PIGMENTS OF COTTON FLOWERS

Part VIII. Constitution of Herbacitrin and Quercimeritrin

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A new flavonol glucoside was recently isolated by Neelakantam and Seshadri from the Uppam (*Gossypium herbaceum*) cotton flowers and was named Herbacitrin.[1] It was shown to occur in the Karunganni (*G. indicum*) flowers also.[2] The glucoside on hydrolysis with acids yielded glucose and an aglucone called Herbacetin (formula I, R = H) whose constitution was arrived at by the above authors[3] from a study of its reactions and decomposition products and confirmed by Goldsworthy and Robinson[4] by synthesis.

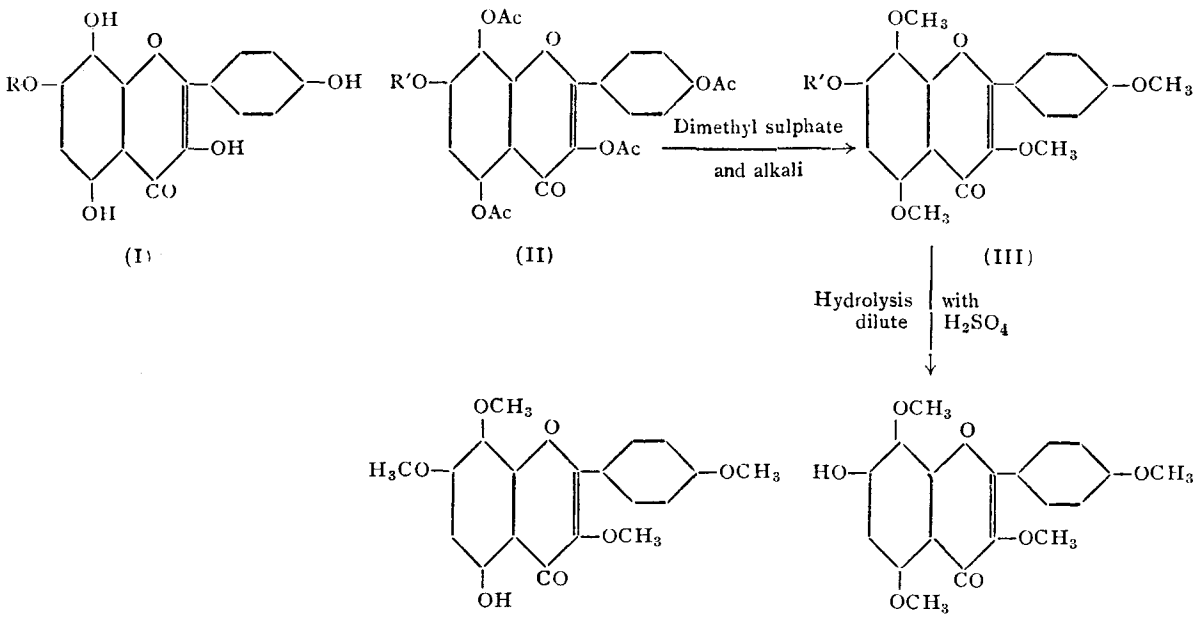
The new glucoside closely resembles Gossypitrin and also occurs along with it. When oxidised in alkaline solution, it yields *p*-hydroxybenzoic acid and hence the hydroxyl group in position 4 should be free. It gives a deep red precipitate with lead acetate and is hydrolysed with difficulty by acids, indicating thereby that it is not a 3-glucoside. The possibility of the 5- or the 8-hydroxyl group being involved in glucoside formation does not exist as the substance is readily oxidised to give the gossypetone reaction. From these considerations and also from the fact that all known glycosides of anthoxanthins are either the 3- or the 7-glycosides, Herbacitrin was tentatively represented by Neelakantam and Seshadri as the 7-glucoside of Herbacetin.[3]

For purposes of definitely orienting the position of the glucose residue, the use of diazomethane as a methylating agent is precluded since it does not methylate even Herbacetin completely,[5] the hydroxyl group in position 5 remaining unaffected. Our object has, however, been achieved by methylating Herbacitrin through its acetyl derivative, hydrolysing the methylated glucoside and identifying the partially methylated Herbacetin obtained thereby. This method of methylation through the acetyl derivatives has been recently used by us very successfully in the case of Gossypitrin.[6] Herbacitrin octa-acetate in acetone solution is treated with dimethyl sulphate and alkali alternately. The acetyl groups are removed and all the free phenolic hydroxyl groups get methylated. Hydrolysis of the product with

dilute sulphuric acid gives rise to an O-tetramethyl-herbacetin. There are two tetramethyl ethers of Herbacetin described in the literature :—(1) 3 : 7 : 8 : 4′-tetramethyl ether (V) recently prepared by us by the methylation of Herbacetin with diazomethane[5]; (2) 3 : 5 : 8 : 4′-tetramethyl ether (IV) obtained by Goldsworthy and Robinson (*loc. cit.*), in the course of the synthesis of Herbacetin. These are very easily distinguished from each other from their reactions with sodium hydroxide and ferric chloride. The product resulting from the hydrolysis of the methylated glucoside agrees with (IV) very closely as shown in the table given below :—

