this mixture could be separated by fractionation of the acetates. The constitution of oroxylin-A as the 6-methyl ether of baicalein is confirmed by ethylating it to the diethyl ether and showing that the product is identical with a synthetic sample of 6-methoxy-5:7-diethoxy flavone. The details of the synthesis are given.

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