SOME IMPORTANT ETHYL AND ETHYL-METHYL ETHERS OF FLAVONOLS

Transformation of Quercimeritrin into O-Tetraethylrhamnetin

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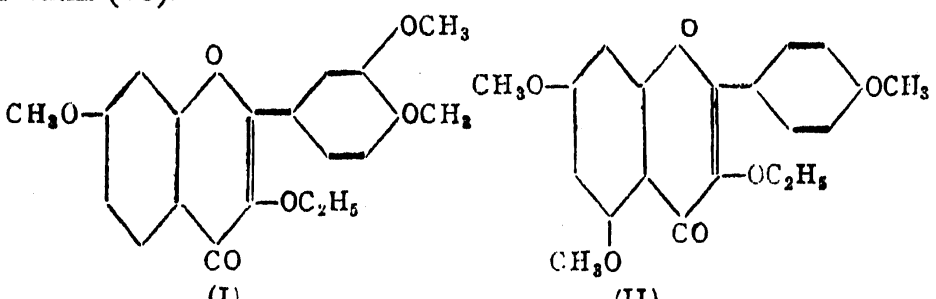
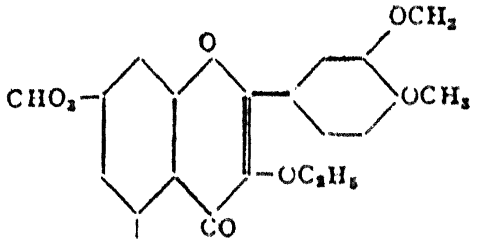
Ethylation as a technique has not been as widely adopted as methylation in the study of naturally occurring organic compounds. However, earlier workers like Herzig[1] and Perkin[2] prepared ethyl ethers of flavonols in a number of cases using ethyl iodide. The special reason might have been that, by using ethyl iodide, ethylation could be carried to completion whereas with the use of methyl iodide the resistant 5-hydroxyl group was left out. The higher boiling point of ethyl iodide and consequently the use of a higher temperature should account for this advantage.

More recently Seshadri and co-workers have employed ethylation as a convenient means for the study of partial methyl ethers and glucosides of hydroxy flavones and this has enabled the work to be carried out with greater ease and definiteness. The main examples are patuletin,[3] calycopterin,[4] tambuletin,[5] gossypin,[6] populnin[7] and hibiscitrin.[8]

There has been some difficulty in preparing synthetically most of the ethyl ethers of flavonols because ethoxyacetonitrile was not easily available. The recent simplified synthesis of this compound by Row and Thiruvengadam[9] has enabled the preparation of various substituted ω-ethoxy acetophenones to be easily made. From these could be readily prepared flavonols with an ethoxyl in the 3-position and methoxyls in the other positions. The following compounds have been made in this connection: 3-ethoxy-7: 3′: 4′-trimethoxy flavone (I), 3-ethoxy-5: 7: 4′-trimethoxy flavone (II) and 3-ethoxy-5: 7: 3′: 4′-tetramethoxy flavone (III). These can serve as reference compounds for the study of 3-glycosides such as kæmferitrin (IV), quercitrin (V*a*) and rutin (V*b*).





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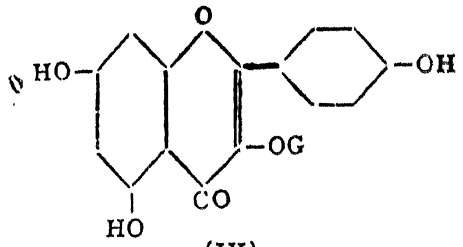
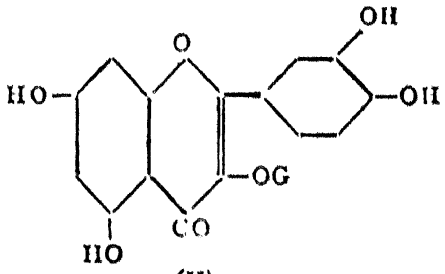


(IV)



(V)

(*a*) G = Rhamnose. (*b*) G = Rutinose.

