COLOURING MATTER OF THE FLOWERS OF *HIBISCUS CANNABINUS* : CONSTITUTION OF CANNABISCETIN

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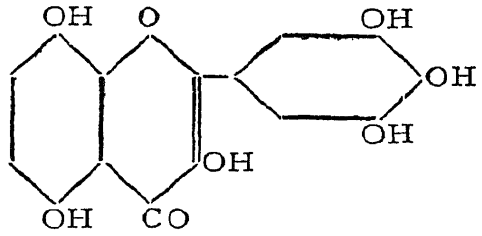
Hibiscus cannabinus (Deccan Hemp) is a small shrub cultivated in India for the sake of its valuable fibre and also as a vegetable. The flowers are light yellow in colour with a purple centre. A preliminary report of the chemical investigation of these flowers was made sometime back by Neelakantam and Seshadri[1] who announced the isolation of a new flavonol and its glucoside. They were named Cannabiscetin and Cannabiscitrin respectively because of their first isolation from the flowers of the cannabinus. Subsequently results have been obtained giving information regarding the constitution of the aglucone. A detailed account of the preparation and properties of the pigments and the determination of the constitution of cannabiscetin is given in this paper.

The dried petals were extracted with methylated spirit and the pigments isolated in various fractions according to the procedure already outlined in connection with other flowers.[2] The first fraction which was sparingly soluble in alcohol and came out on concentrating the alcoholic extract, consisted of the glucoside, cannabiscitrin. The aqueous solution subsequently obtained by diluting the alcoholic mother-liquor and removing the alcohol did not deposit any solid, but, when extracted with ether, yielded the aglucone, cannabiscetin. The neutral lead acetate fraction also seemed to consist mostly of the glucoside, which was, however, difficult to be purified. But pure cannabiscetin could be readily obtained therefrom after hydrolysis. The basic lead acetate fraction was too small to be studied. It could, therefore, be concluded that the colouring matter consists mostly of cannabiscitrin along with small quantities of the aglucone.

Cannabiscitrin has the formula $C_{21}H_{20}O_{13}$ and decomposes at about 245°. With alkaline buffer solutions, the substance yields two characteristic colorations—green and orange, the yellow colour that is first produced being evanescent. On acetylation it produces a colourless nonaacetyl derivative, and on hydrolysis with dilute sulphuric acid, it gives rise to glucose and the aglucone, cannabiscetin.

Cannabiscetin has the formula $C_{15}H_{10}O_8$ and does not melt below 350°. With ferric chloride, it gives a brown-black colour, and with dilute alkali, it undergoes a series of colour changes—yellow, green, blue, then olive green and finally light-brown. With alkaline buffer solutions the characteristic colorations are green, blue, crimson and purple, the initial yellow changing almost instantaneously, and these colour changes enable the flavonol to be easily distinguished from its glucoside. It yields a hexaacetyl derivative and a hexamethyl ether, thereby indicating the presence of six hydroxyl groups. It gives a deep red precipitate with neutral lead acetate solution, and undergoes decomposition with alkali and air; hence it should be a flavonol. It is isomeric with gossypetin, myricetin and quercetagetin. When treated with *p*-benzoquinone, it gives the " gossypetone " reaction just like gossypetin or herbacetin, thereby showing the presence of two hydroxyl groups in 5 and 8 positions. This is further supported by the rapid colour changes in alkaline buffer solutions just as in the case of gossypetin and herbacetin. As the result of oxidation in an alkaline solution exposed to air, it produces gallic acid which could be isolated and identified as its trimethyl ether. The completely methylated ether also yields trimethyl gallic acid, when boiled with 50% potash. These experiments prove the existence of hydroxyl groups in 3′, 4′ and 5′ positions. Hence cannabiscetin may be represented as 3 : 5 : 8 : 3′ : 4′ : 5′-hexahydroxy flavone.



Further details regarding the properties of cannabiscitrin and the position of the sugar group will be published later.

