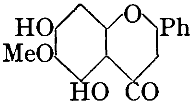
290. *The Constitution of Oroxylin-A. Part II. Attempted Synthesis of Oroxylin-A and the Synthesis of Wogonin.*

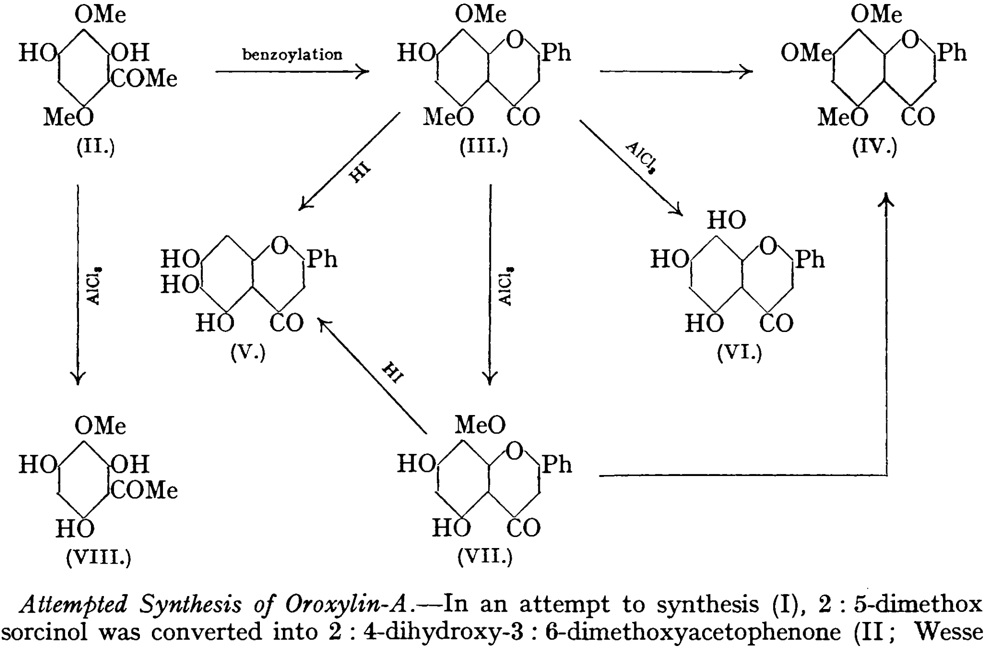
By R. C. Shah, C. R. Mehta, and T. S. Wheeler.

The presence of baicalein in the root bark of *Oroxylum indicum*, Vent, has been confirmed, and sitosterol and galactose have also been isolated. In an attempt to synthesise the previously isolated oroxylin-A (5 : 7-dihydroxy-6-methoxyflavone), it was found that benzoylation of 2 : 4-dihydroxy-3 : 6-dimethoxyacetophenone proceeded normally (with ring closure) to give *7-hydroxy-5 : 8-dimethoxyflavone*, which was demethylated by aluminium chloride to 5 : 7 : 8-trihydroxyflavone, obtained, it is believed, pure for the first time, and to wogonin (5 : 7-dihydroxy-8-methoxyflavone). This is the first synthesis of this naturally occurring flavone. The action of hydriodic acid on 7-hydroxy-5 : 8-dimethoxyflavone and on wogonin under the experimental conditions described gave baicalein (5 : 6 : 7-trihydroxyflavone), and it is suggested that this is due to the opening of the pyrone ring with subsequent rearrangement.

Investigation of the constituents of the root bark of *Oroxylum indicum*, Vent, has been continued, and the presence of baicalein (5 : 6 : 7-trihydroxyflavone), previously suspected on the basis of its specific colour reaction with alkali (dissolution to a reddish-brown solution with subsequent formation of green flocks; Part I, J., 1936, 592), has been confirmed by its isolation. In addition to oroxylin-A, 5 : 7-dihydroxy-6-methoxyflavone (I), sitosterol and galactose have also been isolated. Bhose and Battacharya (*Science and Culture*, 1936, 581) report the presence of baicalein and chrysin (5 : 7-dihydroxyflavone).