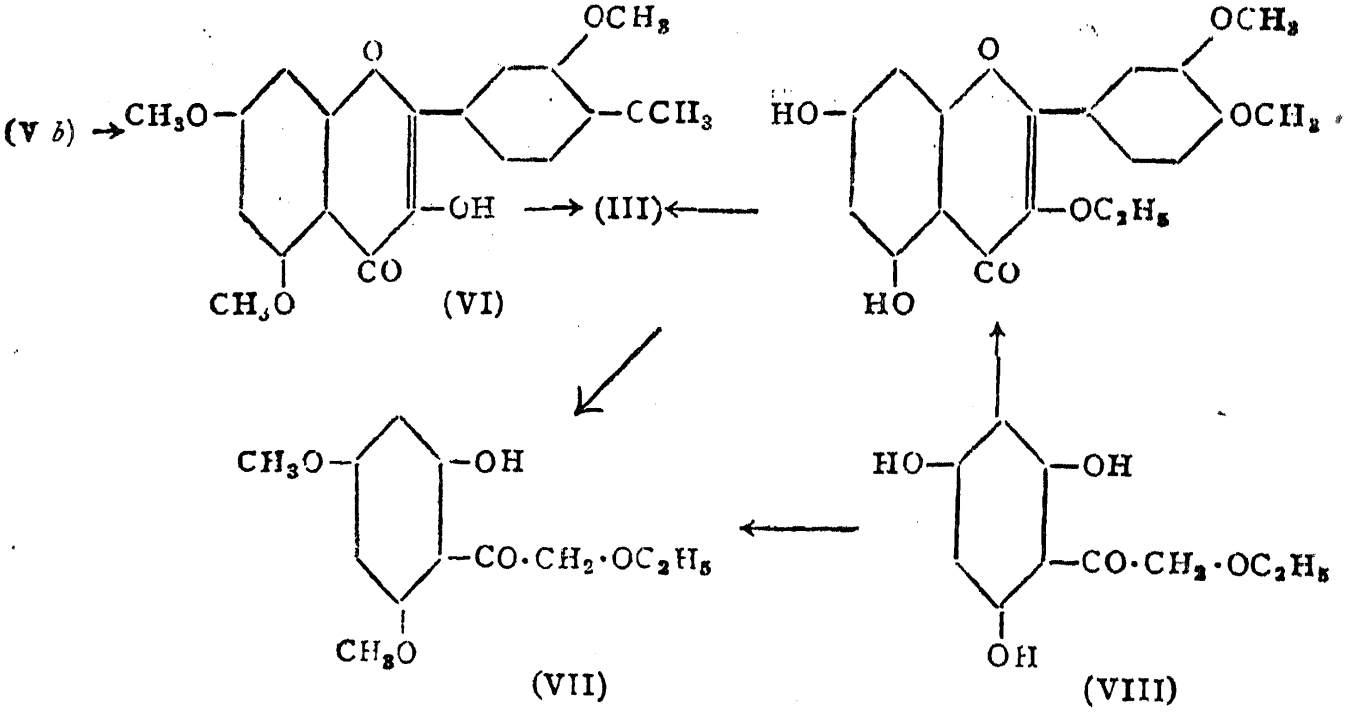
The case of rutin is now studied as a suitable example. It was discovered by Weiss[10] in the leaves of rue (*Ruta graveolens*, Linn.) and has subsequently been isolated from other sources as well, notably from *Sophora japonica*, buckwheat (*Fagopyrum esculentum*) and tobacco. Recently it has assumed great importance on account of its capacity to act as vitamin P and is being produced in U.S.A. in large quantities from buckwheat as the source. Zwenger and Dronke[11] showed that it was not identical with quercitrin but differed from it in that it gave on hydrolysis one molecule of quercetin and two molecules of sugar. Schmidt[12] proved that of the two molecules of sugar one was glucose and the other rhamnose. He assigned to rutin the formula $C_{27}H_{30}O_{16}$. It is now known that the sugar is a biose, termed rutinose.

The position of the sugar residue in rutin was first investigated by Attree and Perkin.[13] They methylated rutin with diazomethane when they obtained a yellowish orange resinous mass. This was hydrolysed with 7% sulphuric acid and the resulting product compared with 3-hydroxy-5: 7: 3′: 4′-tetramethoxy flavone (VI).[14] The two were found to be identical. Hence they concluded that rutin is a 3-glycoside.

