CONSTITUTION OF HIBISCITRIN. PART II

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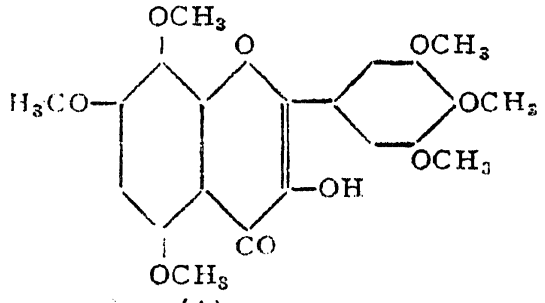
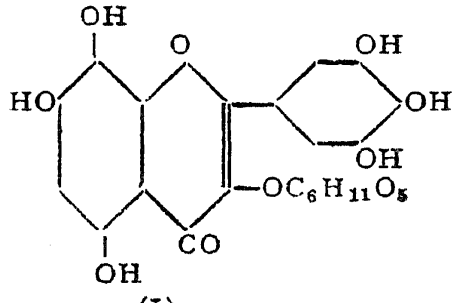
Received December 10, 1947

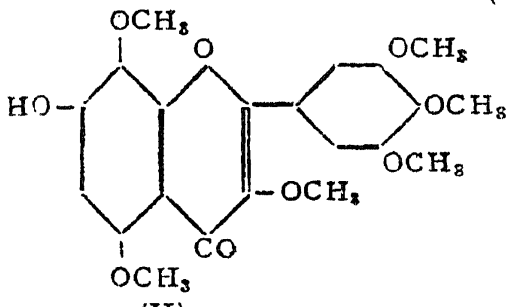
In an earlier publication (Part I[1]) it was shown that hibiscitrin is a monoglucoside of hibiscetin. It undergoes complete methylation with dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium and on further hydrolysis yields O-hexamethyl-hibiscetin (A) with only one hydroxyl group free. This partial methyl ether forms trimethyl-gallic acid by fission with alkali. Hence the free hydroxyl group is not in the side phenyl nucleus. Of the other alternatives, positions 7, 8 and 5 were eliminated from considerations of melting point, colour with ferric chloride and solubility in alkali. The free hydroxyl in (A) was considered therefore to be in position 3 and thus it followed that hibiscitrin is a 3-glucoside of hibiscetin (I).



(A)



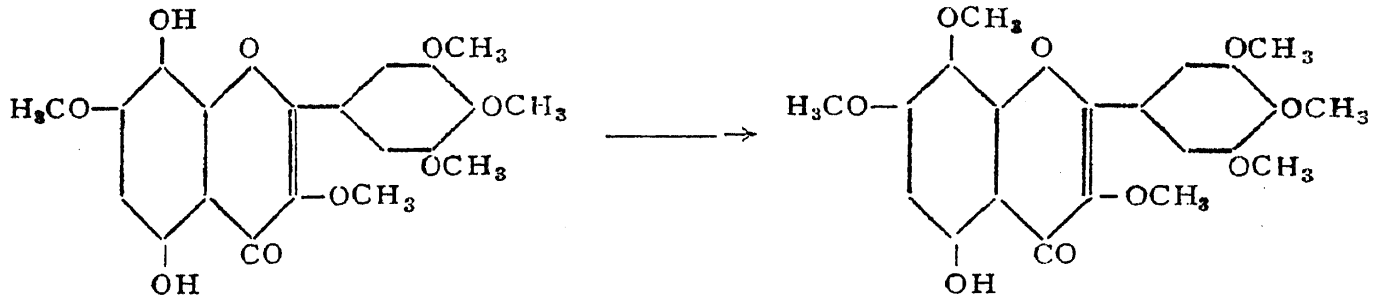
(I)