(I.)



Attempted Synthesis of Oroxylin-A.—In an attempt to synthesis (I), 2 : 5-dimethoxyresorcinol was converted into 2 : 4-dihydroxy-3 : 6-dimethoxyacetophenone (II; Wessely and Moser, *Monatsh.*, 1930, **56**, 97), which was fused with benzoic anhydride and sodium benzoate; since the product (m. p. 287—288°) was a hydroxydimethoxy- and not a dihydroxymethoxy-flavone, it must be *7-hydroxy-5 : 8-dimethoxyflavone* (III) and not (I;

m. p. 231—232°). This is confirmed by the fact that on methylation (III) gave a trimethoxyflavone (m. p. 167—168°), which is not 5 : 6 : 7-trimethoxyflavone (m. p. 163—164° ; Hattori, *Acta Phytochim.*, 1930, **5**, 99), but 5 : 7 : 8-trimethoxyflavone (IV ; m. p. 167—168° ; *idem, ibid.*), obtained for purpose of comparison by methylation of authentic wogonin (VII).

Wessely and Moser (*loc. cit.*) found that anisoylation of (II) afforded 5 : 7-dihydroxy-6 : 4′-dimethoxyflavone, and this was confirmed by Wessely and Kallab (*Monatsh.*, 1932, **60**, 26), but Furukawa and Tamaki (*Bull. Inst. Phys. Chem. Res. Tokyo*, 1931, **10**, 732; cf. Hattori, *Acta Phytochim.*, *loc. cit.*; 1931, **5**, 219) claimed to have obtained the normal product, 7-hydroxy-5 : 8 : 4′-trimethoxyflavone. Baker, Nodzu, and Robinson (J., 1929, 74) and Goldsworthy and Robinson (this vol., p. 56), who have studied respectively the veratroylation and the anisoylation of the ω-methoxy-derivative of (II), also obtained 5 : 7 : 8-derivatives.

Demethylation of O-*Alkylated* 5 : 7 : 8-*Trihydroxyflavones*.—Demethylation of (III) by hydriodic acid proceeded abnormally, the product being 5 : 6 : 7-trihydroxyflavone (V) instead of the expected 5 : 7 : 8-derivative (VI). Rearrangement of a 5 : 7 : 8- to a 5 : 6 : 7-trihydroxyflavone during hydriodic acid demethylation has been observed by Wessely and Moser (*loc. cit.*; cf. Furukawa, quoted by Hattori, *loc. cit.*, 1931), who obtained 5 : 6 : 7 : 4′-tetrahydroxyflavone (scutellarein) from 7-hydroxy-5 : 8 : 4′-trimethoxyflavone. Normal demethylation to gossypetin has, however, been obtained by Baker *et al.* (*loc. cit.*) with 7-hydroxy-3 : 5 : 8 : 3′ : 4′-pentamethoxyflavone; by Hattori (*loc. cit.*, 1930) with wogonin (VII) and 5 : 7 : 8-trimethoxyflavone, and (*loc. cit.*, 1931) with 7 : 8-dimethoxy-5-ethoxyflavone; by Furukawa and Tamaki (*loc. cit.*) with 7-hydroxy-5 : 8 : 4′-trimethoxyflavone, and by Goldsworthy and Robinson (*loc. cit.*) with 7-hydroxy-3 : 5 : 8 : 4′-tetramethoxyflavone. On the other hand, Wessely and Kallab (*loc. cit.*), in a re-examination of the previous work of Wessely and Moser (*loc. cit.*), claim that, contrary to the results of Hattori (*loc. cit.*, 1931), rearrangement to scutellarein occurs on demethylation of 5 : 7 : 8 : 4′-tetramethoxyflavone with hydriodic acid. Further evidence on this point is afforded by the following observations. Demethylation of (III) by means of anhydrous aluminium chloride (1—3 mols.) yielded a trihydroxyflavone (m. p. 250—251°), which gave an alkali colour reaction distinct from that of baicalein; it dissolved in dilute alkali with a scarlet colour, which rapidly changed through green to yellow with a tinge of green, without separation of green flocks. In addition, it gave indications of a positive gossypetone reaction (Perkin, J., 1913, **103**, 656); the quantity available, however, was too small for the complete application of the test. It cannot, therefore, be baicalein, and this view was confirmed by a mixed m. p. comparison; it can only be 5 : 7 : 8-trimethoxyflavone (VI). Hattori's product (cf. Nierenstein, *Acta Phytochim.*, 1932, **6**, 173; Hattori, *ibid.*, p. 176), on the other hand, which was obtained by the action of hydriodic acid on wogonin, 5 : 7 : 8-trimethoxyflavin, 7 : 8-dimethoxy-5-ethoxyflavone, and 2 : 3 : 4 : 6-tetramethoxydibenzoylmethane (the product from this was usually mixed with baicalein), had m. p. 226—227°, and was regarded by him as 5 : 7 : 8-trihydroxyflavone, although only acetyl derivatives were analysed.