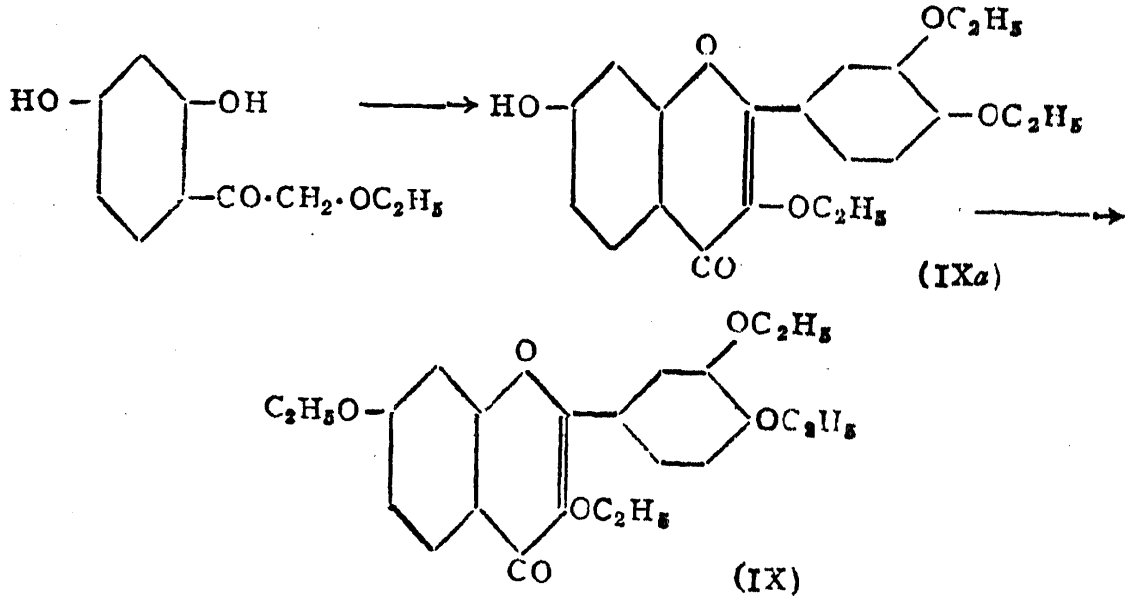
The concerned 3-hydroxy compound does not undergo degradation satisfactorily and was originally synthesised by the method of Kostanecki[14] involving several steps. On the other hand its ethyl ether suffers smooth fission and this mixed ether as well as its fission ketone can be synthesised readily and could therefore serve better as reference compounds.

The methylation of rutin (V*b*) has now been effected conveniently with dimethyl sulphate in acetone solution in the presence of anhydrous potassium

carbonate. The product is subjected to hydrolysis with 7% sulphuric acid and ethylation with diethyl sulphate. The mixed ethyl-methyl ether thus obtained is found to be identical with a synthetic sample of 3-ethoxy-5:7:3′:4′-tetramethoxy flavone (III). Further when this ether is subjected to fission with alcoholic potash, it yields the ketone (VII) which is identical with ω-ethoxy-2-hydroxy-4:6-dimethoxy acetophenone obtained from ω-ethoxy phloracetophenone (VIII) by partial methylation.

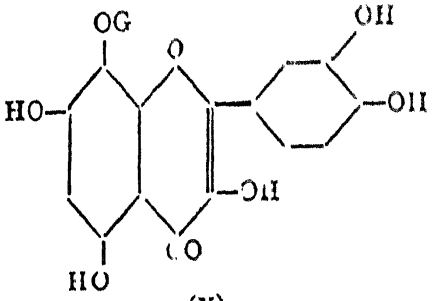


As already mentioned, though earlier workers ethylated certain naturally occurring flavonols, these ethers have not till now been obtained directly by synthetic means probably because the required ω-ethoxy acetophenones were not available. As a typical example of fully ethylated ethers the synthesis of fisetin tetraethyl ether (IX) has been carried out now.

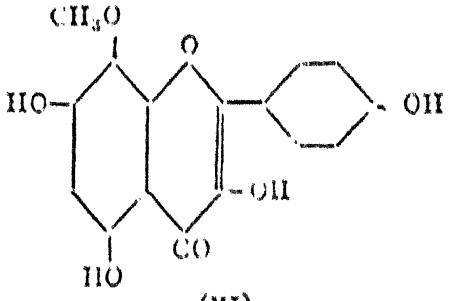


In a recent paper by Rao and Seshadri[6] it has been pointed out how the ethylation of flavonol glycosides is itself a very useful operation and how the

ethylation product, after hydrolysis and further methylation, can be conveniently used for purposes of establishing the constitution of glycosides. This enables also a correlation to be effected between glycosides and partial methyl ethers. The case of gossypin (X) and tambuletin (XI) was studied by them and reported.

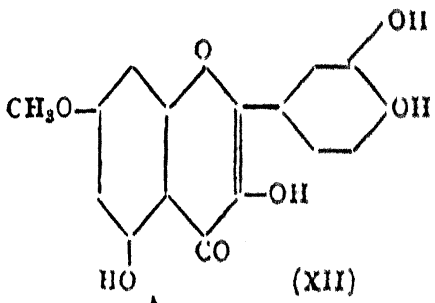


(X)



(XI)

A more closely related pair would be rhamnetin (XII) and quercimeritrin (XIII). The constitution of quercimeritrin as the 7-monoglucoside of quercetin has been discussed in detail in a recent publication from this laboratory.[15] Rhamnetin (XII) is a monomethyl ether of quercetin. The position of the ether group as 7 was arrived at by Perkin and Allison[16] based on the following considerations. Alkali fission gave rise to protocatechuic acid and monomethyl phloroglucinol. This indicated that the methoxyl was in the 5- or 7-position. But the production of quercetin tetramethyl ether (XIV) from rhamnetin (XII) by the action of methyl iodide showed that the resistant 5- hydroxyl was free and that the 7-position therefore carried the ether group.