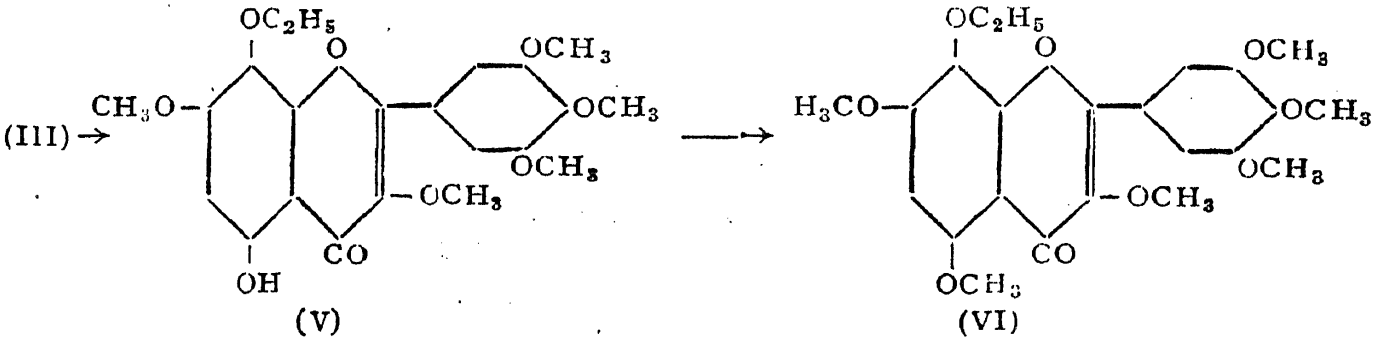
(II)

To support the above conclusion, there was need for samples of the different O-hexamethyl-hibiscetins or their derivatives for comparison with the degradation product (A) or its derivatives. The hexamethyl ether with the 7-hydroxyl alone free (II) was already known as an intermediate stage in the earlier synthesis of hibiscetin[2]; it has a much higher melting point as compared with (A) and further it does not give the ferric chloride colour. The other derivatives of hibiscetin required for the present study have now