A re-examination of the hydriodic acid demethylation of natural wogonin (VII) gave a product with m. p. 247—248°, depressed by admixture with (VI; m. p. 250—251°), but not with baicalein (m. p. 264°). Further, this new product gave the characteristic alkali colour reaction for baicalein. Hattori (*Acta Phytochim.*, 1935, **5**, 109) reports for his product (m. p. 226—227°) an alkali colour reaction similar to that given by baicalein, and he may have had in hand a mixture of baicalein and 5 : 7 : 8-trihydroxyflavone; we do not, however, wish finally to exclude the possibility of 5 : 7 : 8-trihydroxyflavone existing in dimorphic forms (m. p. 226—227°; 250—251°). The fact that Hattori's 5 : 7 : 8-trihydroxyflavone gives an absorption spectrum very similar to wogonin and its monomethyl ether supports this latter view (see *ibid.*, 1931, **5**, 233).

The disagreement among various workers in regard to the action of hydriodic acid on *O*-alkylated 5 : 7 : 8-trihydroxyflavones not containing a 3-methoxy-group is probably due to the difficulty of isolation of pure products when dealing with small quantities of material, and, although the results given above definitely show rearrangement, they are put forward with reserve. It is suggested that demethylation is accompanied by opening of the pyrone ring, followed by ring closure with formation of 5 : 6 : 7- or 5 : 7 : 8-trihydroxyflavones. Of interest in this connection is the fact that 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4 : 6 : 4′-penta-methoxydibenzoylmethanes give mixtures of 5 : 6 : 7- and 5 : 7 : 8-derivatives on treatment with hydriodic acid (Bargellini, *Gazzetta*, 1919, **49**, ii, 47; Hattori, *Acta Phytochim.*, 1931, **5**, 219).

If these contentions are correct, then it is unsafe to assign a 5 : 6 : 7-structure to an alkoxy-flavone because it gives a 5 : 6 : 7-trihydroxyflavone on treatment with hydriodic acid, unless remethylation is carried out for the purpose of further comparison; aluminium chloride would appear to be a safer demethylating agent. In Part I (*loc. cit.*) emphasis was laid on the fact that demethylation of oroxylin-A with hydriodic acid gave baicalein; we no longer regard this evidence of structure as cogent, and prefer to base the constitution assigned to oroxylin-A on its colour reactions, and the correspondence of its *O*-alkylated derivatives with those of baicalein.

Synthesis of Wogonin.—Wogonin, which has not previously been synthesised, was isolated by Takahashi (*Chem. Zentr.*, 1889, II, 620) from the roots of *Scutellaria baicalensis* Georgi. Its constitution was first investigated by Shibata, Iwata, and Nakamura (*Acta Phytochim.*, 1923, **1**, 105), and later by Hattori (*ibid.*, 1930, **5**, 99), who assigned to it the structure 5 : 7-dihydroxy-8-methoxyflavone ($1H_2O$; VII). Partial demethylation of (III) was effected with aluminium chloride under mild conditions (Gulati and Venkataraman, J., 1936, 267) with production of a dihydroxymethoxyflavone, having m. p. 200—201°, not depressed by admixture with a sample of authentic natural wogonin to which it corresponded in analysis and properties. Methylation of the synthetic product with diazomethane gave 5-hydroxy-7 : 8-dimethoxyflavone, and, with methyl sulphate and alkali, 5 : 7 : 8-trimethoxyflavone (IV; identical with the methylation product of III), with properties in agreement with those described for those products by Hattori (*loc. cit.*).

