NUCLEAR OXIDATION IN THE FLAVONES AND RELATED COMPOUNDS

Part XIV. Constitution of Quercetagitrin

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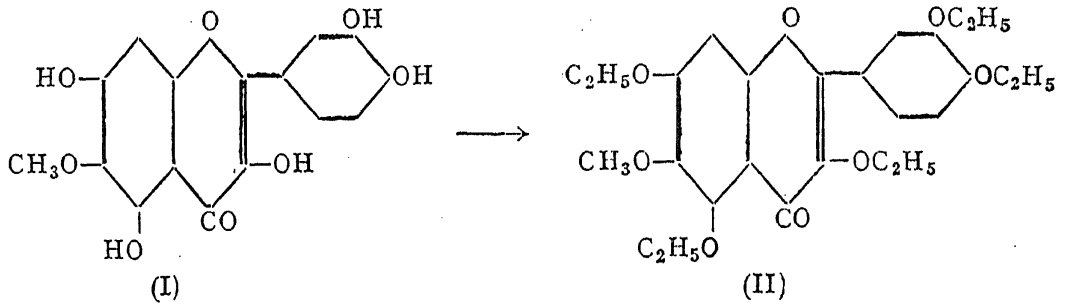
Two important species of the Marigold, *Tagetes erecta*[1] and *Tagetes patula*[2] have been examined in the past with regard to their flower pigments. The difference in species is clearly indicated in the nature of the colouring matter present. The first contains mainly the monoglucoside, quercetagitrin along with some quantity of its aglucone, quercetagetin. On the other hand the flowers of *Tagetes patula* yield only patuletin which is a mono-methyl ether of quercetagetin. Thus the close botanical relationship between the two species and at the same time their species difference is paralleled on the chemical side. The pigments of both flowers are based on quercetagetin, one containing the monoglucoside and the other the monomethyl ether.

The constitution of patuletin was first surmised[3] as the 6-methyl-ether (I) from its properties and reactions and it was later confirmed[4] definitely by the unequivocal synthesis of its penta-ethyl ether (II).