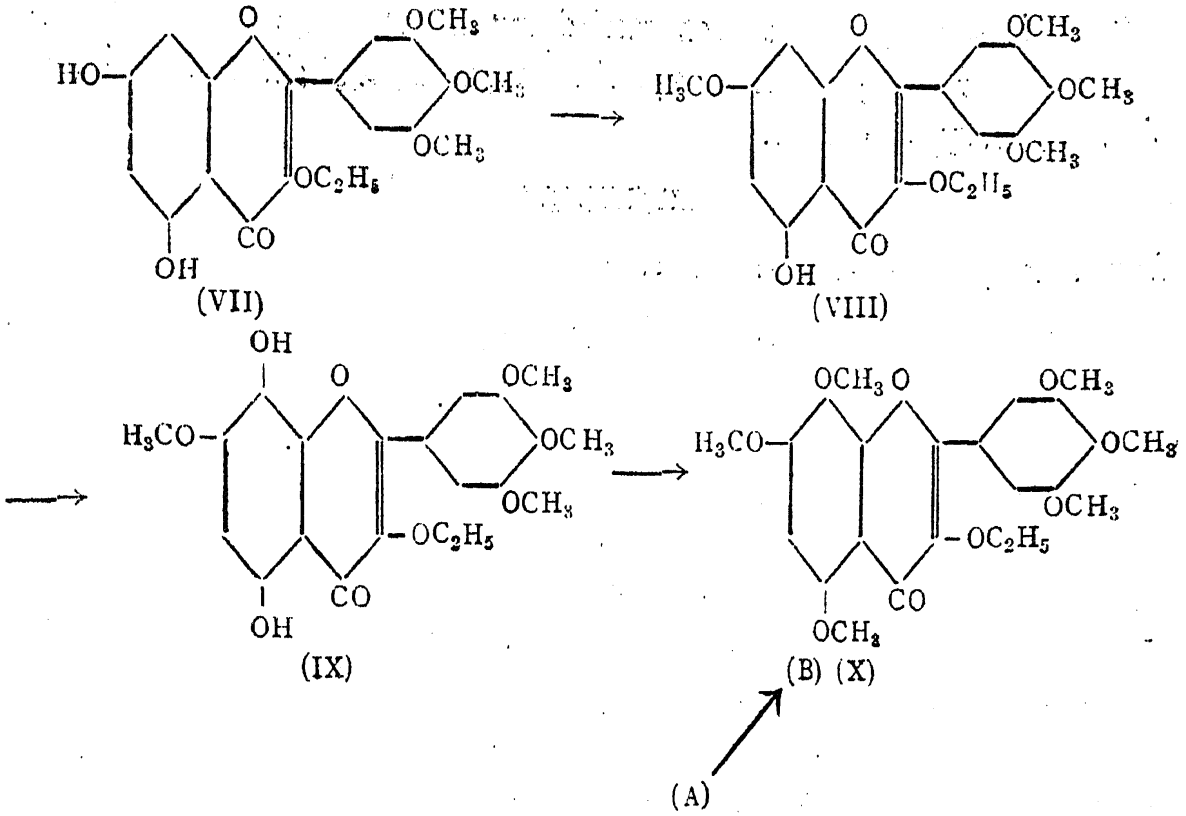
been conveniently prepared by employing the method of nuclear oxidation in the flavone series. The synthesis of 5 : 8-dihydroxy-3 : 7 : 3′ : 4′ :5′ pentamethoxy flavone (III) has been recently described by Rao and Seshadri.[3] Partial methylation of this quinol forms O-hexamethyl hibiscetin (IV) with a free hydroxyl in the 5-position. This compound crystallises from alcohol as pale lemon yellow rectangular prisms and plates and melts at 179–80°. It is sparingly soluble in aqueous alkali and gives a green colour with alcoholic ferric chloride. It is thus different from compound (A) which crystallises from alcohol as bright yellow short needles and melts at 198–200°, readily dissolves in aqueous alkali and gives a dark brown colour with ferric chloride.



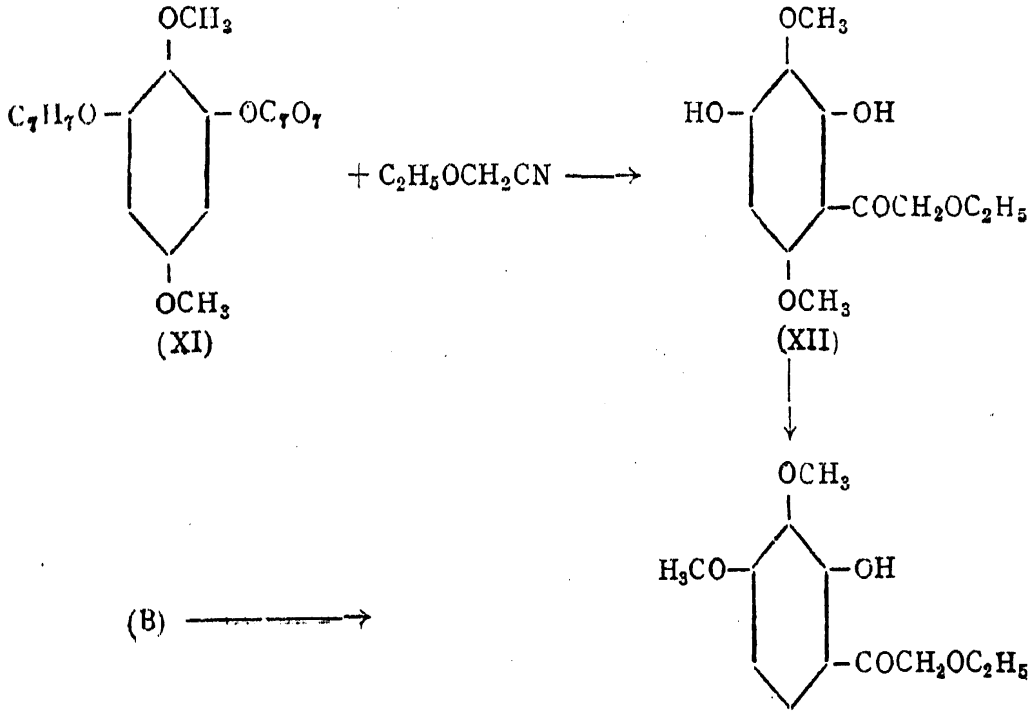
Partial ethylation of the quinol (III) and subsequent methylation produces hexamethyl-monoethyl hibiscetin (VI) with the ethoxyl in 8-position. This has a melting point of 149–50° as against 175–76° for (B), the ethyl ether of (A) and thus the two are different compounds.



