

CONSTITUTION OF CANNABISCITRIN—PART II

BY T. R. SESHADRI AND V. VENKATESWARLU

(From the Department of Chemistry, Andhra University)

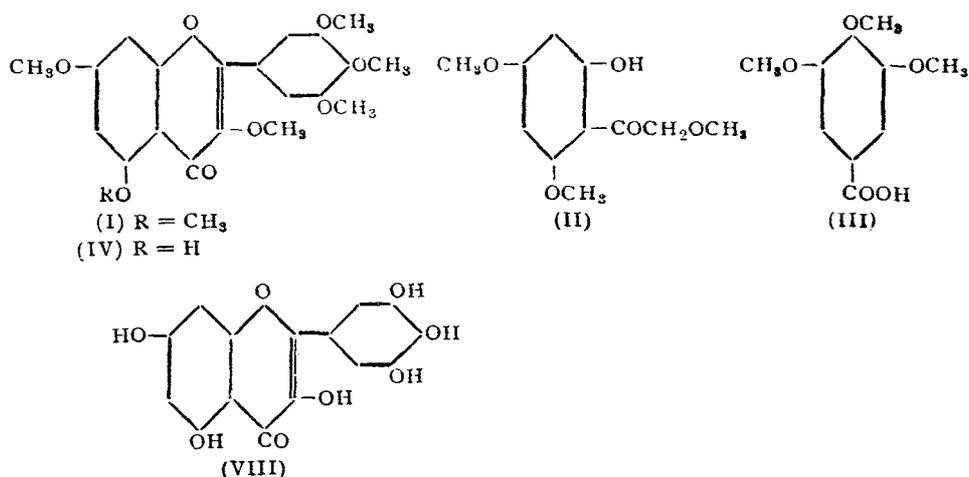
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CANNABISCITRIN¹ is the main crystalline component of the flowers of *Hibiscus cannabinus*. It is a monoglucoside of cannabiscetin, a flavonol having six hydroxyl groups. When decomposed with aqueous alkali its hexamethyl ether yielded *O*-trimethyl-gallic acid. Thus four of the six hydroxyl groups of the flavonol could be definitely allotted to the positions 3, 3', 4' and 5'. The orientation of the other two as 5:8 was tentatively suggested, based on colour reactions. The aglucone was considered to be isomeric with myricetin and not identical with it mainly for the following reasons: (1) it gave the gossypetone reaction though not so readily as gossypetin and this was considered to be characteristic of compounds having hydroxyl groups in the *para*-positions 5 and 8; it further gave a display of colours in alkaline buffer solutions; (2) the methyl ether of cannabiscetin as originally obtained was noticed to have a higher melting point than that of myricetin though the melting points of cannabiscetin and its acetate agreed with those of myricetin and its acetate.

In a subsequent paper² the position of the sugar group was fixed as 3'. This was done by methylation of the glucoside, hydrolysis and fission of the product whereby 3-hydroxy-4:5-dimethoxy-benzoic acid was obtained and identified.

Further work has now been done with a view to obtain more definite information about the nature of the flavonol and its glucoside using improved methods of methylation and fission leading particularly to the isolation and identification of the ketonic decomposition product. The different methods of methylation that have been so far employed by different workers have been applied to the present case. The use of dimethyl sulphate and anhydrous potassium carbonate in anhydrous acetone solution has been found to be the most satisfactory and has given the best yields of the pure ether. The hexamethyl ether of cannabiscetin obtained in this way is found to melt at 156° agreeing with the melting point of myricetin methyl ether.³ The higher melting product originally described could not be obtained again even by adopting the other methods and the older sample was not available for comparison due to loss during the transfer of our laboratories as a war measure. Whether this is another case of dimorphism found in certain other flavonol ethers could not therefore be settled.