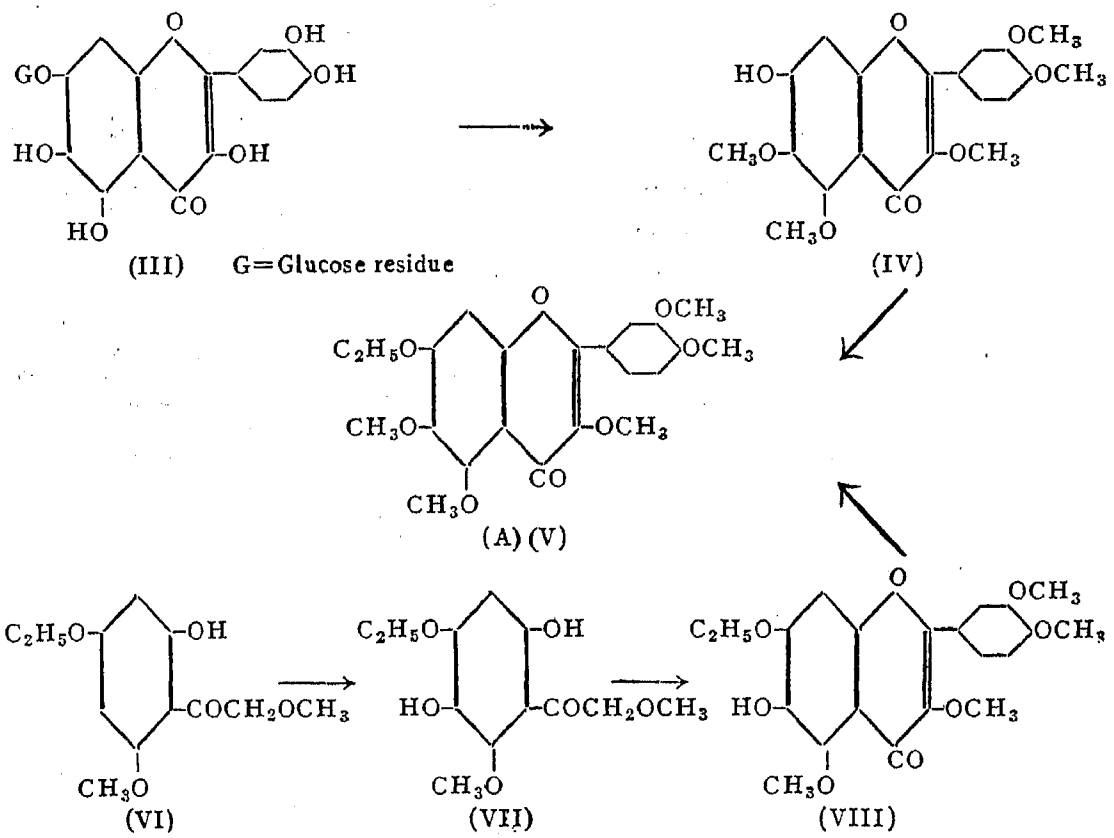


(I) (II)

With regard to quercetagitrin, its constitution as the 7-monoglucoside was originally arrived at only by the elimination of other possibilties.[1] After the complete methylation of the glucoside through the acetate and subsequent hydrolysis, a pentamethyl quercetagetin was obtained which gave, on decomposition with alkali, veratric acid thus eliminating the possibility of existence of the free hydroxyl group in the side phenyl nucleus. Comparison with the already known 5-hydroxy compound showed that it was different and eliminated this position also from consideration. The lack of prominent ferric chloride colour showed that there was no free

