ISOLATION OF HIBISCITRIN FROM THE FLOWERS OF *HIBISCUS SABDARIFFA*: CONSTITUTION OF HIBISCETIN

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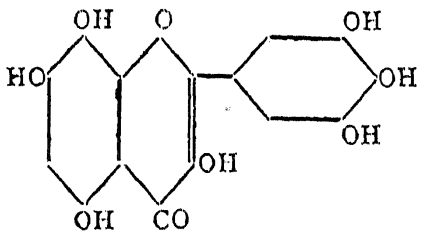
Received November 7, 1941

HIBISCUS SABDARIFFA like the *cannabinus*, is a small plant which grows to a height of about three feet. It is largely cultivated all over India as a food plant and also for the sake of its valuable fibre. The stem, branches, ribs and the calyces are red in colour, while the flower petals are pale yellow. But when once the flowers are plucked from the plant, the petals rapidly acquire a red colour. The petals of the *cannabinus*, on the other hand, do not exhibit this characteristic property, and hence it offers a method of distinguishing between the materials obtained from these closely allied species.

The flowers of *H. sabdariffa* were originally examined by Perkin[1] who employed the entire flowers consisting of the stalk, epicalyx, calyx and corolla. He isolated gossypetin and a new yellow compound named " Hibiscetin " as the result of hydrolysis and subsequent ether extraction of the aqueous extract. Regarding the new substance which was obtained in a small yield the data recorded were its melting point and that of its acetyl derivative and no analytical or other details were given. Following the examination of the flowers of *H. cannabinus*,[2] the study of the flowers of the *sabdariffa* has now been taken up in order to compare the closely related species. The general procedure adopted is the same as that described in some of our past publications. When the alcoholic extract is concentrated and allowed to stand, a yellow crystalline solid separates out gradually. It is a new glycoside melting at 238–40° (decomp.). The yellow crystalline aglycone obtained from it by hydrolysis melts at about 350° with decomposition. Since the melting point of the new flavonol and that of its acetyl derivative agree with those given by Perkin for Hibiscetin and its acetyl derivative, it may reasonably be expected that our compound is identical with that of Perkin, and we, therefore, propose to retain the name Hibiscetin. Consequently the glycoside is designated as Hibiscitrin.

Hibiscetin has the formula $C_{15}H_{10}O_9$, undergoes easy decomposition in 50% potash in the cold, and gives a red precipitate with lead acetate. It is, therefore, a hexahydroxy flavonol. On boiling with acetic anhydride and

anhydrous sodium acetate, it produces a heptaacetyl derivative melting at 242–44,° and the latter, when treated with dimethyl sulphate and alkali according to the method of Rao and Seshadri,[4] yields a heptamethyl ether melting at 194–96°. It readily undergoes oxidation with *p*-benzoquinone to give the " gossypetone " reaction, and hence it should contain two hydroxyl groups in 5 and 8 positions. By the decomposition of the heptamethyl ether with boiling 50% potash, trimethyl gallic acid is obtained ; thus the presence of three hydroxyl groups in 3′, 4′ and 5′ positions of the side phenyl nucleus is established. In its colour reactions with buffer solutions and in other properties, the new flavonol resembles gossypetin and occurs along with it in the flowers in the form of the glycosides. Consequently the benzopyrone part of hibiscetin may be expected to be just the same as that of gossypetin, and hence its constitution is given as 3:5:7:8:3′:4′: 5′-heptahydroxy flavone.



Hibiscetin

Hibiscetin is, therefore, the more highly hydroxylated member in the herbacetin and gossypetin series. A comparison of the melting points of the three flavonols and their derivatives (see table below) support the above conclusion.

Name	Formula	M.P. of the flavonol	M.P. of the acetyl derivative	M.P. of the methyl ether
Herbacetin	$C_{15}H_{10}O_7$	281–83°	192–93°	157–58°
Gossypetin	$C_{15}H_{10}O_8$	310–12°	228–30°	170–72°
Hibiscetin	$C_{15}H_{10}O_9$	About 350°	242–44°	194–96°

Confirmation of the above constitution of the new flavonol is being sought by synthesis. A complete description of the chemical composition of the flowers will be published later.