Experimental

Fraction 1—

Isolation of Cannabiscitrin.—Dry petals of the flowers of *Hibiscus cannabinus* (2 kg.) were extracted twice with methylated spirit, each extraction lasting for about 12 hours. The total alcoholic extract was concentrated to about 500 c.c. On leaving overnight a good amount of resin and wax separated out from the concentrate. They were removed by filtration through fluted filters. When the clear filtrate was allowed to stand for about 10 days, it deposited a large quantity of a yellow crystalline solid. It was filtered, and washed well with water till it assumed a pale yellow colour. It was then dissolved in pyridine and the solution diluted with water till the

impurities just began to separate out in a colloidal form. They were precipitated by adding a few drops of calcium chloride solution and then filtered. The clear filtrate which did not separate out any more impurities on further dilution, was concentrated on a water-bath till solid began to appear on the surface of the liquid. On leaving overnight, a good amount of a deep yellow crystalline substance separated. It was recrystallised from dilute pyridine, when it came down as rods and plates (25 g.). It crystallised from dilute acetic acid as clusters of short yellow needles. On heating, it sintered at 210° fused at 220°, and decomposed at 245°. [Found in air-dried sample: C, 46·7; H, 5·1; and loss on drying 10·0; $C_{21}H_{20}O_{13}$, 3 H_2O requires C, 47·2; H, 4·9; and loss (H_2O) on drying 10·1%. [Found in the sample dried *in vacuo* at 110°: C, 51·9 ; H, 4·3 ; $C_{21}H_{20}O_{13}$ requires C, 52·5; H, 4·2%.]

The glucoside was found to be easily soluble in alcohol, dilute and glacial acetic acids, and pyridine. When an aqueous alcoholic solution of the substance was treated with a drop of alkali, an olive-green colour was obtained; on adding more alkali, a pure yellow solution was produced. An alcoholic solution gave a reddish orange precipitate with neutral lead acetate, and with ferric chloride an olive-green colour which faded rapidly to dark-brown.

On boiling with acetic anhydride and anhydrous sodium acetate, the pigment yielded the nonaacetate. It crystallised from glacial acetic acid (animal charcoal) as fine colourless needles. It was sparingly soluble in alcohol and even dilute acetic acid. A mixture of acetic anhydride and alcohol was found to be a convenient solvent for crystallisation. On heating it melted into a glassy mass at 200–02° and did not flow down even at 260°. [Found: C, 54·3; H, 4·4; $C_{21}H_{11}O_4(OCOCH_3)_9$ requires C, 54·5; H, 4·4%.]

Hydrolysis of the Glucoside : *Preparation of Cannabiscetin.*—The glucoside (2 g.) was hydrolysed by boiling with 7% sulphuric acid (50 c.c.). The solid went into solution on boiling for some time and within half an hour, the aglucone separated out giving rise to bumping. However the boiling was continued carefully over a mild flame for two hours. After cooling, the solid was filtered and crystallised from glacial acetic acid. It did not melt below 350°. When allowed to crystallise slowly, it came out as large yellow rectangular plates and prisms, but on rapid crystallisation it was obtained as flat needles and narrow plates. The yield was 1·2 g. [Found in air-dried specimen : C, 53·7; H, 3·8; loss on drying 5·2%; $C_{15}H_{10}O_8$, H_2O requires C, 53·6; H, 3·6 and loss on drying (H_2O) 5·4%. Found in the sample dried at 110°: C, 56·4; H, 3·5; $C_{15}H_{10}O_8$ requires C, 56·6, H 3·1%.]

From the acid filtrate left after the removal of the aglucone, glucose was isolated in the form of its osazone by neutralising it with barium carbonate, concentrating it on a water-bath and subsequently treating it with phenyl hydrazine.

Fraction II—

Cannabiscetin.—The alcoholic mother-liquor remaining after the separation of the glucoside was concentrated to half its bulk and cooled. As no precipitate was thereby obtained, the solution was considerably diluted with water and the alcohol was removed almost completely on a water-bath. The resin that separated out during the course of this operation was carefully removed by decanting the liquid into a fluted filter. The clear filtrate was further concentrated and cooled. As no precipitate was obtained from this liquor even after several days, it was repeatedly extracted with ether. On distilling off the ether, a yellow crystalline solid was obtained (1 g.). It was purified by crystallisation from dilute alcohol, when it appeared in the form of plates and prisms. The substance did not melt below 350°. It was identical in all its properties with cannabiscetin obtained from the new glucoside as already described. The identity was confirmed by preparing their acetyl derivatives and taking their mixed melting point.

