

# PIGMENTS OF THE FLOWERS OF *HIBISCUS SABDARIFFA*

## Isolation of Sabdaretin, a New Hydroxyflavone

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IN a recent publication of ours,<sup>1</sup> the isolation of hibiscitrin, a glycoside of hibiscetin was described, and the constitution of the aglycone was established as 3:5:7:8:3':4':5'-heptahydroxyflavone. This flavonol was originally prepared along with gossypetin by Perkin<sup>2</sup> who employed as his starting material the entire flowers of *Hibiscus sabdariffa*, consisting of stalk, calyx, epicalyx and corolla. The deep red calyx was later examined by Yamamoto and Osina<sup>3</sup> who isolated therefrom an anthocyanin named hibiscin which was shown to be a delphinidin derivative. A careful analysis of the calyx and the associated parts such as the epicalyx has now shown that they contain very little of anthoxanthins. Hence it is clear that the petals alone are responsible for the anthoxanthin pigments. Consequently we have now made a detailed examination of a pure sample of the flower petals, and the results are presented in this paper.

The procedure followed runs practically on the same lines as described in our previous publications relating to cotton and other flowers. When an alcoholic extract of the petals is concentrated and allowed to stand for a long time, hibiscitrin gradually separates out (Fraction I). After the distillation of the alcohol from the mother-liquor, the material is taken up with water. Ether-extraction of this solution gives no crystalline product. But the aqueous solution deposits a solid which is collected in two fractions (II and III) and these are shown to be hibiscitrin and gossypitrin respectively. The neutral lead acetate precipitate yields a new compound called sabdaritrin, while the basic lead acetate fraction is too small to be studied.

Sabdaritrin has the formula  $C_{21}H_{20}O_{14} \cdot 3H_2O$  and melts at 251–53° (decomp.). On boiling with dilute sulphuric acid, it yields a yellow crystalline substance named sabdaretin. The latter has the formula  $C_{15}H_{10}O_9$ ,

$3\text{H}_2\text{O}$  and does not melt below  $350^\circ$ . It gives prominent colour changes with alkaline buffer solutions, a red colour when reduced with magnesium and hydrochloric acid, and a red precipitate with lead acetate, and so it is considered to be most probably a flavonol. On acetylation, it produces an acetyl derivative melting at  $198\text{--}200^\circ$ .

Besides the analytical results, sabdaritrin differs markedly in many of its properties from both gossypitrin and hibiscitrin with which it is occurring side by side in the flowers. The two latter have several points in common. They dissolve in dilute alkali to form green solutions which change to blue in a moment and further to greenish blue on shaking with air, whereas sabdaritrin forms first a yellow solution which rapidly changes to purple red and fades to reddish brown in a few minutes. With alkaline buffer solutions, gossypitrin and hibiscitrin give emerald green as the characteristic coloration whereas the new compound does not produce any green but yields crimson as the prominent colour. Hibiscetin and gossypetin similarly differ from sabdaretin. The former two produce green and blue as the prominent colours with alkaline buffer solutions; on the other hand, sabdaretin gives only a green and no blue colour. It may also be noted that hibiscitrin, gossypitrin and their aglycones are sensitive to alkaline buffer solutions of even the lower range (10.4 and 11.0) whereas sabdaritrin and sabdaretin respond only at the higher range (12.8 and 13.4).

The flower petals of *Hibiscus sabdariffa* contain, therefore, three definite compounds, hibiscitrin, gossypitrin and sabdaritrin belonging to the anthoxanthin group. This is in sharp contrast to the allied flowers of *Hibiscus cannabinus* from which only cannabiscitrin accompanied by a little of its aglycone could be obtained. A small-scale study of the anthocyanins present in the deep red central spots of the petals of *Hibiscus sabdariffa*, according to the method of Robinson and co-workers, has shown that it is a delphinidin derivative resembling hibiscin present in the calyx.

A more detailed study of sabdaritrin and sabdaretin could not be made due to lack of material and it will be undertaken as early as possible.