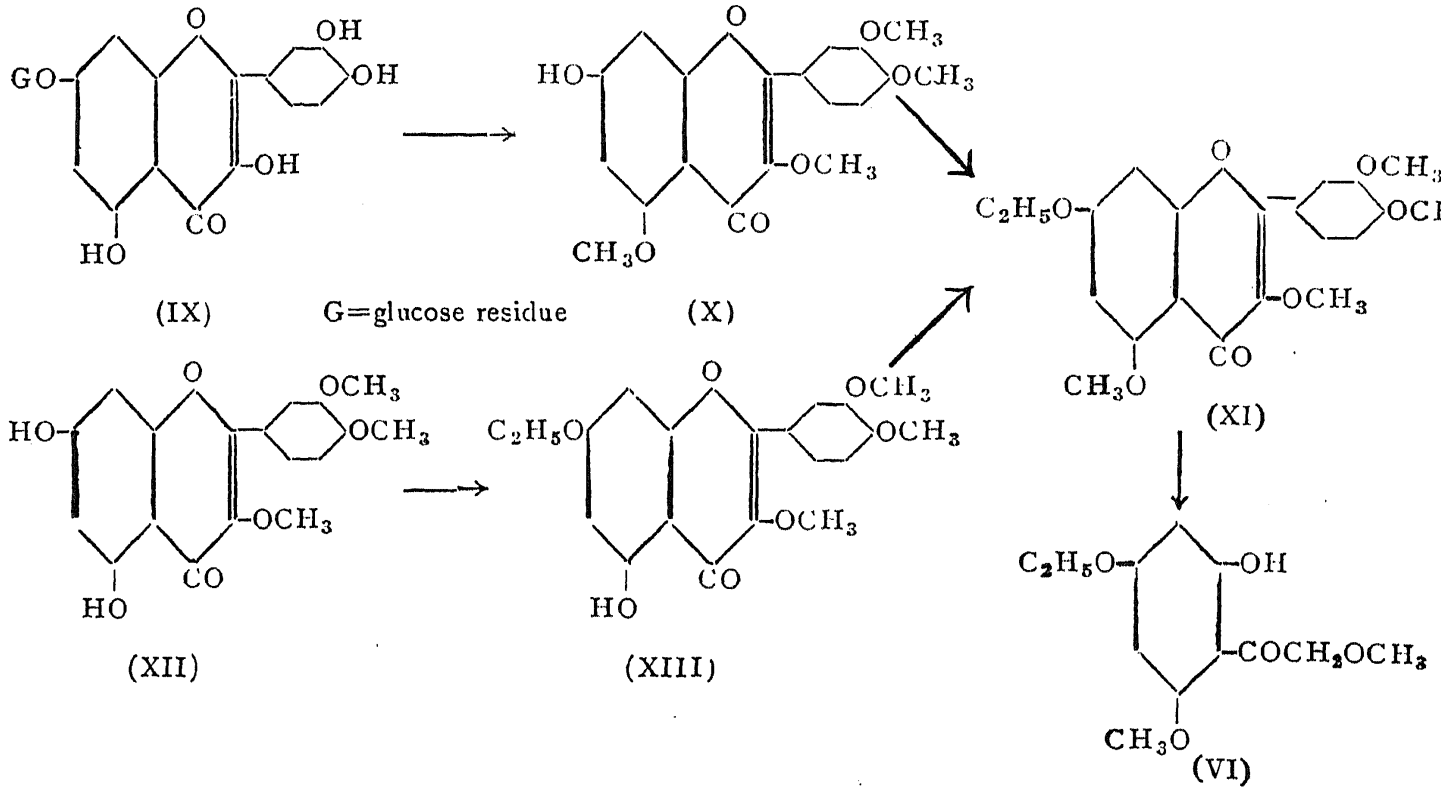
hydroxyl even in the 3-position. Out of the two still left, the position 7 (formula IV) was considered to have the free hydroxyl group because the allyl ether of the compound underwent Claisen migration smoothly. This would not have been possible if the 6-position was concerned. But no confirmation by synthesis was provided for the constitution of this pentamethyl quercetagetin and eventually for the glucoside, quercetagitrin. This has now been supplied by experiments described in this paper.