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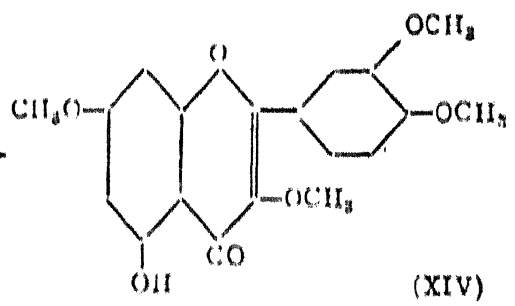

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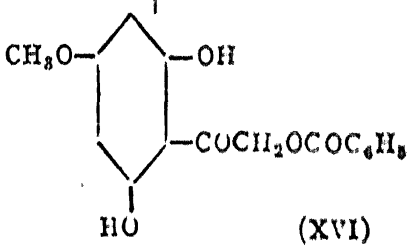

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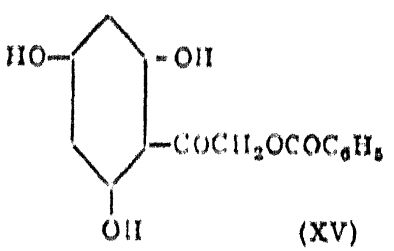
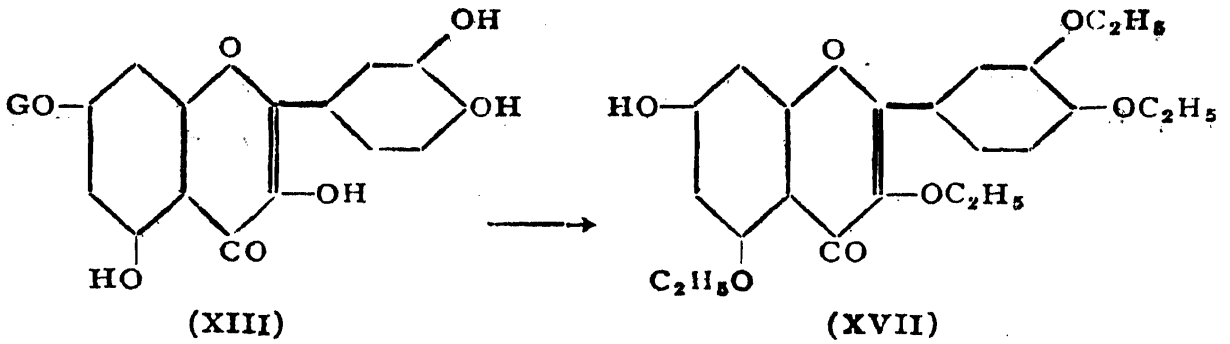


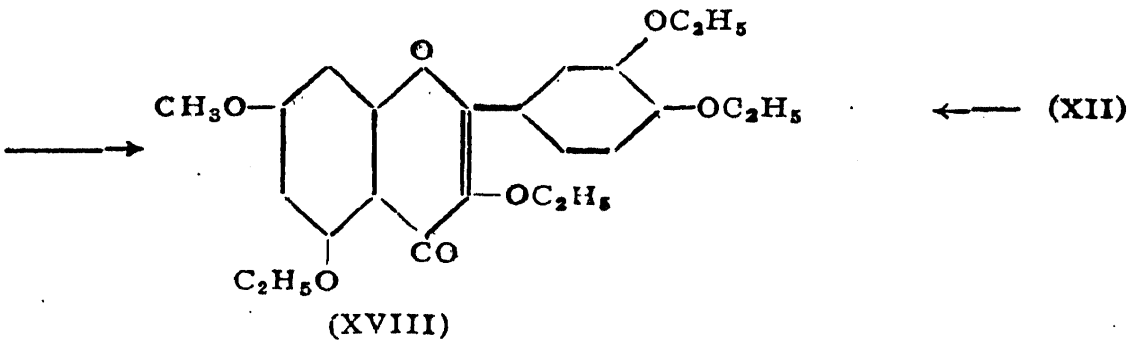
(XV)

Synthetic confirmation of this constitution was lacking for over forty years. Only recently the synthesis of rhamnetin was reported by Kuhn, *et al.*[17] who started with ω-benzoyloxyphloracetophenone (XV). This was