Neutral Lead Acetate Fraction.—The aqueous solution left after extraction with ether, did not deposit any solid on standing even for 15 days. It was then treated with neutral lead acetate solution, when an orange-red precipitate was obtained. From this lead salt the pigment was liberated by passing hydrogen sulphide repeatedly into its suspension in water. The precipitated lead sulphide was filtered off and the filtrate concentrated on a water-bath. But no solid separated out even after several days. Hence the calculated amount of concentrated sulphuric acid was added to make the solution 7% in the acid content and then boiled for two hours in order to hydrolyse any glucosides that might have been present. By this treatment a yellow crystalline substance was obtained (1 g.). It was identified as cannabiscetin by comparison of the pigment and its acetyl derivative with authentic samples.

Properties of Cannabiscetin.—The pigment was sparingly soluble in alcohol but it freely dissolved in glacial acetic acid and pyridine. It produced a deep red precipitate with lead acetate and a dark-brown colour with ferric chloride. It dissolved in very dilute alkali, producing at first a yellow solution which rapidly changed to green and then blue, gradually to olive-green and finally to light-brown.

Colour Reactions of the Aglucone and its Glucoside with Alkaline Buffer Solutions.—Both the aglucone and the glucoside showed prominent colour

changes with alkaline buffer solutions of only high pH value; the following table summarises the changes :

pH	Aglucone	Glucoside
8·0	Slowly dissolved to form a pale yellow solution which changed to yellowish brown after 24 hours.	Dissolved slowly to a pale yellow solution which became yellowish red after 24 hours.
8·6	The dissolution was more quick and the yellow solution acquired an orange tinge within an hour and became yellowish brown after 24 hours.	Same changes as above but more rapid.
9·2	Quickly dissolved, yielding a yellow solution, with a green tinge. The colour rapidly changed to yellowish green, then yellowish brown and to pure brown within 10 minutes. The brown became orange-red which was stable for an hour. The next day, it was still brown.	Same changes as above ; but the final colour was orange-yellow (after 24 hours).
9·8	The initial yellow solution quickly turned to green and became within 5 minutes brown through dirty brown. It became orange-red within 10 minutes and remained so for an hour. After 24 hours it was brown.	Dissolved very quickly to produce a clear yellow solution which became orange after 24 hours.
10·4	Quick succession of changes :—yellow solution—bright emerald green—greenish-brown—brown—reddish brown —bright crimson (within 5 minutes). The crimson colour was stable for 1 hour and faded to brown the next day.	Quick succession of changes :—yellow solution —yellowish green—again yellow. The solution slowly acquired an orange tinge and after 24 hours became definitely orange with a strong green fluorescence.
11·0	Same changes as above but more quick, the crimson appearing even within 2 minutes. Subsequent behaviour was also just the same.	Rapid changes :—Yellow solution—greenish yellow—orange. It became brown after 24 hours.
11·6	The yellow solution changed rapidly to purple which gradually became reddish purple within an hour. It became brown as usual after 24 hours.	The yellow solution rapidly changed to green and became almost colourless within 10 minutes. After 24 hours it was brown.
12·2	Same changes as above ; but the initial purple was mixed up with violet.	Same changes as above.
12·8	Very quick changes :—yellow solution —emerald green—deep pure blue—purple—brown (within 5 minutes). The last colour faded quickly to pale yellowish-brown which was stable for 1 hour, and became dirty brown after 24 hours.	Same changes as above ; but the tinge after 24 hours was green-brown.
13·4	Same changes as above ; but the purple was less prominent.	Same changes as above, the green-brown giving place to faint brown.

Preparation of Hexaacetyl Cannabiscetin.—The flavonol was acetylated by boiling with acetic anhydride and anhydrous sodium acetate. The acetyl

derivative was sparingly soluble in alcohol and freely in acetic anhydride. When crystallised from a mixture of acetic anhydride and alcohol, it came out as fine colourless needles sintering at 210° and melting at 214–15°. One more crystallisation from glacial acetic acid raised the melting point to 215–17°. [Found: C, 56·6; H, 3·9; $C_{15}H_4O_2$ $(OCO\ CH_3)_6$ requires C, 56·8; H, 3·9%.]