Quercetagitrin has now been more conveniently methylated using excess of dimethyl sulphate and potassium carbonate in acetone medium. The pentamethyl quercetagetin obtained by the hydrolysis of the methylated glucoside has been further ethylated and this mixed ether (A) has been shown to be identical with a synthetic sample of 7-ethoxy-3 : 5 : 6 : 3′ : 4′-pentamethoxy flavone (V). The starting point for this synthesis is 2-hydroxy-4-ethoxy-ω : 6-dimethoxy acetophenone (VI) and the stages are given by the following formulæ. Thus it is confirmed that the pentamethyl quercetagetin has formula (IV) and quercetagitrin formula (III).

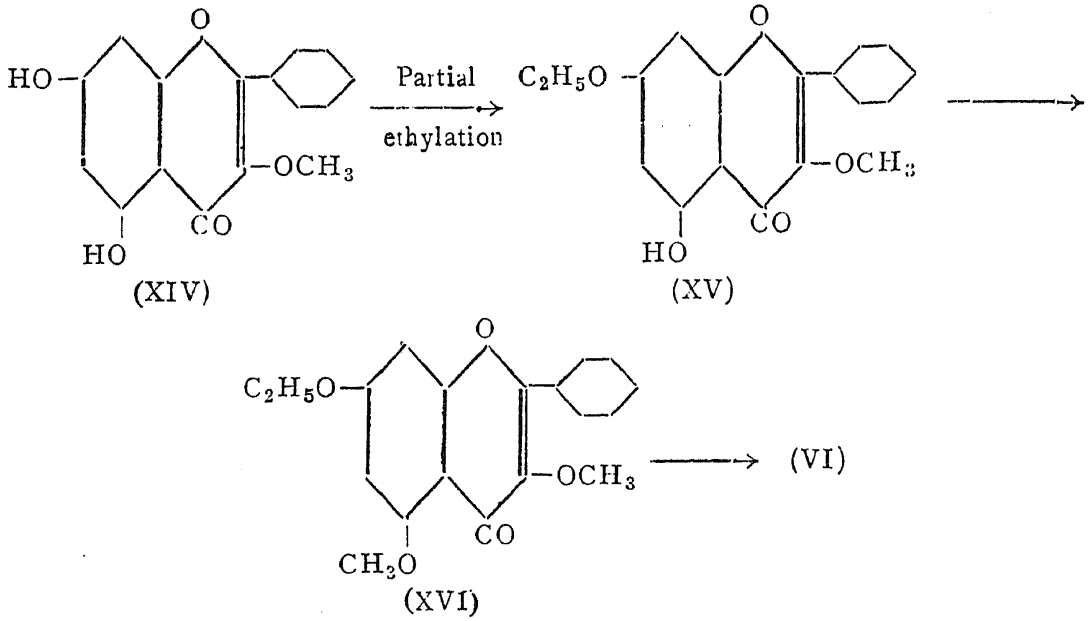


The above mentioned starting material (VI) has been most conveniently obtained from quercimeritrin, the well-known monoglucoside of quercetin. Its constitution as a 7-glucoside (IX) was originally advanced by Attree and Perkin[5] (see also Rao and Seshadri[6]) by a process of elimination. They

showed that the derived tetramethyl quercetin did not have the free hydroxyl group in the side phenyl nucleus by a study of its decomposition products and that it was not in the 3 or the 5-position by comparison with the required synthetic compounds. No confirmation of the constitution (X) by synthesis has been made so far. This has now been done by the ethylation of the tetramethyl quercetin and by the synthesis of the resulting mixed ether (XI) by a straightforward method. 3 : 3′ : 4′-*O*-trimethyl quercetin (XII)[7] which is readily obtained by the Allan-Robinson condensation of ω-methoxy-phloracetophenone with the anhydride and sodium salt of veratric acid, is partially ethylated using just one mol. of ethyl iodide and the product (XIII) is a monoethyl ether whose reactions indicate that the resistant 5-hydroxyl group is still free. Final methylation with excess of dimethyl sulphate yields the required mixed ether of quercetin (XI). The various stages are indicated by the following formulæ:



Fission using alcoholic alkali of the above mixed ether (XI) obtained either from quercimeritrin or by the synthetic method yielded besides veratric acid the required 2-hydroxy-4-ethoxy-ω : 6-dimethoxy acetophenone (VI) and this was employed as the starting material for the synthesis of the above mentioned pentamethyl-monoethyl quercetagetin. As a purely synthetic method of obtaining this ketone, the synthesis of *O*-7-ethyl-3 : 5-dimethyl galangin (XVI) and its fission with alcoholic alkali has also been carried out. This is far more convenient than preparing the quercetin derivative synthetically.



The above experiments emphasise the relationship between quercimeritrin and quercetagitrin, both being 7-glucosides. Further from the results it will be clear that the species difference between *T. erecta* and *T. patula* is not only exhibited in the way a hydroxyl group is protected but this is also exhibited in the particular position involved, 7- in the glucoside and 6- in the methyl ether. This choice of the position for the glucoside formation and methylation in the case of 5:6:7-hydroxy-flavones and flavonols is rather interesting. As somewhat parallel cases may be mentioned the derivatives of scutellarein and baicalein. In their partial methyl ethers the 6- position is used in preference to 7. For example, the 6-methyl ether of baicalein (oroxylin-A) is found in *Oroxylum indicum* and the 6:4'-dimethyl ether of scutellare in occurs in *Linaria vulgaris*. Regarding the glucuronides, baicalin and scutellarin Armstrong and Armstrong in their well-known monograph on glycosides[8] state that the glucuronic acid is in both cases thought to be attached in position 6. No evidence is however mentioned in support of this opinion. On the other hand the experiments of Shibata and Hattori[9] would show that the constitution of baicalin should be as the 7- glucuronide of baicalin.