Thus all the possibilities except 3 for the position of the hydroxyl group in compound (A) are eliminated. In order to provide positive confirmation of this finding, 3-ethyl-hexamethyl hibiscetin (X) has been prepared in the following manner. Starting from ω-ethoxy-phloracetophenone[4] and trimethyl-gallic acid, 3-ethyl-3′ : 4′ : 5′-trimethyl ether of myricetin (VII) is obtained. This is partially methylated to form the O-tetramethyl-monoethyl myricetin (VIII) with the 5-hydroxyl free. This compound is subjected to oxidation with persulphate and the quinol (IX) fully methylated. The product (X) is found to be identical with (B) the ethyl ether of (A) derived from hibiscitrin.



In Part I it was mentioned that from the alkali fission of (A) only trimethyl gallic acid was isolated and that the ketonic product could not be obtained and characterised due to further decomposition. However its ethyl ether (B) is now found to undergo smooth fission and the ketonic part (C) could be isolated. As an independent line of evidence for the constitution of hibiscitrin, this ketone has been shown to be identical with a synthetic sample of ω-ethoxy-2-hydroxy-3 : 4 : 6-trimethoxy acetophenone



(XIII). The synthesis has been carried out in the same way as that of gossypetol-tetramethyl ether[5] except that ethoxy acetonitrile is used instead of the methoxy compound.

Experimental

Ethylation of O-hexamethyl-hibiscetin (A)

The partial methyl ether (A) of hibiscetin (1 g.) was dissolved in anhydrous acetone (50 c.c.), ethyl iodide (1 g.) and anhydrous potassium carbonate (10 g.) added and the mixture refluxed on a water-bath for 20 hours. At the end of the experiment, it was filtered while still hot and the residue washed with hot anhydrous acetone. The filtrate was concentrated and kept in an ice-chest after dilution with water. The solid that separated out was filtered and washed with dilute sodium hydroxide solution to remove any unethylated compound. The product was crystallised from alcohol when a pale brownish yellow crystalline solid was obtained. A colourless sample could be obtained by further repeated crystallisation from alcohol. The ethyl ether (B) then melted at 163–5°. When the dry substance was recrystallised from ethyl acetate, however, it readily separated out as colourless needles melting at 175–76°. (Found: C, 61·7; H, 6·2; $C_{23}H_{26}O_9$ requires C, 61·9; H, 5·8%.)

Alkali fission of ethyl ether (B)

The ethylated product (B) obtained above (0·5 g.) was boiled under reflux with absolute alcoholic potash (20 c.c., 8%) for a period of six hours. As much of alcohol as possible was then removed by distillation; the residue was dissolved in water and the solution filtered to remove any insoluble impurities. The clear solution was acidified with excess of dilute sulphuric acid and ether-extracted. The ether solution was washed three times with 5% sodium bicarbonate solution to extract the acid part.