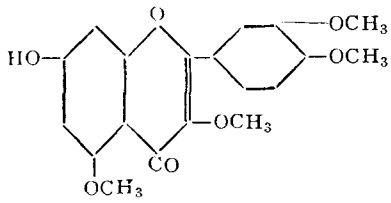
	3 : 7 : 8 : 4′-tetramethyl herbacetin	3 : 5 : 8 : 4′-tetramethyl herbacetin	The tetramethyl ether obtained by the hydrolysis of the methylated glucoside
Melting point	159–60	269–70	269–70
Solubility in sodium hydroxide ..	Difficultly soluble	Easily soluble	Easily soluble
Colour with ferric chloride solution	Bright green colour	No characteristic colour	No characteristic colour

Hence the glucose residue occupies position 7 in Herbacitrin (I, R = $C_6H_{11}O_5$) and the course of the reaction can be represented thus :—

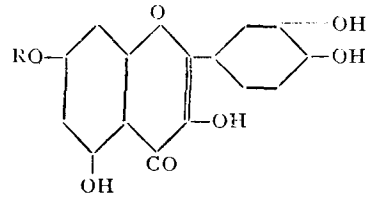


(R′ is acetylated or partially methylated glucose residue)

Quercimeritrin, a glucoside of Quercetin was originally isolated by Perkin[7] from the Egyptian cotton flowers and has since been shown to occur in *Prunus emarginata*,[8] *Helianthus annus*,[9] *G. hirsutum*,[10] *G. indicum*,[2] etc. The constitution of this glucoside was established by Attree and Perkin[11] by methylation with diazomethane and subsequent hydrolysis. We have now examined the suitability of the new method starting with the acetyl derivative of Quercimeritrin.* All the available phenolic hydroxyl groups are easily methylated and the methylated glucoside, after hydrolysis, yields 3 : 5 : 3′ : 4′-tetramethyl quercetin (VI) thus confirming the constitution of Quercimeritrin as a 7-glucoside (VII, R = $C_6H_{11}O_5$).



(VI)



(VII)

Experimental

3 : 5 : 8 : 4′-*Tetramethyl herbacetin from Herbacitrin*

Acetyl herbacitrin was prepared in the usual way by boiling Herbacitrin with acetic anhydride and anhydrous sodium acetate for 4 hours. It crystallised readily from alcohol as long needles melting at 222–24° and not at 214–16° as stated previously.[3]

Herbacitrin acetate (150 mg.) was dissolved in acetone (10 c.c.) and treated with dimethyl sulphate (1·5 c.c.) and 20% sodium hydroxide (1·5 c.c.). After shaking vigorously for sometime, more of methyl sulphate (2 c.c.) and 20% alkali (2 c.c.) were added alternately in small amounts shaking vigorously after each addition. Finally 2 c.c. more of the aklali were added to keep the medium strongly alkaline. As usual heat was gradually developed during the course of the reaction. After leaving overnight, the contents were refluxed on a water-bath for an hour. On driving off the solvent (acetone) almost completely the methylated glucoside separated out as a brown semi-solid mass. As the quantity was small, no attempt was made to isolate and purify it. In order to hydrolyse it, alcohol (25 c.c.) and water (25 c.c.) were added along with sufficient quantity of concentrated sulphuric

* This was felt to be particularly necessary since diazomethane fails to effect complete methylation in many cases.

acid so as to render the solution 7% acid and then boiled under reflux for 2 hours. The substance soon went into solution. After the hydrolysis was over, almost all the alcohol was distilled off, when a pale yellow solid separated out on cooling. It was filtered and crystallised from alcohol twice (using a little animal charcoal). It was thereby obtained in the form of golden yellow rectangular plates melting at 269–70°. (Found in air-dried specimen : OCH_3, 32·7% ; $C_{15}H_6O_3(OCH_3)_4$, H_2O requires : OCH_3, 33·0%.) It corresponded to 3 : 5 : 8 : 4′-tetramethyl herbacetin in all its properties. In alcoholic solution it imparted no colour to ferric chloride and dissolved easily in dilute sodium hydroxide and concentrated hydrochloric acid to produce yellow solutions.

On further methylation with dimethylsulphate and aqueous alkali it yielded the pentamethyl herbacetin melting at 156–58°. Mixed melting point with an authentic sample was undepressed.

3 : 5 : 3′ : 4′-*Tetramethylquercetin from Quercimeritrin*

Quercimeritrin acetate (300 mg.) was dissolved in acetone (20 c.c.) and methylated as described in the case of Herbacitrin acetate, using the proportionate amounts of dimethyl sulphate and 20% alkali. After the removal of the solvent on the water-bath, the methylated glucoside came down as a pale yellow substance. It was then hydrolysed with 100 c.c. of 7% sulphuric acid. On cooling the contents, a pale yellow substance was obtained, which was rendered pure by crystallising twice from acetic acid. It came down in the form of pale yellow needles and melted at 284–85° corresponding to 3 : 5 : 3′ : 4′-tetramethylquercetin. (Found : OCH_3 30·5 ; $C_{15}H_6O_3(OCH_3)_4$, $3H_2O$ requires : OCH_3, 30·1%.) Like the above tetramethyl ether of quercetin the substance easily dissolved in dilute sodium hydroxide to give a yellow solution and gave no characteristic colour with ferric chloride. Further treatment of this substance with dimethyl sulphate and alkali produced pentamethyl quercetin melting at 151–53°.

Summary

The constitution of Herbacitrin is established as the 7-glucoside of Herbacetin by methylating the glucoside through the acetyl derivative and isolating 7-hydroxy-3 : 5 : 8 : 4′tetramethoxy flavone from the hydrolysis of the methylated glucoside. Similarly, the constitution of Quercimeritrin is confirmed as the 7-glucoside of Quercetin.

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