Since it has been our experience that the use of absolute alcoholic potash for the fission of methyl ethers gives much better results than older methods and is particularly suited for dealing with small quantities, this method has now been adopted for the fission of the hexamethyl ether of cannabiscetin (I). Besides trimethyl gallic acid (III) good yields of a ketonic product has been obtained and it has been identified as methoxy fisetol-dimethyl ether (II)⁴ by a detailed study of its reactions and conversion into derivatives and also by comparison with a synthetic sample. This definitely established the position of the two hydroxyl groups in the benzopyrone part as 5:7 and not 5:8 and thus cannabiscetin should be the same as myricetin (VIII). The identity was established by the synthesis of myricetin and its derivatives according to the method of Kalff and Robinson⁵ and comparison with cannabiscetin, its acetate and methyl ether. It should be noted here that the colour reactions with buffer solutions and *p*-benzoquinone which were originally considered to be characteristic of the 5:8 arrangement of hydroxyl groups are also given by flavonols containing three hydroxyls in the 3':4':5' positions.

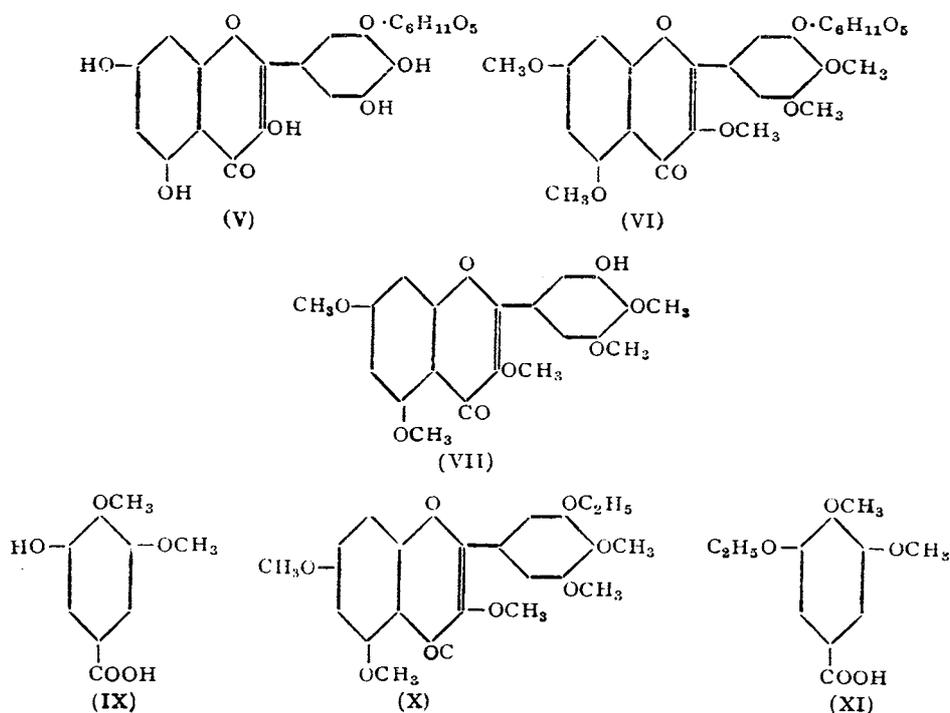


In the course of the experiments on the identification of cannabiscetin, methoxy-fisetol-dimethyl ether (ω :4:6-trimethoxy-2-hydroxy acetophenone) was fused with the anhydride and sodium salt of trimethyl-gallic acid. There was partial demethylation during the condensation and the product was found to be 5-hydroxy-3:7:3':4':5'-pentamethoxyflavone (IV) or pentamethylmyricetin³ obtained by Perkin by the partial methylation of myricetin.