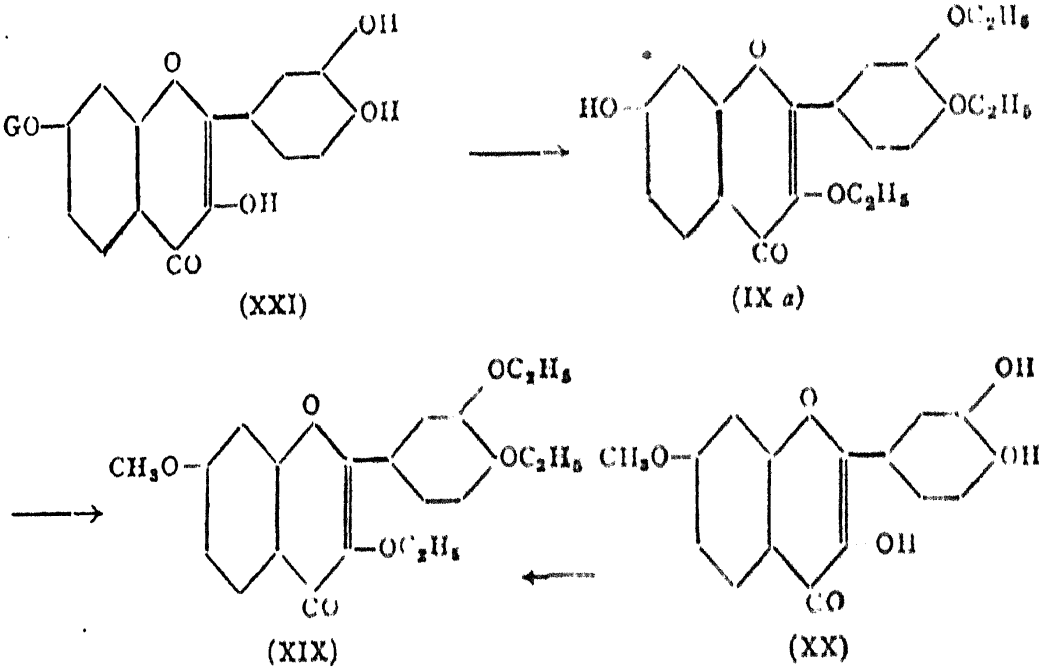
methylated with diazomethane in methyl alcoholic solution whereby a poor yield of the 4-methyl ether (XVI) was obtained. Subsequent condensation with dibenzoyl protocatechuic anhydride in the presence of triethylamine as the condensing agent and hydrolysis of the benzoate yielded rhamnetin (XII).

An elegant method of showing the interrelationship between the above two quercetin derivatives is by ethylation. Quercimeritrin (XIII) obtained from the flowers of the combodia cotton plant is ethylated using excess of diethyl sulphate and anhydrous potassium carbonate in anhydrous acetone medium. The product is directly hydrolysed whereby the quercetin tetraethyl ether (XVII) is obtained melting at 200–201°. This resembles closely tetramethyl quercetin obtained similarly.[15] The melting point of the compound is high; it does not give any colour with ferric chloride and is soluble in alkali. Final methylation of it yields quercetin tetraethyl monomethyl ether (XVIII) melting at 118°. This is found to be identical with O-tetraethyl rhamnetin obtained by the direct ethylation of rhamnetin (XII). It should be mentioned here that for the ethylation of quercimeritrin, the use of diethyl sulphate is quite necessary whereas rhamnetin could be ethylated even by means of ethyl iodide.





A simpler compound useful for this type of work has also been synthesised now, namely 7-methoxy-3 : 3′ : 4′-triethoxy flavone (XIX). It will correlate the 7-methyl ether (XX) with the 7-glycoside of fisetin (XXI). The intermediate 7-hydroxy-3 : 3′ : 4′-triethoxy flavone (IX*a*) has already been mentioned.



(XXI) (IX *a*) (XIX) (XX)

The rhamnetin used for ethylation was kindly provided by Prof. L. H. Briggs of the University of New Zealand. It belonged originally to the preparations of Prof. A. G. Perkin. The rutin sample was kindly supplied by Dr. A. G. Clark of the Scripps Metabolic Clinic, La Jolla, San diego, California. For these gifts we are highly grateful.

Experimental

ω-Ethoxy-2: 4-dihydroxy-acetophenone.—A solution of dry resorcinol (8 g.) in anhydrous ether (75 c.c.) was treated with ethoxy acetonitrile[9] (6 c.c.) and freshly fused zinc chloride (2 g.). The mixture was cooled to 0° and saturated with dry hydrogen chloride during the course of an hour and a half. By this time a colourless precipitate of the ketimine hydrochloride had separated out. However the current of gas was continued for two hours more. The next morning the ether was decanted off and the ketimine hydrochloride washed thrice with anhydrous ether. It was hydrolysed by dissolving it in water (75 c.c.) and heating the solution at 80° for 30 minutes. On cooling a reddish brown liquid separated. It was extracted with ether and when the ether solution was evaporated a semi-solid residue was obtained. It was crystallised from hot water when the ketone was obtained in the form of stout elongated rectangular prisms and octagonal rods. Purification of the semi-solid ketone was also effected by distilling it under reduced pressure (10 mm.) when it passed over as a pale reddish brown oil between 195–200°; this solidified to a colourless crystalline solid almost immediately, m.p. 135–6°. Yield, 4 g. (Found: C, 61·0; H, 6·2; $C_{10}H_{12}O_4$ requires C, 61·2; H, 6·1%).