Preparation of Hexamethyl Cannabiscetin.—(*a*) The flavonol (1·5 g.) dissolved in methyl alcohol (30 c.c.) was treated with excess of methyl iodide (15 g.) and refluxed on a water-bath. After sometime, methyl alcoholic potash (7 g. of potash in 100 c.c. of methyl alcohol) was slowly added over a period of six hours. The solvent and the excess of the reagent were then distilled off, and water was added to the residue. After cooling, the alkaline liquid was just made acidic, when the ether separated out gradually as a sticky mass. It was purified through repeated crystallisations from alcohol using a little animal charcoal, and was obtained as colourless needles having the appearance of cotton wool.

(*b*) The pigment was also methylated through its acetyl derivative according to the method of Rao and Seshadri.[3] Acetyl cannabiscetin (1 g.) was dissolved in acetone (50 c.c.) and treated with dimethyl sulphate (10 c.c.) and 20% sodium hydroxide (10 c.c.) alternately in small quantities, shaking vigorously after each addition. Finally the mixture was made strongly alkaline by the addition of 5 c.c. more of the alkali. The next day it was refluxed on a water-bath for an hour. The solvent was then driven off and the alkaline solution acidified with dilute hydrochloric acid, when the ether separated out. The solid was filtered and crystallised from dilute alcohol, using a little animal charcoal. The yield was 0·6 g.

The samples as obtained from the above two experiments were found to be identical and melted at 175–76°. [Found in the sample dried at 110°: C, 62·3; H, 5·4; OCH_3 46·1%; $C_{15}H_4O_2(OCH_3)_6$ requires C, 62·7; H, 5·5; OCH_3 46·3%.]

Gossypetone Reaction with the Flavonol.—The substance (0·5 g.) was dissolved in absolute alcohol (8 c.c.) and *p*-benzoquinone (0·2 g.) dissolved in a small amount of alcohol was added to the solution. A dull red crystalline substance began to separate out gradually. When the separation was complete, the solid was filtered and washed with a small quantity of absolute alcohol. [Found: C, 56·8; H, 2·4; $C_{15}H_8O_8$ requires C, 57·0; H, 2·5%.] It had the appearance of red phosphorus. When treated with a drop of dilute alkali, it dissolved producing a blue solution which became red on acidification. On reduction with sulphurous acid, it gave back the original pigment.

Oxidation of the Flavonol: Isolation of Trimethyl Gallic Acid.—The flavonol (1 g.) was dissolved in 50% potassium hydroxide (15 c.c.) and left exposed to air for 24 hours with occasional stirring. It was then diluted until the strength of the alkali solution was about 20% and then treated with excess of dimethyl sulphate (15 c.c.) in small quantities. After some time, the excess of the methylating agent was decomposed by heating on the water-bath and the solution acidified with hydrochloric acid. The mixture looked brown and contained a good amount of resin. It was extracted with ether twice and the extract after evaporation left behind a brown viscous liquid which, on leaving in a refrigerator overnight, deposited a colourless crystalline solid. But the substance was still mixed up with a brown viscous resin, and the separation of the two by the ordinary methods of crystallisation was unsuccessful. The mixture was, therefore, pressed on a porous plate, when all the brown viscous liquid was absorbed by the plate, leaving free the colourless crystalline solid. It was scraped out and crystallised from water using a little animal charcoal. It was obtained as fine needles melting at 168–69°, and was found to be identical with the trimethyl ether of gallic acid.

Alkaline Hydrolysis of Hexamethyl Cannabiscetin.—The methyl ether (0·5 g.) was refluxed for 6 hours with 50% potash (20 c.c.) in a silver flask. At the end of this period, a major part of the solid was decomposed. The clear alkaline solution was diluted and then acidified with hydrochloric acid. On extraction with ether, the solution yielded a small amount of trimethyl gallic acid melting at 167–68°. The identification was confirmed by taking the mixed melting point with an authentic sample.

Summary

The colouring matter of the flower petals of *Hibiscus cannabinus* consists mostly of the glucoside cannabiscitrin along with small quantities of the corresponding aglucone cannabiscetin. The properties and reactions of these two substances are described along with those of some of their derivatives.

Cannabiscetin forms a hexaacetate and a hexamethyl ether. It is a flavonol exhibiting similarities with gossypetin and herbacetin particularly in regard to alkali colour reactions and gossypetone reaction. It yields gallic acid on being subjected to alkali fission, its methyl ether producing trimethyl gallic acid. It is, therefore, concluded to be 3 : 5 : 8 : 3′ : 4′ : 5′ hexahydroxy flavone.

REFERENCES

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