2 : 4-Dihydroxy-3 : 6-dimethoxyacetophenone (II) was partly demethylated with aluminium chloride to a monomethoxy-ketone which is presumably the iretol derivative 2 : 4 : 6-*trihydroxy*-3-*methoxyacetophenone* (VIII), the 5-methoxy-group being, it is assumed, preferentially attacked. This ketone was benzoylated by the fusion method in the hope of obtaining (I), but no definite product could be isolated.

EXPERIMENTAL.

Isolation of Baicalein, Sitosterol, and Galactose from the Root-bark of Oroxylum indicum, *Vent.*—*Baicalein* (V). The aqueous-alcoholic mother-liquor, obtained after crystallisation of oroxylin-A (Part I, *loc. cit.*), was concentrated to small bulk; the resulting precipitate, after frequent crystallisation from dilute alcohol (charcoal) and from xylene, formed yellow needles, m. p. 264—265°, not depressed by authentic baicalein, to which it corresponded in properties and analysis.

Sitosterol. The fatty matter obtained by extraction of the root-bark with light petroleum was saponified with alcoholic potassium hydroxide, alcohol was removed under diminished pressure, and the residue dissolved in water. Ether extraction of the aqueous solution yielded a product which, after crystallisation from dilute alcohol (charcoal), had m. p. 136—137° (acetate, m. p. 127°) (Found, after drying at 100° : C, 81·8; H, 12·0. Calc. for $C_{27}H_{46}O,\frac{1}{2}H_2O$: C, 82·0; H, 11·9%); it gave the Burkhardt–Liebermann test and corresponded in properties to sitosterol (m. p. 137°; acetate, m. p. 127°).

Galactose. The crystalline solid which was deposited in the Soxhlet flask during extraction of the root-bark with acetone was washed with light petroleum and with ether, and dissolved in the minimum quantity of water. Alcohol was added to turbidity, and the solution kept at 0° overnight. The crystals thus obtained had m. p. 164°, not depressed by authentic galactose. This result was confirmed by preparation of the osazone and by oxidation of the sugar to mucic acid (mixed m. p. with acid from authentic galactose).

Preparation of 2 : 4-*Dihydroxy*-3 : 6-*dimethoxyacetophenone* (II).—2 : 6-Dibenzyloxyquinol was prepared as described by Baker, Nodzu, and Robinson (*loc. cit.*), but the reaction was accelerated by using three times the quantity of sulphuric acid (25%) and of alcohol. These authors' preparation of 2 : 5-dimethoxyresorcinol was modified as follows. A mixture of 2 : 6-dibenzyloxy-1 : 4-dimethoxybenzene (10 g.), prepared from 2 : 6-dibenzyloxyquinol as described by them, glacial acetic acid (70 c.c.), and hydrochloric acid (*d* 1·16; 30 c.c.) was kept at 65—70° for an hour; an equal volume of water was added, and the solution evaporated under diminished pressure to a syrup, which was extracted thrice (3 × 100 c.c.) with boiling water. The aqueous solution, after concentration to one-third of its original volume, was saturated with ammonium sulphate, and extracted several times with ether. After removal of the ether, 2 : 5-dimethoxyresorcinol was obtained as a light-brown, uncrystallisable oil (3·5 g.); its aqueous solution gave a weak bluish-violet coloration with aqueous ferric chloride.

The last compound, when treated according to Wessely and Moser (*loc. cit.*), afforded (II), with m. p. (129°) and properties as described by them (Found: C, 57·2; H, 5·6; OMe, 27·2. Calc. for $C_{10}H_{12}O_5$: C, 56·6; H, 5·7; OMe, 29·3%).