In the light of the identity of cannabiscetin with myricetin, the constitution of the glucoside, cannabiscitrin has been further investigated using the simpler

method of methylation. Though the glucoside is sparingly soluble in dry acetone, it could be successfully and fully methylated in this medium in a finely powdered condition by boiling with excess of dimethyl sulphate and potassium carbonate. The methylated glucoside could be obtained in a pure condition and hydrolysed to give a pentamethyl ether of cannabiscetin (myricetin) in good yield. Fission of this substance with alcoholic potash has yielded as one product methoxy-fisetol-dimethyl ether (II) showing conclusively that the sugar group is not present in the benzopyrone part. The acid decomposition product was identified to be 4:5-dimethyl gallic acid (IX) by comparison with a sample synthesised according to the method of Shriner and McCutchen⁶ thus establishing the constitution of the penta-methyl ether as (VII) and the constitution of the glucoside as (V) with the sugar unit in the side phenyl nucleus in position 3'.

A more convenient method of establishing the exact constitution of the glucoside is to ethylate the above penta-methyl ether to give ethyl-penta-methyl-cannabiscetin (myricetin) (X) and subject this to fission. The reaction goes more smoothly yielding the same ketone (II) and 3-ethyl-4:5-dimethyl gallic acid (XI) which is identical with the ethylation product of 4:5-dimethyl gallic acid. These reactions are represented below:



Cannabiscitrin is, therefore, a new mono-glucoside of myricetin, the glucose group being in the 3'-position. Myricitrin⁸ isolated by Perkin from *Myrica nagi* is a rhamnoside and the rhamnose unit is considered to be linked to the 3-position of the flavonol.

It is interesting to compare the flavonols present in the flower petals of the two closely related species of *Hibiscus*, *H. cannabinus* and *H. sabdariffa*. Botanically the plants resemble closely and they are also used for the same purposes, as acid vegetables and as sources of fibre. The *cannabinus* flowers contain myricetin as the flavonol, whereas hibiscetin is the main component of *sabdariffa* petals. Since the latter is 8-hydroxymyricetin, it appears that the species difference rests in the oxidation of the 8-position of the flavonol molecule.

EXPERIMENTAL

Hexamethyl cannabiscetin (I).—Cannabiscetin (1.0 g.), dissolved in dry acetone (100 c.c.), was treated with dimethyl sulphate (2.5 c.c.) and anhydrous potassium carbonate (20 g.) and the mixture boiled under reflux for 30 hours. After the completion of the reaction, the potassium salts were filtered off and washed with more acetone. The filtrate was concentrated and the residue treated with water (100 c.c.); the methyl ether then separated out completely during the course of one hour. It was twice crystallised from dilute alcohol when it came out in the form of colourless rectangular plates and prisms melting at 155–56°. It was insoluble in dilute sodium hydroxide solution and did not give any colour with alcoholic ferric chloride. The methyl ether exhibited a weak blue fluorescence in alcoholic solution. Yield: 1.0 g. (Found: C, 62.9; H, 5.7; OCH₃, 46.1; C₂₁H₂₂O₈ requires C, 62.7; H, 5.5; OCH₃, 46.3%.) The mixed melting point with myricetin hexamethyl ether was undepressed.

The use of benzene as the solvent in the above experiment also yielded the same methyl ether; the only difficulty was that cannabiscetin was not easily soluble in this solvent. Still the yield and the quality of the product were unaffected.

Other methods using (i) methyl iodide and anhydrous potassium carbonate in acetone solution, (2) methyl iodide and alcoholic potash and (3) dimethyl sulphate and alkali on cannabiscetin acetate, were examined in order to see if any higher melting ether could be obtained. But all of them yielded the same substance melting at 155–56°.

Decomposition of hexamethyl cannabiscetin with alcoholic potash: Isolation of trimethyl gallic acid (III) and the ketone (II).—Hexamethyl canna-