Experimental

Quercetin-tetramethyl-ether (X) *from Quercimeritrin.*

Quercimeritrin required for this work was obtained from the flower petals of the Cambodia cotton plant (*Gossypium hirsutum*).[10] It (1·0 g.) was suspended in dry acetone (150 c.c.) and anhydrous potassium carbonate (5 g.) and dimethyl sulphate (3 c.c.) were added and the mixture was heated under reflux for a period of 30 hours. The potassium salts were filtered and washed with warm acetone. From the filtrate the solvent was distilled

off and water added to the residue. The methyl ether separated as a semi-solid mass; it gave no colour with alcoholic ferric chloride. It was directly boiled with 7% aqueous sulphuric acid for two hours. It first went into solution and a colourless solid soon separated out. The product was filtered and washed with water. On crystallisation from alcohol the tetramethyl ether was obtained as narrow rectangular plates, melting at 284–85°; Attree and Perkin[5] and Rao and Seshadri[6] recorded the same melting point. It dissolved in aqueous sodium hydroxide to give an yellow colour but did not give any colour with alcoholic ferric chloride. Yield, 0·4 g. (Found: C, 63·7; H, 5·4; $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0%.) Its acetate melted at 174–75°.

Monoethyl-tetramethyl-quercetin (*XI*)

The above tetramethyl quercetin (0·2 g.) was dissolved in dry acetone (50 c.c.), freshly ignited potassium carbonate (5 g.) and ethyl iodide (0·5 c.c.) added and the mixture refluxed for 15 hours. The potassium salts were filtered and washed with warm acetone. On removing the solvent from the filtrate and adding water, the ethyl ether separated as a colourless solid. It crystallised from ethyl acetate as broad rhombohedral plates and prisms melting at 159–60°. It was insoluble in aqueous sodium hydroxide and did not give any colour with alcoholic ferric chloride. The mixed melting point with a synthetic sample of 7-ethoxy-3 : 5 : 3′ : 4′-tetramethoxy-flavone was undepressed. (Found: C, 65·6; H, 5·8; $C_{21}H_{22}O_7$ requires C, 65·3; H, 5·7%).

7-Ethoxy-5-hydroxy-3 : 3′ : 4′-trimethoxy-flavone (*XIII*)

5 : 7-Dihydroxy-3 : 3′ : 4′-trimethoxy-flavone[7] (0·5 g.) in dry acetone (50 c.c.) was treated with anhydrous potassium carbonate (5·0 g.) and ethyl-iodide (1·1 mol.; 0·3 c.c.) and refluxed for 8 hours. After filtration, the solution was concentrated whereby the crude partially ethylated compound was obtained as a pale yellow solid. It crystallised from absolute alcohol as narrow rectangular plates (pale yellow) melting at 134–35°. It gave a reddish-brown colour with alcoholic ferric chloride, was sparingly soluble in aqueous sodium hydroxide and dissolved in concentrated sulphuric acid to give a bright yellow solution. Yield, 0·4 g. (Found: C, 64·7; H, 5·0; $C_{20}H_{20}O_7$ requires C, 64·5; H, 5·4%).

7-Ethoxy-3 : 5 : 3′ : 4′-tetramethoxy-flavone (*XI*)

The above 5-hydroxy compound (0·2 g.) was further methylated in dry acetone (50 c.c.) solution using anhydrous potassium carbonate (5 g.) and freshly distilled dimethyl sulphate (0·5 c.c.) and heating under reflux for

20 hours. The product was readily obtained as a colourless solid. It crystallised from ethyl acetate as broad rectangular plates melting at 158–60°. Yield, 0·2 g. (Found: C, 65·1; H, 5·3; $C_{21}H_{22}O_7$ requires C, 65·3; H, 5·7%). It was identical with the monoethyl-tetramethyl quercetin obtained from quercimeritrin.

Pentamethyl quercetagetin from quercetagitrin (IV)

The glucoside required for this purpose was isolated from the flowers of *Tagetes erecta* using a modification of the method adopted by Rao and Seshadri.[1] The alcoholic extract of the petals was concentrated removing as much alcohol as possible. The residue was treated with excess of water, heated and resinous matter filtered off. The clear solution was concentrated to a small bulk and repeatedly extracted with ether in order to remove quercetagetin. When the aqueous solution was allowed to stand saturated with ether, the glucoside separated in the course of a few weeks. It was filtered and washed with ether and recrystallised from pyridine.