Experimental

Isolation of Hibiscitrin.—The dried petals (3 kg.) were extracted with boiling methylated spirits, and the combined alcoholic extract was concentrated to about 800 c.c. After removing the wax and resin that had separated by filtration through fluted filters, the clear concentrate was allowed

to stand. A yellow solid began to appear in about a month, and the deposition seemed to be complete at the end of three months. The solid was filtered and washed well with water till it assumed a lemon-yellow colour. It was, however, not pure, and was sticky to the touch. Therefore a solution of it in a small quantity of pyridine was diluted with water till the impurities began to separate in a colloidal form. They were completely precipitated by the addition of a little calcium chloride, and filtered. On concentrating the clear filtrate to a small bulk a yellow crystalline solid was obtained. One more crystallisation from alcohol rendered the substance pure and it appeared as yellow rectangular plates. On heating, it sintered at about 225°, became a semi-fluid a few degrees higher and decomposed at 238–40°. The yield was 6 g. (Found in the air-dried sample : C, 47·5, H, 4·7; loss on drying at 150° *in vacuo* for 5 hours : 3·0%. $C_{27}H_{30}O_{19}$, H_2O requires C, 47·9, H, 4·7; loss on drying (H_2O), 2·7%. Found in the sample dried at 150° *in vacuo* : C, 48·8, H. 4·2 ; $C_{27}H_{30}O_{19}$ requires C, 49·2, H, 4·6%.)

Preparation of Hibiscetin.—The glycoside (2 g.) was boiled under reflux with 100 c.c. of 7% sulphuric acid. After about 5 minutes the solid completely dissolved forming a clear solution, and another yellow crystalline solid began to separate out within 15 minutes. As there was severe bumping, the boiling had to be carried out over a low flame for the rest of the period (2 hours) to complete the hydrolysis. After cooling, the aglycone was filtered and washed with a little water. It was a deep yellow shining crystalline substance, and was almost pure.

Properties.—It was very sparingly soluble in almost all the organic solvents except pyridine from which it crystallised as glistening rectangular plates melting at about 350° with decomposition. (Found in the air-dried sample : C, 50·8 ; H, 3·8 ; $C_{15}H_{10}O_9$, H_2O requires C, 51·1, H, 3·4%. Found in the specimen dried at 120° *in vacuo*: C, 53·6, H, 3·3 ; $C_{15}H_{10}O_9$ requires C, 53·9; H, 3·0%.) When treated with dilute alkali the substance dissolved immediately producing a red solution which rapidly changed to brown. In alcoholic solution it gave a deep red precipitate with neutral lead acetate, and an olive brown colour with ferric chloride. It dissolved in 50% potash producing a deep red solution which, on being left exposed to air for 24 hours with occasional shaking, became opaque and brown. On acidification, the alkaline solution gave no precipitate.

Colour Changes with Alkaline Buffer Solutions.—With alkaline buffer solutions the flavonol exhibited a beautiful and characteristic display of colours as shown in the table given below, and the colour changes were just similar to those given by gossypetin under the same conditions.

pH	Hibiscetin
8·0	Dissolved slowly to form a pale yellow solution which acquired a violet tinge in about 15 minutes. In half an hour the colour was pale violet. The dissolution was only partial and not complete.
8·6	The dissolution was more rapid, and the initial yellow colour changed to dirty green in 3 minutes, to bluish green in 5 minutes and to pure, though not bright blue within a minute more. The final colour which was stable for about 45 minutes began to fade subsequently and was only half the original intensity after an hour. After 2 hours the solution was almost colourless, and the next day it assumed a pale yellow tinge.
9·2	Same changes as above but more rapid ; Yellow solution → greenish yellow (1 min.) → blue (2 min.). The final colour was stable for about half an hour and began to fade as in the former case. After 24 hours it was pale yellow.
9·8	Same changes as above but more quick. The blue colour appeared even within a minute.
10·4	Quick succession of changes: deep yellow → green (15 sec.) → deep blue (30 sec.) → rapid fading → pale yellow (24 hours).
11·0	Same but more rapid changes, the deep blue being observed even within 15 seconds. Within another half a minute the blue began to fade and became pale bluish green in about 2 minutes. It further faded away rapidly, and became almost colourless after 10 minutes. The next day the solution was pale yellow.
11·6	Same changes as above. The disappearance of the final deep blue (10 seconds) was almost complete in about 5 minutes. The colour after 24 hours was pale yellow.
12·2	Immediately dissolved to produce a green solution, and the initial yellow could not be noticed. On shaking the green changed to blue in a moment. The colour began to fade away even within 15 seconds, and almost completely disappeared in a minute. The next day it was brownish yellow.
12·8	Quick succession of changes:— Green → blue → almost colourless (½ min.) → pale brown (2 min.) → brownish yellow (24 hours).