biscetin (1.0 g.) was boiled under reflux with absolute alcoholic potash (30 c.c. of 8%) for six hours under anhydrous conditions. At the end of the period as much of the alcohol as possible was distilled off and the residue dissolved in water. The aqueous solution was then filtered from a little insoluble impurity and the clear filtrate acidified with dilute sulphuric acid with cooling. The precipitated solid was then thrice extracted with ether and the combined ether solution shaken with 5% sodium bicarbonate to remove the acid part (A). On evaporating the ether solution the required ketone was obtained. It was crystallised twice from dilute alcohol when it came out in the form of thin colourless plates melting at 104–05°. The yield of the pure ketone was 0.34 to 0.38 g. It could also be crystallised from hot water. It dissolved easily in aqueous sodium hydroxide forming a pale yellow solution and gave a greenish brown colour with alcoholic ferric chloride. The mixed melting point with an authentic sample of 2-hydroxy- ω -4:6-trimethoxy-acetophenone was undepressed. (Found: C, 58.6; H, 6.5; OCH₃, 40.9; C₁₁H₁₄O₅ requires C, 58.4; H, 6.2; and OCH₃, 41.2%.)

The *dinitrophenylhydrazone* was obtained by heating a mixture of the above ketone (0.1 g.) and 2:4-dinitrophenylhydrazine (0.2 g.) dissolved in alcohol (10 c.c.), on a water-bath for half an hour. The phenylhydrazone separated out on cooling; it was washed with dilute hydrochloric acid and crystallised from alcohol when it came out in the form of orange-red micro-crystals melting at 160–62°. It was soluble in aqueous alkali to give an orange-red solution. (Found: C, 49.9; H, 4.7; C₁₇H₁₈O₈N₄ requires C, 50.2; H, 4.4%.)

The clear bicarbonate solution (A) obtained above was neutralised with dilute hydrochloric acid. The precipitated solid was repeatedly crystallised from hot water using a little animal charcoal. It came out in the form of colourless rectangular plates, melting at 167–68° and was identified as trimethyl gallic acid. The mixed melting point with an authentic sample of trimethyl gallic acid was undepressed. (Found: C, 56.7; H, 5.7; OCH₃, 44.1; C₁₀H₁₂O₅ requires C, 56.6; H, 5.7 and OCH₃, 43.9%.)

ω : 2:4:6-*Tetramethoxy-acetophenone*.—2-Hydroxy- ω -4:6-trimethoxy-acetophenone (0.2 g.) was methylated in acetone solution with dimethyl sulphate (0.5 c.c.) and anhydrous potassium carbonate (5.0 g.). The product was crystallised from dilute alcohol when it came out in the form of colourless rectangular plates melting at 151–52°. It was insoluble in dilute sodium hydroxide solution and did not give any colour with alcoholic ferric chloride. (Found: C, 60.1; H, 6.9; OCH₃, 51.9; C₁₂H₁₆O₅ requires C, 60.0; H, 6.7 and OCH₃, 51.7%.)

Comparison of cannabiscetin and myricetin (synthetic).—The following properties were compared and found to be identical. The flavonols decomposed above 350° and gave a dark brown colour with alcoholic ferric chloride; the hexamethyl ethers melted at 155–56° and the hexa-acetates at 220–21°. The colour reactions in alkaline buffer solutions were also identical. With freshly made buffer solutions, the appearance of pure blue has now been noticed even with a slightly lower pH. This may be due to changes in the buffer solutions during storage.

Condensation of hydroxy-fisetol trimethyl ether (II) with the anhydride and the sodium salt of trimethyl gallic acid: Preparation of 5-Hydroxy-3:7:3':4':5'-pentamethoxy-flavone (IV).—2-Hydroxy- ω :4:6-trimethoxy acetophenone (1.0 g.) was intimately mixed with the dry anhydride (6 g.) and the sodium salt of trimethyl gallic acid. The product was heated at 180° for 6 hours *in vacuo*. The hard mass was then broken up and dissolved in alcohol (150 c.c.) and while boiling, an aqueous potassium hydroxide solution (8 g. in 15 c.c.) was added during the course of 20 minutes to decompose the excess of the anhydride. The alcohol was then completely removed under reduced pressure and the residue dissolved in water. A small quantity of a solid (S) separated out. It was filtered and the filtrate was then saturated with carbon dioxide. The pale yellow product was repeatedly crystallised from alcohol when it came out as very pale yellow stout needles melting at 140–41°. This product corresponded to the pentamethyl ether of myricetin (m.p. 138–39°) recorded by Perkin.³ In bulk, the substance appeared yellow. It gave an olive green colour changing to brown with alcoholic ferric chloride and did not exhibit any visible fluorescence in alcoholic solution. (Found: C, 62.0; H, 5.3; OCH₃, 40.1; C₂₀H₂₀O₈ requires C, 61.9; H, 5.2 and OCH₃, 40.0%.) On working up solid (S) some more of the above 5-hydroxy compound was obtained.