The ketone was easily soluble in alcohol, ethyl acetate and acetone but sparingly in benzene. Its alcoholic solution developed a reddish violet colour with ferric chloride.

7-Hydroxy-3-ethoxy-3′: 4′-dimethoxy-flavone.—An intimate mixture of ω-ethoxy-2: 4-dihydroxy-acetophenone (1 g.), veratric anhydride (5 g.) and sodium veratrate (3 g.) was heated under reduced pressure at 170-80° for three hours. The product was refluxed with 10% alcoholic potash (80 c.c.) for 20 minutes. After the removal of alcohol, the residue was dissolved in water and the solution saturated with carbon dioxide. The flavone separated out in the form of a flocculent brown precipitate. This was repeatedly extracted with ether. The ether extract gave a pale yellow crystalline solid on evaporation. It crystallised from alcohol (charcoal) in the form of straw coloured elongated rectangular plates and needles melting at 194–5°. Yield, 0·6 g. (Found: C, 67·0; H, 5·0; $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3%.) It developed a bright purplish red colour in alcoholic solution on reduction with magnesium and hydrochloric acid. Its solution in concentrated sulphuric acid was bright yellow with no fluorescence. It gave no colour with alcoholic ferric chloride.

3-Ethoxy-7: 3′: 4′-trimethoxy-flavone (I).—The above flavone (0·2 g.) was dissolved in dry acetone (50 c.c.) and dimethyl sulphate (0·2 c.c.) and anhydrous potassium carbonate (1 g.) added. The mixture was refluxed for six hours. The solvent was then distilled off and the residue treated with water. The methyl ether separated out as a colourless crystalline solid. It crystallised from alcohol in the form of colourless rectangular prisms melting at 125–7°. (Found: C, 67·1; H, 5·5; $C_{20}H_{20}O_6$ requires C, 67·4; and H, 5·6%.)

7-Hydroxy-3: 3′: 4′-triethoxy-flavone (IX a).—ω-Ethoxy-?: 4-dihydroxy acetophenone (0·6 g.) was condensed as in a previous experiment with diethyl protocatechuic anhydride (4 g.) and sodium diethyl protocatechuate (2·5 g.). After hydrolysis of the condensation product, the alkali solution was saturated with carbon dioxide and the precipitated flavone separated by ether extraction. It recrystallised from alcohol in the form of pale yellow aggregates of rectangular plates melting at 192–3°. Yield, 0·9 g. (Found: C, 68·4; H, 6·3; $C_{21}H_{22}O_6$ requires C, 68·1; and H, 6·0%). When reduced with magnesium and hydrochloric acid in alcoholic solution, it developed a scarlet red colour. It dissolved in concentrated sulphuric acid yielding a bright yellow solution with no fluorescence. No colour developed with alcoholic ferric chloride.

3: 7: 3′: 4′-Tetraethoxy-flavone: Fisetin tetraethyl ether (IX).—A solution of the triethoxy-hydroxy flavone (0·5 g.) in anhydrous acetone (50 c.c.)

was refluxed with diethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (1 g.) for 12 hours. The solvent was then removed by evaporation and water added to the residue. The tetraethyl ether was filtered, washed and crystallised from alcohol. After two crystallisations it was obtained as colourless shining needles melting at 106–7° (*cf.* Perkin and Everest[18]). (Found: C, 69·0; H, 6·2; $C_{23}H_{24}O_6$ requires C, 69·3 and H, 6·5%.) It was soluble in concentrated sulphuric acid yielding a bright yellow solution with feeble green fluorescence. With magnesium and hydrochloric acid, it rapidly developed a bright orange colour.

7-Methoxy-3 : 3′ : 4′-triethoxy-flavone (*XIX*).—7-Hydroxy-3 : 3′ : 4′-triethoxy-flavone (0·2 g.) was refluxed for 8 hours with dimethyl sulphate (0·3 c.c.) and anhydrous potassium carbonate (1 g.) in dry acetone solution (50 c.c.). The methyl ether was precipitated as a colourless product when water was added to the residue after the removal of acetone. It crystallised from aqueous alcohol as colourless thin rectangular plates melting at 131–2°. (Found: C, 68·6; H, 6·0; $C_{22}H_{24}O_6$ requires C, 68·7; H, 6·3%.)