It (0·5 g.) was methylated as in the case of quercimeritrin using dry acetone (100 c.c.), anhydrous potassium carbonate (7 g.) and freshly distilled dimethyl sulphate (3 c.c.) and refluxing the mixture for 30 hours. When the filtrate was concentrated to remove the solvent and water added no solid separated. Hence the solution was rendered 7% acid by the addition of the requisite amount of sulphuric acid and gently boiled for 2 hours, when the aglucone separated out slowly. It was filtered and washed with water. On crystallisation from alcohol the monohydroxy compound was obtained as colourless long plates and needles, melting at 234–35° (Rao and Seshadri[1]). Yield, 0·2 g.

Monoethyl-pentamethyl-quercetagetin (A)

The above pentamethyl ether (0·15 g.) was ethylated using dry acetone (50 c.c.), anhydrous potassium carbonate (3 g.) and ethyl iodide (0·3 c.c.) and refluxing the mixture for 20 hours. The potassium salts were filtered off and washed with warm acetone. When the filtrate was concentrated to remove the solvent and water added, a brown sticky solid separated out. It was left in the ice-chest overnight when the product came out as an amorphous powder. Crystallisation from aqueous alcohol yielded colourless plates melting at 127–28°. The melting point was not depressed by admixture with a synthetic sample of 7-ethoxy-pentamethoxy flavone (V). Yield, 0·15g. It was insoluble in aqueous sodium hydroxide and did not give any colour with alcoholic ferric chloride. (Found: C, 63·2; H, 5·6; $C_{22}H_{24}O_8$ requires C, 63·5; H, 5·8%).

2-*Hydroxy*-4-*ethoxy*-ω: 6-*dimethoxy-acetophenone* (*VI*)

7-Ethoxy-3 : 5 : 3′ : 4′-tetramethoxy-flavone obtained from quercimeritrin or synthetically (1 ·0 g.) was treated with absolute alcoholic potash (40 c.c., 8%) and the solution refluxed for 6 hours. As much of the alcohol as possible was removed under reduced pressure and the remaining solution diluted with water. It was then rendered acidic with dilute sulphuric acid and ether extracted. The ether extract was shaken with aqueous sodium bicarbonate in order to remove veratric acid and then evaporated when the ketone separated as a colourless solid. Crystallisation from alcohol yielded it as thin rectangular plates and needles melting at 105–6°. It gave a reddish brown colour with alcoholic ferric chloride and dissolved in aqueous sodium hydroxide to give an yellow solution. Yield 0 ·5 g. (Found: C, 60 ·0; H, 6 ·5; $C_{12}H_{16}O_5$ requires C, 60 ·0; H, 6 ·7%.)

2 : 5-*Dihydroxy*-4-*ethoxy*-ω: 6-*dimethoxy-acetophenone* (*VII*)

To a stirred solution of the above ketone (1 ·0 g.) in aqueous sodium hydroxide (1 ·0 g. in 15 c.c.) was added dropwise a solution of potassium persulphate (1 ·2 g. in 30 c.c. of water) in the course of two hours, the temperature being kept at 15–20°. After 24 hours the brown aqueous solution was neutralised with dilute hydrochloric acid to congo red and ether extracted to remove the unreacted ketone. To the aqueous solution was then added concentrated hydrochloric acid (20 c.c.) and sodium sulphite (2 g.) and the mixture heated in a boiling water-bath for half an hour. It was then cooled and extracted with ether. On distilling off the ether a viscous liquid was obtained which did not solidify even after leaving in the refrigerator for a number of days. Hence it was employed directly for the subsequent condensation. Yield, 0 ·25 g.

It gave a greenish brown colour with alcoholic ferric chloride and an yellow solution with aqueous sodium hydroxide.