The ketonic part (C)

The ether solution was then washed with water and on distilling off the solvent, the residue was found to be a pale yellow viscous liquid which solidified during the course of a few hours when treated with water and kept in an ice-chest. The ketone was thrice recrystallised from alcohol and it finally separated out as almost colourless flat needles melting at 135–36°. (Found: C, 57·4; H, 6·5; $C_{13}H_{18}O_6$ requires C, 57·8; and H, 6·7%.)

The acid part

The bicarbonate solution on acidification with concentrated hydrochloric acid gave rise to a precipitate which on recrystallisation from hot water was found to be identical with a synthetic sample of trimethyl gallic acid,

5-*Hydroxy*-3 : 7 : 8 : 3′ : 4′ : 5′-*hexamethoxy-flavone* (*IV*)

A solution of the 5 : 8-dihydroxy-3 : 7 : 3′ : 4′ : 5′-pentamethoxy-flavone (III)[3] (0·4 g.) in anhydrous acetone (20 c.c.) was treated with dimethyl sulphate (0·13 g.) and anhydrous potassium carbonate (5 g.). The mixture was boiled under reflux for 5 hours. At the end of the experiment the acetone was distilled off and the residue taken up with water when a light yellow solid separated out. The product was filtered and recrystallised from rectified spirits when it came out as pale lemon yellow rectangular prisms and plates melting at 179–80°. It was sparingly soluble in alkali and gave an olive green colour with alcoholic ferric chloride. (Found: C, 60·0; H, 5·0; $C_{21}H_{22}O_9$ requires C, 60·3 and H, 5·3%.)

5-*Hydroxy*-8-*ethoxy*-3 : 7 : 3′ : 4′ : 5′-*pentamethoxy-flavone* (*V*)

The dihydroxy compound (III) (0·5 g.) was dissolved in anhydrous acetone (30 c.c.) ethyl iodide (0·2 c.c.) and anhydrous potassium carbonate (5 g.) added and the mixture boiled under reflux for 5 hours. At the end of the experiment, acetone was distilled off and the residue taken up with water when the partially ethylated flavonol separated out. It was filtered and recrystallised from dilute alcohol. It came out as pale yellow rectangular plates and prisms melting at 113–15°. (Found: C, 61·3; H, 5·4; $C_{22}H_{24}O_9$ requires C, 61·1 and H, 5·6%.) The substance was sparingly soluble in alkali and gave an olive green colour with alcoholic ferric chloride.

8-*Ethoxy*-3 : 5 : 7 : 3′ : 4′ : 5′-*hexamethoxy-flavone* (*VI*)

The above compound (V) (0·4 g.) was dissolved in acetone (30 c.c.) and methylated by boiling for 30 hours with dimethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (5 g.). The solvent was then removed by distillation and the residue treated with water when the methylated product separated out. It was filtered and washed with aqueous sodium hydroxide to remove any unmethylated product. The substance was recrystallised from dilute alcohol when it came out as almost colourless (pale brown) rectangular rods tending to be needles melting at 148–50°. (Found: C, 61·6; H, 5·4; $C_{23}H_{26}O_9$ requires C, 61·9 and H, 5·8%.)

5 : 7-*Dihydroxy*-3-*ethoxy*-3′ : 4′ : 5′-trimethoxy-flavone (VII)

ω-Ethoxy-phloracetophenone (1·0 g.) was well mixed with dry trimethyl-gallic anhydride (6 g.) and dry sodium salt of trimethyl-gallic acid (3 g.). The mixture was heated at 180° for 4 hours *in vacuo*. The hard mass was then broken up and dissolved in alcohol (150 c.c.) and aqueous potassium hydroxide solution (8·0 g. in 15 c.c.) was added during the course of 20 minutes while the solution was being refluxed. The alcohol was then

completely removed under reduced pressure and the residue dissolved in water. The solution was filtered to remove impurities and the filtrate was saturated with carbon dioxide. The pale yellow compound that separated out was repeatedly crystallised from alcohol. It came out as colourless (very pale yellow) rectangular plates and rods melting at 228–30°. It gave an olive green colour with alcoholic ferric chloride. It did not exhibit any visible fluorescence in alcoholic solution. (Found: C, 61·8; H, 4·9; $C_{20}H_{20}O_8$ requires C, 61·9 and H, 5·2%.)