3-Ethoxy-5 : 7-dihydroxy-4′-methoxy-flavone.—This was obtained by heating ω-ethoxy phloroacetophenone (0·6 g.) with anisic anhydride (4·5 g.) and sodium anisate (3 g.) at 170–80° for three hours under reduced pressure. The condensation product was hydrolysed as in the previous cases and the flavone obtained by extraction with ether after saturating the alkali solution with carbon dioxide. It was crystallised from alcohol when it was obtained as bright yellow stout rectangular prisms and tablets melting at 194–6°. Yield, 0·8 g. (Found: C, 66·2; H, 4·8; $C_{18}H_{16}O_6$ requires C, 65·8 and H, 4·9%.) It dissolved in concentrated sulphuric acid yielding a bright yellow solution with green fluorescence. With neutral ferric chloride in alcohol, it developed a brow colour and with magnesium and hydrochloric acid, a pinkish red colour.

3-Ethoxy-5 : 7 : 4′-trimethoxy-flavone (*II*).—Methylation of the above flavone (0·5 g.) was carried out by refluxing it in acetone solution with dimethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (2 g.) for 20 hours. The product was worked up as in previous cases. The methyl ether crystallised from alcohol in the form of colourless elongated rectangular prisms and rods melting at 102–3°. (Found: C, 67·2; H, 5·5; $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·6%.)

Its solution in concentrated sulphuric acid was bright yellow exhibiting strong green fluorescence. A bright red colour was obtained when it was reduced in alcoholic solution with magnesium and hydrochloric acid.

3-Ethoxy-5 : 7-dihydroxy-3′ : 4′-dimethoxy-flavone.—Condensation of ω-ethoxy phloroacetophenone (1 g.) with veratric anhydride (8 g.) and sodium veratrate (2 g.) was effected as in previous cases by heating the mixture under

reduced pressure for three hours at 170-80° and the condensation product worked up. The flavone crystallised from alcohol in the form of very pale yellow short needles melting at 189–90°. Yield, 0·9 g. (Found: C, 64·0; H, 5·0; $C_{19}H_{18}O_7$ requires C, 63·7 and H, 5·0%.) It gave a bright yellow solution with concentrated sulphuric acid with feeble green fluorescence. With ferric chloride in alcoholic solution, it yielded a reddish brown colour and with magnesium and hydrochloric acid, an orange red colour.

3-*Ethoxy*-5: 7: 3′: 4′-*tetramethoxy-flavone* (*III*).—Methylation of the above flavone (0·3 g.) was effected with dimethyl sulphate (0·2 c.c.) and anhydrous potassium carbonate (1 g.) in dry acetone solution (50 c.c.). After refluxing for six hours, the solvent was distilled off and water added. The colourless precipitate was filtered and crystallised from alcohol when it was obtained as colourless narrow rectangular needles and plates melting at 154–5°. (Found: C, 65·0; H, 6·0; $C_{21}H_{22}O_7$ requires C, 65·3 and H, 5·7%.)

ω-Ethoxy-4: 6-*dimethoxy*-2-*hydroxy-acetophenone* (*VII*).—(*a*) ω-Ethoxyphloroacetophenone (1 g.; 1 mol.) was treated in anhydrous acetone solution (50 c.c.) with dimethyl sulphate (1 c.c.; 2·2 mol.) and anhydrous potassium carbonate (5 g.) and the mixture refluxed for 12 hours. The solvent was then distilled off, water added and the solid product filtered off. It was macerated with 5% aqueous potash (25 c.c.) and filtered. The filtrate on acidification yielded the colourless dimethyl ether which crystallised from aqueous alcohol in the form of colourless rectangular prisms melting at 103–4°. Yield, 0·7 g. (Found: C, 59·9; H, 7·0; $C_{12}H_{16}O_5$ requires C, 60·0; and H. 6·7%.)

(*b*) 3-Ethoxy-5: 7: 3′: 4′-tetramethoxy flavone (III) (0·5 g.) was refluxed with 8% absolute alcoholic potash (25 c.c.) for four hours. At the end of this period the solvent was distilled off and water added to the residue. The alkaline solution was then saturated with carbon dioxide and extracted with ether several times. On removing the ether from the extract a colourless crystalline solid was obtained. It was recrystallised from aqueous alcohol using animal charcoal. The ketone was thus obtained in the form of colourless rectangular prisms melting at 103–4°. A mixed melting point with the sample obtained in (*a*) above was not depressed.

