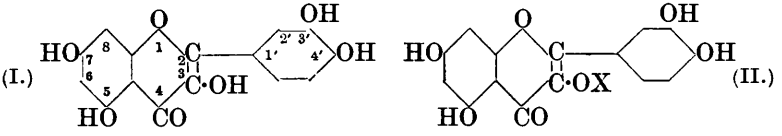
XXXVI.—*The Position of the Sugar Nucleus in the* Quercetin *Glucosides.*

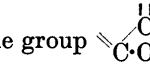
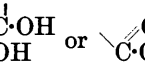
By Gordon Frederick Attree and Arthur George Perkin.

A knowledge of the position of the sugar nucleus in the flavonol glucosides is of special interest, on account of the inter-relationship between these compounds and the naturally occurring anthocyanins. From an examination of the dyeing properties of the hydroxyflavone and -flavonol colouring matters, especially towards aluminium and tin mordants, it has been possible to estimate the tinctorial effect of each of the hydroxyls present in these compounds and thus to predict to some extent the position of the sugar nucleus in their glucosides.

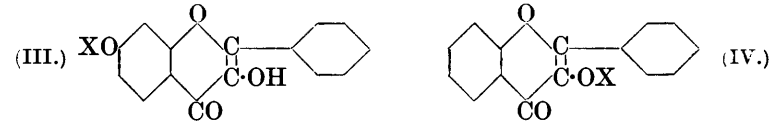
It is now known in regard to quercetin (I) that three factors influence its dyeing property in this respect: (*a*) the hydroxyls 5 and 7, the effect of which is almost negligible (pale yellow); (*b*) the hydroxyls 3′ and 4′, which jointly are powerful auxochromes

(deep yellow); and (*c*) the hydroxyl 3, which itself creates dyeing property (pale yellow) and enhances that given by (*b*) to deep orange.



Interesting again is the fact that the pale yellow shade due to hydroxyl 3 is much strengthened by the presence of hydroxyl 7 or 4′, and if *p*-quinonoid formulæ are adopted, this effect may be assumed to originate in the group  or  present.

Mainly from these considerations, it was predicted (J., 1899, **75**, 826; 1902, **81**, 589) that as quercitrin gives yellow tones, the rhamnose nucleus present therein will be in union with either the 3- or the 3′-hydroxyl group, and of these, the former suggestion (II) is now known to be correct. Thus, by the action of diazomethane on quercitrin, Herzig and Schönbach (*Monatsh.*, 1912, **33**, 678) obtained a crude quercitrin pentamethyl ether, which when hydrolysed gave the 5 : 7 : 3′ : 4′-tetramethoxyflavonol previously synthesised by v. Kostanecki, Lampe, and Tambor (*Ber.*, 1904, **37**, 1402), thus indicating that the rhamnose nucleus is present in the 3-position. More recently, Shibata and Kimotsuki (*Acta Phytochim*, 1923, **1**, 91) have studied the ultra-violet absorption spectra of hydroxy-flavones and -flavonols, and find that by this method it is possible to distinguish the one from the other. Tasaki (*ibid.*, 1925, **2**, 129) has similarly examined the hydroxy-flavone and -flavonol glucosides and as a result considers that these fall into the groups (III) and (IV) in regard to the position of the sugar group (X) present.



In group (III) occur the flavone glucosides, and rutin, whereas in group (IV) are placed the flavonol compounds, robinin, kæmpferitrin, quercitrin, and myricitrin. Such a formulation (IV) is in full agreement with the dyeing properties of the latter, and is certainly correct in regard to quercitrin (*loc. cit.*).

On the other hand, the inclusion of the quercetin glucoside rutin in group (III) does not harmonise with the tinctorial behaviour of this compound.

The experiments herein described are concerned with the methyl-

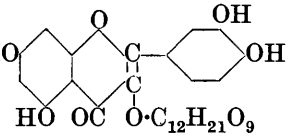
ation of rutin, xanthorhamnin, *iso*quercitrin, and quercimeritrin with diazomethane, and have clearly indicated the position of the sugar group in these glucosides.