Gossypetone Reaction with the Flavonol.—On account of the sparing solubility of the flavonol in absolute alcohol, the gossypetone reaction had to be carried out with a solution of the substance in a mixture of alcohol and pyridine. A suspension of it (0·25 g.) in absolute alcohol (20 c.c.) was treated with a few drops of pyridine till a clear solution was obtained, and was then mixed with a solution of *p*-benzoquinone in absolute alcohol (0·1 g. in 20 c.c.). The mixture immediately assumed a dull red colour, and within 5 minutes a chocolate red precipitate began to separate out. After half an hour the solid was filtered and washed with a little more absolute alcohol. It was sparingly soluble in water and alcohol, and did not melt below 350°. (Found: C, 54·0, H, 2·6; $C_{15}H_8O_9$ requires C, 54·2, H, 2·4%.) With dilute alkali it produced a brown red solution which changed to reddish yellow on acidification with hydrochloric acid. When a suspension of the substance in water was treated with sulphur dioxide, the original flavonol was generated.

Acetylation of Hibiscetin: Preparation of Heptaacetyl Derivative.—The substance was acetylated by boiling with acetic anhydride and anhydrous sodium acetate for 4 hours. On crystallisation from glacial acetic acid using a little animal charcoal, the acetyl derivative was obtained as colourless narrow rectangular plates. It became a glassy mass at 234–35° and flowed down into a liquid at 242–44°. Mixed melting points with acetyl cannabiscetin and acetyl gossypetin were depressed. (Found: C, 55·7, H, 3·9; $C_{15}H_3O_2(OCOCH_3)_7$ requires C, 55·4; H, 3·8%.)

Methylation of Hibiscetin: Preparation of the Heptamethyl Ether.—Acetyl hibiscetin (1 g.) was dissolved in acetone (50 c.c.) and treated alternately with dimethyl sulphate (10 c.c.) and 20% sodium hydroxide (10 c.c.) in small quantities. There was a gradual rise in temperature throughout the process, and occasionally the contents had to be cooled. Further quantities of dimethyl sulphate (5 c.c.) and the alkali (5 c.c.) were then added and the medium was finally made definitely alkaline by the gradual addition of more alkali (10 c.c.). After leaving overnight, the mixture was boiled under reflux for an hour, and the solvent was then driven off completely. On diluting the solution and adding acid, a pale brown solid was precipitated. It was treated with a small amount of ether to remove the adhering impurities and was then crystallised from alcohol, when it came out as colourless needles and narrow rectangular plates, melting at 194–96°. (Found: OCH_3, 45·8; $C_{15}H_3O_2(OCH_3)_7$, $2H_2O$ requires OCH_3, 46·4%.)

Alkaline Oxidation of the Heptamethyl Ether: Isolation of Trimethyl Gallic Acid.—Heptamethyl hibiscetin (1 g.) was boiled under reflux with 50% alkali (30 c.c.) for six hours in a silver flask. The decomposition mixture was diluted to about 150 c.c. and the clear alkaline solution was acidified with concentrated hydrochloric acid. On ether-extraction an almost colourless crystalline acid was obtained. It was purified by dissolution in sodium bicarbonate, subsequent reprecipitation with hydrochloric acid and final recrystallisation from dilute alcohol. It was thus obtained as colourless long rectangular plates melting at 168–70°, and was found to be identical with trimethyl gallic acid.

Summary

A new flavonol glycoside has been isolated from the flower petals of *Hibiscus sabdariffa*, and has been named Hibiscitrin. Its aglycone, Hibiscetin is a hexahydroxy flavonol forming a heptaacetyl derivative on acetylation. When decomposed with boiling 50% alkali, its heptamethyl ether produces trimethyl gallic acid, indicating thereby the existence of hydroxyl groups in 3′, 4′ and 5′ positions in hibiscetin. On oxidation with *p*-benzoquinone,

the pigment yields the corresponding quinone, and hence it should contain two hydroxyl groups in positions 5 and 8. It resembles gossypetin in many of its properties especially the colour changes with alkaline buffer solutions, and also occurs along with it in the flowers. It is, therefore, expected that the benzopyrone part of hibiscetin would be just the same as in gossypetin, and hence it is assigned the structure of 3 : 5 :7 : 8 : 3′ : 4′ : 5′-heptahydroxy-flavone.

REFERENCES

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