14. *Anthoxanthins. Part XVI. Synthesis of Herbacetin.*

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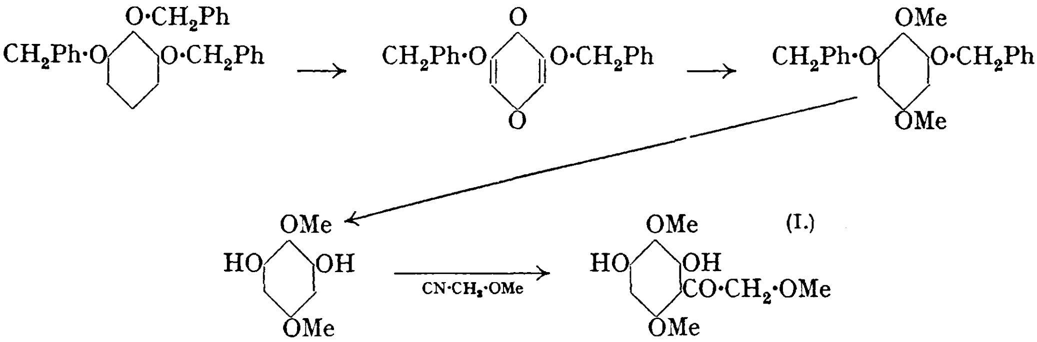
The new glucoside, herbacitrin, isolated by Seshadri and his collaborators from flowers of certain cotton plants is isomeric with quercimeritrin but has a closer resemblance to gossypitrin in its chemical behaviour. The related aglucone, herbacetin, isomeric with quercetin, is similar to gossypetin, especially in its facile aerial oxidation in alkaline solution. Because herbacitrin could be degraded to anisic acid, and taking into account the analogies already mentioned, the suggestion was made that herbacetin bears to gossypetin the relation that kaempferol bears to quercetin.

This view that herbacetin is 3 : 5 : 7 : 8 : 4′-pentahydroxyflavone has now been confirmed by unambiguous synthesis following the lines of the method used for the synthesis of gossypetin.

2 : 4-DIHYDROXY-ω : 3 : 6-TRIMETHOXYACETOPHENONE (I) was obtained (Baker, Nodzu, and Robinson, J., 1929, 79) by a method, indicated in the annexed scheme, which gave no scope for doubt as to the constitution of the product.

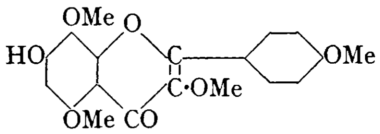


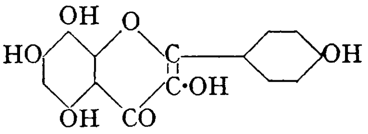
(I.)

The point is an important one, because the synthetical proof of the constitution of quercetagetin (there is no complete analytical proof) rests on the exclusion of the alternative structure first assigned to gossypetin. The intermediate ketone (I) is also constructed fortunately in that the aroylation and ring-closure to a flavonol derivative can proceed in one direction only.

Treatment with veratric anhydride and sodium veratrate afforded a hydroxypentamethoxyflavone which was a pentamethyl ether of gossypetin. Using, in a similar reaction, anisic anhydride and sodium anisate, and working in the usual manner by hydrolysis of the aroylated product, we have now obtained 7-*hydroxy*-3 : 5 : 8 : 4′-*tetramethoxyflavone* (II).

(II.)





(III.)

On demethylation by means of boiling hydriodic acid, 3 : 5 : 7 : 8 : 4′-*pentahydroxyflavone* (III) was obtained, and this flavone proved to be identical with herbacetin as described by Neelakantam and Seshadri (*Proc. Indian Acad. Sci.*, 1937, **5**, Sec. A, No. 4, 357). We are grateful to Dr. T. R. Seshadri for the provision of specimens of herbacetin and its penta-acetyl derivative which have enabled us to establish the identity by direct comparison.

EXPERIMENTAL.