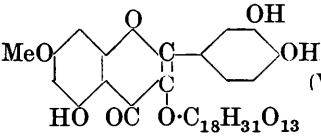
The rutin employed was obtained from the leaves of the *Eucalyptus macrorhyncha* (Smith, J., 1898, **73**, 697; Perkin, J., 1902, **81**, 478) and yielded with diazomethane a dull orange resin, $C_{27}H_{25}O_{11}(OMe)_5$.

Although ter Meulen (*Rec. trav. chim.*, 1923, **42**, 280) has stated that rutin is $C_{33}H_{42}O_{20},4H_2O$ and is derived from quercetin and the trisaccharide rhamninose, Charaux (*Compt. rend.*, 1924, **178**, 1312) finds that the older formula $C_{27}H_{30}O_{16}$ is in reality correct and that the sugar group present therein is the disaccharide rutinose.

When hydrolysed with dilute acid, rutin pentamethyl ether gave 5 : 7 : 3′ : 4′-tetramethoxyflavonol, the identity of which was confirmed by comparison with a specimen derived from quercitrin according to the directions of Herzig and Schönbach (*loc. cit.*). Rutin thus possesses the formula (V), and the statement of Tasaki that the sugar nucleus of this glucoside is present in the 7-position is therefore incorrect.

(V.)





(VI.)

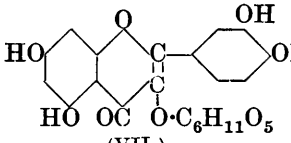
Although it is usually possible to ascertain the positions of the methoxy-groups in methoxy-flavones and -flavonols by means of alcoholic potash, at 170° 5 : 7 : 3′ : 4′-tetramethoxyflavonol is but little attacked in this manner, owing most likely to the sparing solubility of its potassium salt. After some hours at 200°, small amounts of phloroglucinol monomethyl ether and veratric acid were produced, but the presence of phloroglucinol dimethyl ether could not be detected. It is thus probable that at the high temperature involved, a preliminary conversion of the 5 : 7 : 3′ : 4′-tetramethoxyflavonol into the 5-hydroxy-7 : 3′ : 4′-trimethoxy-compound takes place, and that this then suffers hydrolysis as indicated above.

Xanthorhamnin, $C_{34}H_{42}O_{20}$, a yellow-dyeing glucoside obtained from Persian berries, consists of rhamnetin (quercetin 3-monomethyl ether) in union with the trisaccharide rhamninose (Tanret, *Compt. rend.*, 1899, **129**, 725). This, with excess of diazomethane, gave a yellowish-brown resin, which by hydrolysis with acid yielded similarly to quercitrin and rutin the 5 : 7 : 3′ : 4′-tetramethoxy-flavonol. Xanthorhamnin thus has the structure (VI). When less diazomethane is employed, a methylation product can be isolated

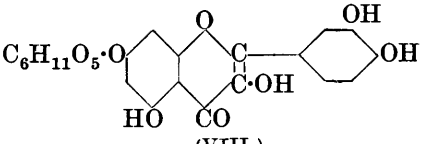
as pale yellow needles, which approximates in composition to a xanthorhamnin trimethyl ether, $C_{34}H_{39}O_{17}(OMe)_3,3H_2O$. Hydrolysis gave yellow needles consisting mainly of a quercetin trimethyl ether, evidently the 7 : 3′ : 4′-compound, but containing a trace of the 5 : 7 : 3′ : 4′-tetramethoxyflavonol.

iso*Quercitrin*, $C_{21}H_{20}O_{10}$, which consists of quercetin in union with glucose, is present in cotton flowers (Perkin, J., 1909, **95**, 2190) and in brown husked maize (Sando and Bartlett, *J. Biol. Chem.*, 1922, **54**, 636).

The small amount available gave with diazomethane pale yellow, flat needles of a methylated glucoside, m. p. 150—152°, and from this by hydrolysis 5 : 7 : 3′ : 4′-tetramethoxyflavonol was produced. *iso*Quercitrin (VII) is thus analogous to rutin and xanthorhamnin.



(VII.)



(VIII.)

Quercimeritrin, $C_{21}H_{20}O_{12}$, occurs in cotton flowers (Perkin, *loc. cit.*), in *Prunus emarginata* (Finnemore, *Pharm. J.*, 1910, **31**, 604), and in *Helianthus annuus* (Sando, *J. Biol. Chem.*, 1925, **64**, 74).