Methylation of Rutin.—Rutin (1 g.) was refluxed in acetone solution (150 c.c.) with dimethyl sulphate (4 c.c.) and anhydrous potassium carbonate (10 g.) for 45 hours. The acetone was then distilled off and water added to the residue. The resulting colourless precipitate was used directly for the next experiment.

3-*Hydroxy*-5: 7: 3′: 4′-*tetramethoxy flavone* (*VI*).—The above precipitate was hydrolysed by refluxing for one and a half hours with 7% aqueous-alcoholic sulphuric acid (30 c.c.). The alcohol was then distilled off and

more water added. A bright yellow precipitate was obtained. It was filtered and crystallised from alcohol when it came out in the form of bright yellow elongated needles and prisms. It melted at 197-8° (*cf.* Kostanecki and Tambor[14]).

3-*Ethoxy*-5:7:3′:4′-*tetramethoxy flavone* (*III*).—The above hydroxy flavone (0·5 g.) was refluxed for six hours in anhydrous acetone solution (50 c.c.) with diethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (2 g.). The acetone was then distilled off and water added to the residue. A colourless crystalline precipitate was obtained which when recrystallised from alcohol was obtained as colourless narrow rectangular needles and plates melting at 154–5°. A mixed melting point with a synthetic sample was not depressed.

Ethylation of quercimeritrin and hydrolysis of the ethyl ether.—Dried and finely powdered quercimeritrin (1 g.) was suspended in anhydrous acetone (75 c.c.) and treated with anhydrous potassium carbonate (10 g.) and diethyl sulphate (4·5 c.c. added in three lots). The mixture was refluxed for a period of 40 hours. It was then filtered and the potassium salts washed thrice with hot acetone. The acetone filtrate and washings were evaporated when a semi-solid mass was obtained. This was directly hydrolysed by refluxing with 7% aqueous alcoholic sulphuric acid (75 c.c.) for two hours. The alcohol was then evaporated off. The tetraethyl quercetin (XVII) that separated was filtered and crystallised from alcohol when it was obtained in the form of pale yellow stout rhombic prisms melting at 200–201°. Yield, 0·6 g. It dissolved readily in aqueous alkali yielding a pale yellow solution and gave no colour with ferric chloride. (Found: C, 66·3; H, 6·6; $C_{23}H_{26}O_7$ requires C, 66·7; and H, 6·3%.)

7-*Methoxy*-3:5:3′:4′-*tetraethoxy-flavone* (*XVIII*).—The foregoing O-tetraethyl-quercetin (0·2 g.) was methylated by means of dimethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (2 g.) in dry acetone medium. After refluxing for 8 hours the contents were filtered and the potassium salts washed with hot acetone. The filtrate and the washings were evaporated to dryness. The colourless crystalline solid obtained was recrystallised from aqueous alcohol when the methyl ether came out as colourless aggregates of thin micacious plates melting at 119–20°. The melting point was not depressed on admixture with O-tetra-ethyl-rhamnetin given below. (Found: C, 67·0; H, 6·7; $C_{24}H_{28}O_7$ requires C, 67·3 and H, 6·5%.)

O-Tetra-ethyl-rhamnetin.—Rhamnetin (0·2 g.) was ethylated by refluxing in acetone solution with diethyl sulphate (1·5 c.c. added in 3 lots) and anhydrous potassium carbonate (5 g.) for 36 hours. The acetone was then distilled off and water added to the residue. A colourless solid separated

out. It was filtered and crystallised from aqueous alcohol when it wa obtained in the form of colourless stout rectangular prisms melting at 119–20°. The mixed melting point with 7-methoxy-3:5:3′:4′-tetraethoxy-flavone (XVIII) obtained from quercimeritrin was not depressed.

Summary

The use of ethylation in the study of naturally occurring glycosides and partial methyl ethers of hydroxy flavones is discussed. This procedure renders the work simpler and more definite. Starting from ω-ethoxy res- and phloracetophenones, the preparation of mixed ethyl methyl ethers of the flavonols, fisetin, kæmpferol, and quercetin, with an ethoxyl in the 3-position and methoxyls in the other positions, is described and it is shown that these compounds are useful in the study of the 3-glycosides of these flavonols. The case of rutin has been used as a typical example.

As an illustration of the synthesis of fully ethylated compounds fisetin tetraethyl ether is made and as an example for the use of ethylation in the study of both 7- glycosides and 7- methyl ethers, O-3:3′:4′-triethyl-7-methyl-fisetin is synthesised.

The use of ethylation for establishing interrelationship between glycosides and partial methyl ethers of hydroxy flavones is illustrated with the case of quercimeritrin and rhamnetin. Quercimeritrin has been ethylated with diethyl sulphate and the ethylated glucoside hydrolysed and methylated. The product is a mixed ether of quercetin and is shown to be identical with O-tetraethyl rhamnetin.

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