5-*Hydroxy*-3-*ethoxy*-7 : 3′ : 4′ : 5′-*tetramethoxy-flavone* (*VIII*)

The above 5 : 7-dihydroxy compound (VII) (1·8 g.) was partially methylated using dry acetone (50 c.c.), dimethyl sulphate (0·55 c.c.) and anhydrous potassium carbonate (5 g.) and boiling for six hours. At the end of the experiment, the acetone was distilled off and the residue treated with water when the partially methylated flavone separated out. It was very sparingly soluble in ether and also in aqueous alkali. Hence purification was effected by repeated crystallisation from a large volume of rectified spirit. It came out finally from absolute alcohol as pale yellow flat needles and narrow rectangular plates melting at 143–44°. It gave an olive green colour with alcoholic ferric chloride. (Found: C, 63·1; H, 5·3; $C_{21}H_{22}O_8$ requires C, 62·7; and H, 5·5%.)

5 : 8-*Dihydroxy*-3-*ethoxy*-7 : 3′ : 4′ : 5′-*tetramethoxy-flavone* (*IX*)

The 5-hydroxy compound (VIII) (1·4 g.) was dissolved in 20 c.c. of pyridine and aqueous potash (1·0 g. in 250 c.c. of water) was gradually added taking care to see that the solution remained clear without the separation of any precipitate. The mixture was then slowly treated with aqueous potassium persulphate (1·4 g. in 50 c.c. of water) while the mixture was kept vigorously stirred by means of an electric stirrer. The addition was completed during the course of two hours. The yellow colour of the solution gradually changed to green in the first half hour and remained deep green till the end of the experiment. After 24 hours, the solution was just acidified and filtered to remove any unreacted substance. The filtrate was also ether extracted thrice to remove the last traces. To the aqueous solution were added sodium sulphite (2·0 g.) and concentrated hydrochloric acid (25 c.c.). The mixture was then heated on a boiling water-bath for half an hour when an yellow solid separated out. This was collected by filtration and a further yield was obtained by ether extraction (total yield, 0·7 g.). The product was recrystallised from absolute alcohol from which it separated out slowly as deep yellow short needles melting at 190–92°. An alcoholic solution of the substance gave a reddish brown colour with ferric chloride. The sub-

stance dissolved in aqueous alkali to form immediately a deep red solution changing gradually to reddish violet. (Found: C, 60·4; H, 4·9; $C_{21}H_{22}O_9$ requires C, 60·3; H, 5·3%.)

3-*Ethoxy*-5 : 7 : 8 : 3′ : 4′ : 5′-*hexamethoxy-flavone* (X)

The 5 : 8-dihydroxy compound (IX) (0·5 g.) was methylated with dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (5·0 g.) in acetone solution by refluxing for a period of 25 hours. The methyl ether was crystallised from alcohol when it came out as colourless needles melting at 175–76°. (Found: C, 62·2; H, 5·6; $C_{23}H_{26}O_9$ requires C, 61·9 and H, 5·8%.) It was insoluble in alkali and did not give any colour with alcoholic ferric chloride. Mixed melting point of the substance with the mono-ethyl-hexamethyl ether of hibiscetin (B) obtained from hibiscitrin by methylation, hydrolysis and subsequent ethylation was undepressed.