It differs from the yellow-dyeing *iso*quercitrin in giving orange shades similar to those obtained from quercetin itself, and mainly on this account was considered most probably to possess the formula (VIII) (Perkin, *loc. cit.*). With diazomethane, it gave almost colourless needles, m. p. 203—205°, sintering at 197°, which there is little doubt consist of the pentamethyl ether, $C_{21}H_{15}O_7(OMe)_5,2H_2O$. By hydrolysis, colourless needles of a sparingly soluble quercetin tetramethyl ether, m. p. 284—285°, were obtained, the acetyl derivative of which melts at 174—176°. As this, when digested with alcoholic potash at 180°, readily gives veratric acid and phloroglucinol monomethyl ether, it is evidently the hitherto unknown 5 : 3 : 3′ : 4′-tetramethoxy-compound. Thus of the five possible quercetin tetramethyl ethers, three only can give veratric acid. These are now known, and as the 7 : 3 : 3′ : 4′- and the 5 : 7 : 3′ : 4′-compounds melt respectively at 156—157° and 195—198°, the quercetin tetramethyl ether derived from quercimeritrin must possess the constitution here stated. Quercimeritrin therefore has the formula (VIII), which, as already suggested, is in harmony with its dyeing properties.

Of the five known quercetin glucosides, therefore, four contain the sugar group in union with the pyrone hydroxyl, and if, as appears likely (compare Tasaki), kæmpferitrin, robinin, and myricitrin are

analogously constituted, quercimeritrin will be the only known flavonol glucoside which differs in this respect.

EXPERIMENTAL.

Rutin (2 g.) in methyl alcohol (30 c.c.) was treated with diazomethane prepared from nitrosomethylurethane (8 c.c.) in ether (80 c.c.) and 25% methyl-alcoholic potash (25 c.c.); precipitation of the rutin by the ether was prevented by additions of methyl alcohol (5 c.c.) from time to time (compare Herzig and Schönbach, *loc. cit.*). On the following day, the solution was again treated as before with diazomethane; then, after 48 hours, it was concentrated, diluted with boiling water to remove the last traces of alcohol, and evaporated to dryness (Found: CH_3, 11·4. Rutin pentamethyl ether, $C_{32}H_{40}O_{16}$, requires CH_3, 11·0%). The yellowish-brown resin was digested with boiling 0·5% sulphuric acid (200 c.c.) for 2 hours, yielding a precipitate which crystallised from benzene in colourless needles; these, alone or mixed with 5 : 7 : 3′ : 4′-tetramethoxyflavonol prepared from quercitrin by Herzig and Schönbach's method, melted at 194—196° (Found: C, 63·6; H, 5·1; CH_3, 16·5. Calc. for $C_{19}H_{18}O_7$: C, 63·7; H, 5·1; CH_3, 16·75%). The acetyl derivative melted at 160—162° (Herzig and Schönbach give m. p. 160—163°).

Xanthorhamnin (2 g.) in methyl alcohol (50 c.c.) was treated with diazomethane as in the case of rutin; after being kept over-night, the liquid, containing crystals in suspension (A), was evaporated to half its volume, and again treated with diazomethane from nitrosomethylurethane (4 c.c.). The solution was evaporated to dryness and the residue digested with boiling 0·5% sulphuric acid (100 c.c.). The precipitate obtained crystallised from benzene in colourless needles, m. p. 194—196°, and, admixed with the 5 : 7 : 3′ : 4′-tetramethoxyflavonol derived from rutin, showed no alteration in melting point.