Methylation of the above 5-hydroxy-flavone with excess of dimethyl sulphate and anhydrous potassium carbonate in acetone solution yielded the hexamethyl ether of myricetin which crystallised from dilute alcohol as colourless rectangular plates and prisms and melted at 155–56°.

Acetate of cannabiscitrin.—This was conveniently prepared by boiling the glucoside with acetic anhydride and a drop of pyridine, and crystallising the colourless product by dissolving in cold acetone and adding enough ethyl alcohol to start crystallisation. When prepared in this manner, it appeared as colourless needles and melted to a transparent liquid at 194°. (Found: C, 54.4; H, 4.4; C₃₉H₃₈O₂₂ requires C, 54.5; H, 4.4%.)

Methylation of cannabiscitrin: Preparation of the methylated glucoside (VI).—Finely powdered cannabiscitrin (4.0 g.) was suspended in dry acetone (200 c.c.), treated with dimethyl sulphate (6.0 g.) and anhydrous potassium carbonate (25 g.) and the mixture boiled under reflux for 30 hours. It was occasionally shaken to bring any unreacted cannabiscitrin into solution. The acetone solution was filtered, washed with more acetone and then concentrated when colourless crystals of the methylated glucoside separated out. It was recrystallised from methyl alcohol when it came out as shining needles and rectangular plates melting at 149–50°. It was insoluble in dilute alkali and did not give any colour with alcoholic ferric chloride. (Found: C, 56.4; H, 5.3; $C_{26}H_{30}O_{13}$ requires C, 56.7; H, 5.5%.)

Hydrolysis of the methylated glucoside: Isolation of pentamethyl cannabiscetin (myricetin) (VII).—The whole of the product obtained above (VI) was boiled under reflux with 7% sulphuric acid (200 c.c.) for 2 hours. The hot solution was filtered through a plug of cotton-wool to remove a small quantity of insoluble impurity that had separated out. The pentamethyl ether crystallised out almost completely on cooling. It was recrystallised twice from alcohol when it came out in clusters of colourless needles. The correct melting point of this compound is 220–22°. It did not give any colour with alcoholic ferric chloride, but dissolved easily in dilute sodium hydroxide forming a pale yellow solution with no fluorescence. Yield, 1.76 g. from 4 g. of cannabiscitrin. It developed a pale pink fluorescence in neutral alcoholic solution after some time. (Found: C, 61.5; H, 4.8; OCH_3 , 40.2; $C_{20}H_{20}O_8$ requires C, 61.9; H, 5.2 and OCH_3 , 40.0%.)

The above pentamethyl cannabiscetin (0.5 g.) was dissolved in acetone (20 c.c.) and methylated by boiling for 10 hours with excess of dimethyl sulphate and anhydrous potassium carbonate. The product was crystallised from dilute alcohol when it came out as colourless rectangular plates melting at 154–56° and was found to be identical with the hexamethyl cannabiscetin obtained by direct methylation.

Decomposition of the above pentamethyl ether (VII) with alcoholic potash: Isolation of 4:5-dimethyl gallic acid (IX) and the ketone (II).—Pentamethyl cannabiscetin (1.0 g.) was boiled under reflux with absolute alcoholic potash (30 c.c. of 8%) for a period of six hours. The product was worked up as before into the sodium bicarbonate soluble part (acid part) and the ketonic part. On crystallisation from alcohol, the ketone came out as colourless rectangular plates, melting at 104–05°. Mixed melting point with 2-hydroxy- ω :4:6-trimethoxy acetophenone was undepressed.