2 : 4-*Dihydroxy*-3 : 6-*dimethoxy*-ω-*ethoxy-acetophenone* (*XII*)

2 : 6-Dibenzyloxy-1 : 4-dimethoxy benzene (XI) (3·5g.) prepared from pyrogallol according to the procedure adopted by Baker, Nodzu and Robinson[6] was treated with anhydrous ether (50 c.c.), dry ethoxy acetonitrile (2 c.c.) and finely powdered fused zinc chloride (1 g.). A rapid stream of dry hydrogen chloride gas was passed through the mixture for 5 hours, the reaction flask being cooled in an ice-salt mixture. The ketimine hydrochloride began to separate out as a dark brown semi-solid and the reaction was completed by keeping the flask in a refrigerator for two days. The ether was then decanted and the residue of the ketimine hydrochloride washed twice with 20 c.c. portions of dry ether. The original ether solution and the washings contained mainly benzyl chloride. The ketimine hydrochloride was dissolved in water (50 c.c.) and the solution extracted with ether to remove impurities. The hydrolysis was effected by heating the solution on the wather-bath for an hour. On cooling, the solution deposited the crude ketone as a practically colourless mass along with a small quantity of resinous matter. Further quantities could be obtained by concentration of the mother liquor and extraction with ether. The compound was soluble in sodium carbonate solution while the accompanying impurities were not. This property was made use of in the preliminary purification of the compound. It was finally crystallised from hot water when it came out as long almost colourless needles melting at 130–32°. Yield, 0·5 g. The compound was freely soluble in alcohol and dissolved with a pale yellow colour in aqueous sodium hydroxide. Its alcoholic solution developed a bluish-violet colouration with a drop of ferric chloride. (Found: C, 56·3; H, 6·3; $C_{12}H_{16}O_6$ requires C, 56·3 and H, 6·3%.)

2-Hydroxy-3 : 4 : 6-trimethoxy-ω-ethoxy-acetophenone (XIII)

A mixture of the above dihydroxy acetophenone (XII) (0·5 g.), dry acetone (25 c.c.), anhydrous potassium carbonate (5 g.) and pure dimethyl sulphate (0·3 g.) was refluxed on the water-bath for 8 hours. Acetone was then distilled off and the residue taken up with water and ether extracted. From the ether solution the partially methylated ketone was extracted by means of dilute alkali repeatedly The combined alkali extract was acidified and then ether extracted. The solvent was distilled off from the ether extract and the residue crystallised from alcohol. The ketone came out as colourless flat needles melting at 136–37°. An alcoholic solution of the substance gave an olive green colour with ferric chloride. (Found: C, 57·4; H, 6·5; $C_{13}H_{18}O_6$ requires C, 57·8 and H, 6·7%.) The mixed melting point with ketone (C) obtained from (B) was found to be undepressed.

Summary

The constitution of hibiscitrin proposed in Part I as the 3-glucoside of hibiscetin is confirmed here by synthetic experiments. The O-hexamethyl hibiscetin (A) has been ethylated to (B). Using the method of nuclear oxidation a hexamethyl hibiscetin with a free hydroxyl in position 5 is now prepared and shown to be different from (A). Ethyl ethers of the isomeric 8- and 3-hydroxy compounds are also prepared. The former is different from (B) whereas the latter is identical with it.

Though alkali fission of (A) does not yield the ketonic part, the ethyl ether (B) can be satisfactorily employed for this fission. The ketonic product is found to be identical with ω-ethoxy-2-hydroxy-3 : 4 : 6-trimethoxy-acetophenone obtained synthetically, thus confirming again the above constitution of (B) and of hibiscitrin.

References

1. Rao and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1948, **27**, 104.
2. Rao, Rao and Seshadri .. *Ibid.*, A, 1944, **19**, 88.
3. Rao and Seshadri .. *Ibid.*, A, 1947, **25**, 444.
4. ———— .. *Ibid.*, A, 1946, **23**, 143.
5. Murti, Row and Seshadri .. *Ibid.*, A, 1946, **24**, 233.
6. Baker, Nodzu and Robinson .. *J. C. S.*, 1929, 74.