7-*Hydroxy*-3 : 5 : 8 : 4′-*tetramethoxyflavone* (II).—An intimate mixture of 2 : 4-dihydroxy-ω : 3 : 6-trimethoxyacetophenone (I) (1·0 g.), anisic anhydride (4·0 g.), and sodium anisate

(2·0 g.) was heated for 3 hours at 180—185° (oil-bath) under 20 mm. pressure. The cooled product was hydrolysed by refluxing it for 30 minutes with a solution of potassium hydroxide (4·0 g.) in alcohol (30 c.c.) and water (2 c.c.). The bulk of the alcohol was removed on the steam-bath, and the residue shaken with water (50 c.c.). The portion of the residue (0·76 g.) insoluble in water was unhydrolysed material: it was powdered and refluxed with a further quantity (2·0 g.) of potassium hydroxide, dissolved in alcohol (15 c.c.) and water (1 c.c.). After the removal of the alcohol, the residue dissolved completely in water. When carbon dioxide was passed into the combined aqueous solutions, diluted to 100 c.c., the *flavone* was eventually precipitated. No precipitate was formed until a rapid stream of the gas had been passed through the solution for ½ hour. The product was collected, washed well with water, and dried at 100°; m. p. *ca.* 254° with previous sintering. The substance crystallised readily from alcohol in glistening, golden-yellow, thin, rectangular plates, m. p. 268—269° with slight sintering. A second crystallisation from alcohol raised the m. p. to 269—270° (Found in material dried at 100° and finally in a vacuum over sulphuric acid at room temperature: C, 63·9; H, 4·9. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·1%). The flavone dissolved in concentrated hydrochloric acid and in aqueous alkalis to yellow solutions. It gave no characteristic coloration when treated in alcoholic solution with ferric chloride.

3 : 5 : 7 : 8 : 4′-*Pentahydroxyflavone* (*Herbacetin*, III).—7-Hydroxy-3 : 5 : 8 : 4′-tetramethoxyflavone (0·6 g.) was demethylated by heating for 3 hours at 135—140° with pure hydriodic acid (9 c.c., *d* 1·7). The red crystalline hydriodide, which separated on cooling, was collected on sintered glass and washed with dilute sulphurous acid. The crystals were then decomposed by heating for 15 minutes on the steam-bath. The yellow product separated from aqueous alcohol in microscopic crystals, m. p. 278—280° (Found in air-dried material: loss at 130° in a vacuum, 5·6. Calc. for $C_{15}H_{10}O_7,H_2O$: H_2O, 5·6%). Neelakantam and Seshadri (*loc. cit.*) cited elementary analyses of the natural product, crystallised from aqueous alcohol and dried in the air, showing that herbacetin forms a monohydrate.

Although the separation from aqueous alcohol is usually in the form of extremely minute crystals, slow deposition from a more dilute solution affords flat needles as described for herbacetin. These needles can also be obtained by long digestion of the microcrystalline powder with 50% acetic acid (Found in material dried in a vacuum over sulphuric acid: C, 58·4; H, 3·5. Found for material dried at 110° in high vacuum: C, 59·4; H, 3·4. Calc. for $C_{15}H_{10}O_7$: C, 59·6; H, 3·3%). It is very difficult to remove the last traces of water and the dried product is hygroscopic.

Pure herbacetin is stated to have m. p. 280—283° (*loc. cit.*), but the specimen supplied to us, after recrystallisation, had m. p. 279—281° under our conditions, and the best synthetic specimen and a mixture of the natural and the synthetic specimen showed the same behaviour on heating. The sintering, range of temperature during fusion, and the extent of decomposition were identical.

The synthetic flavonol, like the naturally occurring herbacetin, gives a deep red precipitate with lead acetate in aqueous alcohol, and the ferric reaction is dull green. The yellow solution in sulphuric acid is devoid of fluorescence.

The colour reactions shown in buffered solutions prepared from " B.D.H. Buffer Salt " (containing phosphoric, boric, and phenylacetic acids) were found to resemble generally those described by Neelakantam and Seshadri (*loc. cit.*, p. 362), but there were slight divergences in the observed rates of colour changes. As the processes involved depend on aerial oxidation, they are affected by the quantity taken, the size of the crystals, the shape of the tubes employed, the frequency of shaking, and especially by the temperature.