Fusion of (II) *with benzoic anhydride and sodium benzoate*: 7-*hydroxy*-5 : 8-*dimethoxyflavone* (III). An intimate mixture of (II) (0·5 g.), benzoic anhydride (2·5 g.), and sodium benzoate (1·25 g.) was heated at 180—185° (oil-bath) for 8 hours. The product was boiled under reflux for 20 minutes with alcohol (70 c.c.) and sodium hydroxide (1·2 g. in 2 c.c. of water). Alcohol was removed under reduced pressure, and the residue dissolved in water (50 c.c.). The aqueous solution was saturated with carbon dioxide, and the precipitated crude *flavone* was collected, washed with water, and crystallised twice from methyl alcohol; m. p. 287—288°; yield 0·25 g. (Found: C, 67·7; H, 4·9; OMe, 20·0. $C_{17}H_{14}O_5$ requires C, 68·4; H, 4·8; OMe, 20·8%). The substance dissolved in alkali with a deep yellow colour, and its alcoholic solution did not develop any coloration with alcoholic ferric chloride, showing the absence of (I). It was insoluble in most organic solvents. In methyl-alcoholic solution it gave a yellowish-white coloration with alcoholic lead acetate, and after an interval, a yellowish-white precipitate. It developed an orange coloration with magnesium and hydrochloric acid in aqueous-alcoholic solution. The above condensation was repeated at 155—160° and at 199—200°, but the yield in both cases was only 60% of the above.

Methylation of (III): 5 : 7 : 8-*trimethoxyflavone* (IV). The foregoing flavone (0·05 g.), dissolved in aqueous potassium hydroxide (20%; 0·5 c.c.), was treated with methyl sulphate (0·5 c.c.), and the resulting precipitate dissolved by heating at 100°. Methyl sulphate and potassium hydroxide solution (20%) in large excess were added alternately, dropwise, with heating, the mixture being kept alkaline throughout. The solution was diluted to three times its volume, whereby crude, completely methylated flavone was precipitated. After crystallisation from water and alcohol, it formed long colourless needles, corresponding in m. p. (167—168°), analysis (Found, after drying at 150—160°/2—3 mm.: C, 69·4; H, 5·5. Calc. for $C_{18}H_{16}O_5$: C, 69·2; H, 5·1%), and properties (absence of ferric chloride coloration; insolubility in dilute alkali) to 5 : 7 : 8-trimethoxyflavone. Its m. p. was not depressed by admixture with a sample of fully methylated wogonin, but was markedly lowered by addition of dimethyloroxylin-A.

Demethylation of (III) *with hydriodic acid*: 5 : 6 : 7-*trihydroxyflavone* (V). A mixture of (III; 0·05 g.), acetic anhydride (1·25 c.c.), and hydriodic acid (*d* 1·5; 1·25 c.c.) was heated under reflux at 145—150° (oil-bath) for 2 hours, and the cooled mixture poured into a saturated solution of sodium hydrogen sulphite. The precipitate was boiled with water, and twice crystallised from dilute alcohol and once from boiling xylene, from which it separated (0·01 g.) in yellow shining needles, m. p. 265—266°, not depressed by admixture with authentic baicalein, to which it corresponded in analysis (Found: C, 66·5; H, 3·9. Calc. for $C_{15}H_{10}O_5$: C, 66·7; H, 3·7%) and properties.

Demethylation of (III) *with anhydrous aluminium chloride* (1—3 *mols.*): 5 : 7 : 8-*trihydroxyflavone*. A mixture of (III; 0·1 g.) and anhydrous aluminium chloride (0·14 g.) with dry nitrobenzene (2 c.c.) was heated at 100° for 70 minutes. The resulting viscous mass was treated with ice and hydrochloric acid, and nitrobenzene removed in steam. The residual aqueous solution deposited on cooling a flocculent yellow mass which, when collected and crystallised thrice from dilute alcohol, formed deep golden-yellow needles, m. p. 250—251°, depressed by admixture with baicalein (Found: C, 66·0; H, 3·7; OMe, 0. Calc. for $C_{15}H_{10}O_5$: C, 66·7; H, 3·7%). The substance dissolved in dilute alkali with a scarlet colour, which rapidly changed through green to yellow with a tinge of green, without any separation of green flocks. Alcoholic lead acetate gave an orange-red precipitate, and alcoholic ferric chloride developed a deep green colour, which finally changed to bluish-green. With *p*-benzoquinone in alcoholic solution, it gave a reddish-brown coloration, followed by separation of a precipitate (cf. Perkin, *loc. cit.*); the minute quantity available prevented further testing of the precipitate.