If the mixture (A) was evaporated to half its volume and kept, fine, colourless needles (1·7 g.) separated. These melted at 175—178° (those from a second experiment at 172—178°), became yellow when dried over sulphuric acid, were readily soluble in water, and gave with ferric chloride a brown coloration (Found: H_2O at 140°, 6·7; CH_3, 6·8. Xanthorhamnin trimethyl ether, $C_{37}H_{48}O_{20},3H_2O$, requires H_2O, 6·2; CH_3, 6·8%). On hydrolysis with boiling 1% sulphuric acid, fine, yellow needles separated which, after recrystallisation from alcohol, melted at 170—172° (Found: CH_3, 14·1. $C_{18}H_{16}O_7$ requires CH_3, 13·1%). It was evidently quercetin 7 : 3′ : 4′-trimethyl ether (rhamnetin dimethyl ether) contaminated with a small amount of the 5 : 7 : 3′ : 4′-tetramethoxy-compound.

iso*Quercitrin* (0·6 g.) in methyl alcohol was treated with diazomethane from nitrosomethylurethane (4 c.c.); crystals separated over-night. The ether was distilled off, and the liquid again treated with diazomethane as before. Pale yellow needles slowly separated (0·4 g.) which sintered slightly at 140° and melted at 150—152°. This compound, on hydrolysis with boiling 1% sulphuric acid, gave needles which, after recrystallisation from benzene, melted at 193—195°, and, as a mixed melting-point determination showed, consisted of 5 : 7 : 3′ : 4′-tetramethoxyflavonol.

Quercimeritrin (2 g.) in methyl alcohol (80 c.c.) was methylated with ethereal diazomethane from nitrosomethylurethane (8 c.c.), methyl alcohol (20 c.c.) being added gradually at the same time. On the following day, the ether was removed by distillation, methyl alcohol (50 c.c.) added, and the solution again treated with the same amount of diazomethane. When the latter proved to be absent, the liquid was evaporated to a small bulk and diluted with a few drops of water; almost colourless needles (1 g.) then slowly separated. These sintered at 197°, melted at 203—205°, and could be recrystallised from water. With ferric chloride solution, no coloration was produced, indicating complete methylation (Found :* C, 54·05; H, 6·0; H_2O, 6·7. $C_{26}H_{30}O_{12},2H_2O$ requires C, 54·7; H, 6·0; H_2O, 6·3%). This compound, apparently quercimeritrin pentamethyl ether, on hydrolysis (1 g.) with boiling 1% sulphuric acid for 4 hours, gave a semi-gelatinous precipitate (0·635 g.). It was dissolved in much boiling alcohol in presence of animal charcoal, and the solution evaporated until crystallisation commenced. The fine, colourless needles obtained were evidently pure, as the melting point, 284—285°, was unaltered after recrystallisation (Found:* C, 63·6; H, 5·1. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0%).

This new *quercetin tetramethyl ether*, which is very sparingly soluble in alcohol, dissolves in alkaline solutions with a pale yellow colour. The *acetyl* derivative forms minute, colourless needles, m. p. 174—176° (Found :* C, 62·8; H, 5·3; CH_3, 14·2. $C_{21}H_{20}O_8$ requires C, 63·0; H, 5·0; CH_3, 15·0%). In order to determine the positions of the methoxy-groups, the tetramethyl ether (0·6 g.) in 20% alcoholic potash (5 c.c.) and water (1 c.c.) was heated at 170—180° for 3 hours. The pale yellow liquid was evaporated to dryness, the residue dissolved in water, and the solution saturated with carbon dioxide. Extraction with ether removed a colourless, viscid product, the solution of which in dilute aqueous sodium carbonate was treated with benzenediazonium chloride. The orange-red precipitate was dried and extracted with a little alcohol, and the residue was crystallised from alcohol–acetic acid. The crystals, m. p. 250—252°, evidently consisted of benzeneazophloro-

glucinol monomethyl ether. Addition of acid to the bicarbonate solution remaining after the extraction with ether gave a deposit of veratric acid, m. p. 174—176°. The quercetin methyl ether, m. p. 284—285°, derived, as above stated, from quercimeritrin, is accordingly 5 : 3 : 3′ : 4′-tetramethoxyflavonol.

We are greatly indebted to Mr. Arthur Bennett of the Chemistry Department of the University of Manchester for the numerous micro-analyses (marked*) which he has carried out in connexion with this work, and we desire to acknowledge a grant from the Department of Scientific and Industrial Research which enabled one of us (G. F. A.) to take part in this investigation.

CLOTHWORKERS RESEARCH LABORATORY,
THE UNIVERSITY, LEEDS. [*Received, November 29th*, 1926.]