Small specimens of the synthetic and the naturally occurring flavonol were crystallised under similar conditions so as to obtain a fine microcrystalline powder. This was shaken with a buffered solution of p_H 8·0, and approximately equal volumes (*ca.* 1 c.c.) of the suspensions were added to 20 c.c. of the various buffered solutions in boiling tubes (1 in. diameter) at 14° with only one shaking to mix. Under these conditions the two specimens gave identical results when compared side by side.

p_H 6·8 : The solutions remained colourless during 24 hours.

p_H 8·0 : The solid dissolved very slowly to a light yellow solution and after ¾ hour a faint green shade had developed. Later this changed to a weak dull purple.

p_H 8·6 : A light yellow solution was quickly formed; this became green in a few minutes and then very slowly went through slaty grey to a violet-blue.

p_H 9·2 : The same colour changes as the last-mentioned occurred more rapidly. This solution becomes purplish in tone more rapidly than those in the ranges on either side because the yellow background is not so much in evidence as in the solution of p_H 9·8.

p_H 9·8 : The change from yellow to green was much quicker and the solution then became gradually bluer and eventually pure blue. After about 1 hour the colour deteriorated to a slaty blue and thereafter progressively diminished in intensity and after 24 hours all coloration had disappeared. It was found in other experiments that the use of relatively more of the flavonol resulted in retention of a dull purple colour even on long keeping.

At p_H 10·4 and p_H 11·0 the same changes occurred but were greatly accelerated. The deep blue solutions obtained at p_H 9·8—11·0 became red on acidification and doubtless contained " herbacetone " analogous to " gossypetone " (A. G. Perkin, J., 1913, **103**, 650).

Identical dyeings on mordanted cotton were obtained from the " natural " and the synthetic specimens. These are doubtless wholly or partly due to herbacetone, because similar colorations were observed on dyeing from an oxidised bath (an alkaline solution kept until it became blue and then neutralised with acetic acid). The dyeings were : weak iron, grey ; strong iron, brownish-black ; iron and aluminium, dark dull brown ; strong aluminium, brown ; weak aluminium, fawn.

3 : 5 : 7 : 8 : 4′-*Penta-acetoxyflavone*.—The synthetic pentahydroxyflavone (0·1 g.) was refluxed with acetic anhydride (0·5 c.c.) and a drop of pyridine for 3 hours. After cooling and keeping for a few hours, the derivative crystallised ; it was collected and washed with cold alcohol ; m. p. 187—190° with slight sintering. On recrystallisation from alcohol, colourless needles, m. p. 189—191°, were obtained (Neelakantam and Seshadri, *loc. cit.*, give m. p. 192—193° for penta-acetylherbacetin) and two further crystallisations did not raise the m. p., which was not depressed on admixture of the specimen with authentic *O*-penta-acetylherbacetin (Found in synthetic derivative : C, 58·5 ; H, 4·0. Calc. for $C_{25}H_{20}O_{12}$: C, 58·6 ; H, 3·9%).

3 : 5 : 7 : 8 : 4′-*Pentamethoxyflavone*.—The above tetramethyl ether dissolved readily in warm 10% aqueous sodium hydroxide, but a sparingly soluble sodium salt soon separated in yellow needles and did not redissolve on heating. On shaking with methyl sulphate the substance was slowly methylated and it was found best to add some acetone ; the methyl ether was then quickly formed. It crystallised from aqueous methyl alcohol in slender woolly needles or from ethyl acetate in colourless, stouter, prismatic needles, m. p. 156—158° (Found : C, 64·3 ; H, 5·4. $C_{20}H_{20}O_7$ requires C, 64·5 ; H, 5·4%). The *substance* is a good oxonium base and is soluble in cold concentrated hydrochloric acid (or in N-hydrochloric acid on heating) to a bright yellow solution. The formation of a sparingly soluble, crystalline ferrichloride, platinichloride and picrate was observed.

The woolly needles absorb iodine from aqueous solutions and are coloured dark greenish-blue.

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[*Received, November 24th*, 1937.]