The bicarbonate solution was acidified and repeatedly extracted with ether; the residue obtained on evaporating the ether solution was twice crystallised from hot water using a little animal charcoal when it came out as rectangular plates and prisms melting at 192–94°. It was found to be identical with 4:5-dimethyl gallic acid, the mixed melting point with an authentic sample of 4:5-dimethyl gallic acid being undepressed. (Found: C, 54.7; H, 5.4; OCH₃, 31.2; C₉H₁₀O₅ requires C, 54.5; H, 5.1 and OCH₃, 31.3%.)

3'-Ethyl-3:5:7:4':5'-pentamethyl cannabiscetin (myricetin) (X).—The above pentamethyl cannabiscetin was ethylated with ethyl iodide and anhydrous potassium carbonate in acetone solution. The product was worked up as before and the ethyl ether was twice crystallised from dilute alcohol when it came out as stout rods and rectangular plates, melting at 155–56°. A mixed melting point with hexamethyl myricetin was considerably depressed. The product was insoluble in aqueous alkali and did not give any colour with alcoholic ferric chloride.

Decomposition of ethyl-pentamethyl cannabiscetin (X) with alcoholic potash: Isolation of the ketone (II) and 3-ethyl-4:5-dimethyl gallic acid (XI).—The ethyl pentamethyl ether (1.0 g.) was subjected to fission with alcoholic potash following the procedure already described. The ketonic part was found to be identical with 2-hydroxy- ω :4:6-trimethoxy-acetophenone. The acid was crystallised from hot water using a little animal charcoal when it came out as colourless rectangular plates and prisms melting at 164°. It was identified as 3-ethoxy-4:5-dimethoxy-benzoic acid. The mixed melting point with an authentic sample of 3-ethoxy-4:5-dimethoxy-benzoic acid, prepared as given below was undepressed. (Found: C, 58.6; H, 6.4; C₁₁H₁₄O₅ requires C, 58.4; H, 6.2%.)

4:5-Dimethyl gallic acid⁶ (1.0 g.) was ethylated using ethyl iodide (2 c.c.), anhydrous potassium carbonate and anhydrous acetone. The ether ester was obtained as a viscous liquid and it was hydrolysed by boiling with sodium hydroxide solution (20 c.c. of 20%) for one hour. The clear solution was then acidified and the solid product crystallised from hot water when it came out as colourless rectangular plates and prisms melting at 164°. (Found: C, 58.5; H, 6.4; C₁₁H₁₄O₅ requires C, 58.4; H, 6.2%.)

SUMMARY

The methylation of the glucoside, cannabiscitrin and of the aglucone, cannabiscetin has now been effected by a more efficient method and the fission products examined. As the result cannabiscetin has been identified

as myricetin and the identity has been confirmed by comparison of the flavonol and its derivatives with synthetic samples. It is noted that the reactions of flavonols having the pyrogallol side-phenyl nucleus exhibit many similarities with those given by flavonols having the 5:7:8-arrangement of hydroxyl groups. The condensation of hydroxy-fisetol-trimethyl ether with the anhydride and sodium salt of gallic acid yields pentamethyl-myricetin instead of the expected hexamethyl ether, partial demethylation having taken place in the 5 position during the course of the condensation.

Methylation of the glucoside, cannabiscitrin yields a pentamethyl ether which forms on hydrolysis pentamethyl cannabiscetin (myricetin). Alkali fission of this compound gives the same ketone as the hexamethyl ether. The acid part is identified as 4:5-dimethyl gallic acid, thus locating the position of the sugar group definitely in the 3' position of the flavonol. This result has been confirmed by ethylating the pentamethyl cannabiscetin and subjecting the ethyl ether to fission with alkali. Besides the ketone already mentioned, the acid decomposition product is found to be 3-ethyl-4:5-dimethyl gallic acid by comparison with a synthetic sample. The synthesis of the ethyl-dimethyl gallic acid is described.

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