7-*Ethoxy*-3 : 5 : 6 : 3′ : 4′-*pentamethoxy flavone* (*V*)

An intimate mixture of the ketone (VII) (1 ·0 g.), veratric anhydride (6 ·0 g.) and sodium veratrate (2 ·5 g.) was heated under reduced pressure at 170–80° for 4 hours. The dark brown product was dissolved in hot alcohol (25 c.c.) and refluxed with an aqueous solution of potassium hydroxide (3 g. in 3 c.c. of water) for 30 minutes. The solvent was removed under reduced pressure and the residue dissolved in water. After saturation with carbon dioxide the solution was extracted with ether. On evaporating the ether solution the flavone was obtained as a viscous oil. It gave an olive green colour with ferric chloride indicating partial demethylation in the

5-position and an alcoholic solution of the substance gave a deep red colour with magnesium and hydrochloric acid. It was directly employed for the subsequent methylation by dissolving in dry acetone (30 c.c.) and boiling with anhydrous potassium carbonate (6 g.) and dimethyl sulphate (2·5 c.c.) for 20 hours. After filtration, the filtrate was concentrated and treated with water. The semi-solid mass thus obtained was taken up in ether and the ether solution evaporated. The residue was crystallised from benzene-petroleum ether when the 7-ethoxy-pentamethoxy flavone was obtained as rectangular plates melting at 127–28°. Yield, 0·5 g. It was insoluble in aqueous sodium hydroxide and did not give any colour with alcoholic ferric chloride. (Found: C, 63·6; H, 5·8; $C_{22}H_{24}O_8$ requires C, 63·5; H, 5·8%.)

7-Ethoxy-5-hydroxy-3-methoxy-flavone (XV)

Galangin-3-methyl ether[11] (2·0 g.) in dry acetone (100 c.c.) was treated with anhydrous potassium carbonate (2 g.) and ethyl iodide (1 mol., 1 c.c.) and the contents were refluxed for 6 hours. The potassium salts were filtered and washed with warm acetone. On concentrating the filtrate a semi-solid mass was obtained which when treated with a little alcohol yielded a yellow solid. It crystallised from alcohol as narrow rectangular plates (yellow) melting at 125–6°. It gave a reddish-brown colour with alcoholic ferric chloride. Yield, 1·5 g. (Found: C, 69·3; H, 5·1; $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1%.)

7-Ethoxy-3: 5-dimethoxy flavone (XVI)

The above 5-hydroxy compound (0·5 g.) was methylated in dry acetone (50 c.c.) with excess of dimethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (5 g.) by boiling the mixture for 10 hours. The methyl ether crystallised from alcohol as fine needles melting at 128–29°. It did not give any colour with alcoholic ferric chloride and was insoluble in aqueous sodium hydroxide. Yield, 0·4 g. (Found: C, 69·5; H, 5·2; $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·5%).

This flavone (1·0 g.) was subjected to fission using absolute alcoholic potash (40 c.c., 8%) and the product worked up just as in the case of the quercetin derivative. 2-Hydroxy-4-ethoxy-ω: 6-dimethoxy acetophenone was obtained in an yield of 0·6 g.

Summary

Synthetic confirmation for the constitution of quercetagitrin is provided. The pentamethyl-quercetagetin obtained by the complete methylation and hydrolysis of the glucoside is finally ethylated and the product shown to be identical with a synthetic sample of 7-ethoxy-3: 5: 6: 3′: 4′-pentamethoxy

flavone (V) made from 2-hydroxy-4-ethoxy-ω : 6-dimethoxy-acetophenone (VI) using oxidation with persulphate, Allan-Robinson condensation and final methylation as stages. The ketone (VI) is conveniently made by the alkali fission of *O*-7-ethyl-3 : 5 : 3′ : 4′-tetramethyl-quercetin obtained from quercimeritrin. This mixed ether of quercetin is also made independently by synthesis and this constitutes a synthetic confirmation of the constitution of quercimeritrin. Further, the transformations show the relationship between quercimeritrin and quercetagitrin as 7-glucosides. The most convenient synthetic route for the ketone (VI) is through the galangin derivative (XVI).

References

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