Variations in the proportion of aluminium chloride between 1 and 3 mols. did not alter the nature of the product.

Demethylation of Wogonin by Means of Hydriodic Acid.—A sample of wogonin, treated with hydriodic acid in the manner described for (III), gave a product which, on recrystallisation from dilute alcohol, had m. p. 247—248°, depressed by admixture with (VI), but not by addition of baicalein, to the specific alkali coloration of which it also answered.

Partial Demethylation of (III) : *Synthesis of Wogonin* (VII).—A mixture of (III; 0·23 g.), anhydrous aluminium chloride (0·075 g.), and nitrobenzene (2 c.c.) was heated at 100° for 105 minutes, additional nitrobenzene (1 c.c.) added, and heating continued for 45 minutes. After addition of ice and hydrochloric acid, nitrobenzene was removed in steam, and the aqueous residue kept at 0° overnight. The resulting precipitate was extracted with boiling water (1000 c.c.), the residue was dissolved in alcohol, and the solution treated with water to turbidity, deep yellow needles separating. After two recrystallisations from dilute alcohol, the product (0·07 g.) had m. p. 200—201°, not depressed by natural wogonin, to which it corresponded in properties and analysis (Found : C, 65·5; H, 4·2; OMe, 10·5. Calc. for $C_{16}H_{12}O_5,\frac{1}{2}H_2O$: C, 65·5; H, 4·4; OMe, 10·6%. Found, in material dried at 155—165°/1 mm. : C, 67·4; H, 4·6. Calc. for $C_{16}H_{12}O_5$: C, 67·6; H, 4·2%).

Methylation of Synthetic Wogonin.—(i) *With diazomethane* : 5-*hydroxy*-7 : 8-*dimethoxyflavone*. A solution of (VII; 0·02 g.) in dry ether (5 c.c.) was added to an ethereal solution of diazomethane, and the solvent allowed to evaporate spontaneously. The pale yellow residue, after two recrystallisations from dilute alcohol, had m. p. 178—179° (lit., 183°).

(ii) *With methyl sulphate and alkali* : 5 : 7 : 8-*trimethoxyflavone* (IV). A solution of (VII; 0·02 g.) in aqueous potassium hydroxide (0·2 c.c.; 20%) was completely methylated as described for (III). The product on recrystallisation from dilute alcohol and water was identical with (IV).

Partial Demethylation of (II) *with Anhydrous Aluminium Chloride* : 2 : 4 : 6-*Trihydroxy*-3-*methoxyacetophenone* (VIII).—A mixture of (II; 1·5 g.), anhydrous aluminium chloride (0·6 g.), and dry nitrobenzene (6 c.c.) was heated at 100° for 1 hour, and the product treated with ice and hydrochloric acid and steam-distilled. The residual aqueous solution deposited on cooling yellowish needles, m. p. 188° after crystallisation from dilute alcohol (Found : C, 54·6; H, 5·0; OMe, 15·4. $C_9H_{10}O_5$ requires C, 54·5; H, 5·1; OMe, 15·7%); these gave in alcoholic solution with ferric chloride a deep reddish-violet coloration, and with alkali a yellow coloration. Benzoylation by fusion under the usual conditions at 180° yielded no satisfactory product.

We are indebted to Prof. S. Hattori, of the Botanical Institute, Imperial University, Tokyo, Japan, for a specimen of natural wogonin, and to Dr. Jivraj Mehta and Prof. A. S. Paranjpe, of Seth G.S. Medical College, Bombay, for providing facilities for most of this work in the Pharmacological Laboratories of that College.

ISMAIL COLLEGE, SETH G.S. MEDICAL COLLEGE, AND
ROYAL INSTITUTE OF SCIENCE, BOMBAY.

[*Received, July* 12*th*, 1938.]