### *Experimental*

#### *Fraction I. Hibiscitrin:*

The dried petals were extracted with boiling methylated spirits, and the combined alcoholic extract was concentrated to small bulk. After removing the wax and resin that had separated out, 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 small bulk, hibiscitrin crystallised out. After one more crystallisation from alcohol it appeared as yellow rectangular plates. On heating, it sintered at about 225°, became a semifluid a few degrees higher and decomposed at 238–40°. A yield of 6 g. was obtained from 1 kg. only and not from 3 kg. of the petals as mentioned in our former publication.<sup>1</sup>

*Fractions II and III. Hibiscitrin and Gossypitrin:*

The alcoholic mother-liquor left after the removal of Fraction I was diluted with a large volume of water, and the alcohol was removed almost completely by heating on a water-bath. After filtering off the resins that had separated out and concentrating to small bulk, the clear solution was extracted with ether and allowed to stand. The ether extract did not yield any pigment, but from the aqueous solution a yellow substance crystallised out within two or three days. After one crystallisation from alcohol, this substance also sintered at about 225°, became semifluid a few degrees higher and decomposed at 238–40°. It was therefore identified as hibiscitrin and this was confirmed by hydrolysis when it yielded an aglycone which melted at about 350° (decomp.) and gave an acetyl derivative identical with acetyl hibiscetin melting at 242–44°. After fifteen days some more pigment separated out from the aqueous solution. It was filtered, washed well with water and crystallised from glacial acetic acid, when it appeared as yellow needles. It was different from the glycoside, hibiscitrin, since it melted at 250–52° (decomp.) and produced an acetyl derivative melting at 242–44°. It was identified as gossypitrin from a study of its colour reactions with buffer solutions and a comparison of its acetyl derivative with acetyl gossypitrin. In this fraction hibiscitrin and gossypitrin were isolated in an yield of 2 g. and 1 g. respectively from 1 kg. of the petals.

*Neutral Lead Acetate Fraction—Sabdaritrin:*

The mother-liquor left after removing Fractions II and III, was treated with lead acetate solution when a brownish red precipitate was formed. It was filtered, suspended in water and decomposed in the usual way with hydrogen sulphide. The aqueous solution thereby obtained was concentrated and left aside. After nearly four months, some yellowish brown solid was deposited. It was filtered and purified by crystallisation from

aqueous pyridine. It appeared as yellow rectangular plates swelling and decomposing at  $251-53^{\circ}$  with sintering a few degrees earlier. (Found in the air-dried sample: C, 45.3; H, 4.8;  $C_{21}H_{29}O_{14}$ ,  $3H_2O$  requires: C, 45.8; H, 4.7%.) It was sparingly soluble in water, alcohol and even acetic acid but it freely dissolved in pyridine. When treated with ferric chloride, its alcoholic solution gave a brownish green colour, and with sodium hydroxide, it immediately assumed a yellow colour which later changed to brownish red, then red, then scarlet red and finally purple red. After five minutes the colour was reddish brown.

*Sabdaretin* :

(a) *Preparation*.—The new pigment, sabdaritrin (0.5 g.) was boiled under reflux with 7% sulphuric acid (50 c.c.). During the course of half an hour the substance went completely into solution, and no fresh solid separated out even after one hour. After boiling for two hours, the solution was filtered hot in order to remove some extraneous impurities and cooled when sabdaretin crystallised out as shining golden yellow needles. It was subsequently recrystallised from boiling water. (Found in the air-dried sample: C, 45.8; H, 3.5;  $C_{15}H_{10}O_9$ ,  $3H_2O$  requires C, 46.4; H, 4.1%.)

(b) *Properties*.—This substance, unlike sabdaritrin, was freely soluble in boiling water and could be conveniently crystallised from it. It was easily soluble in alcohol, acetic acid and also pyridine. When its aqueous or alcoholic solution was treated with a drop of alkali, immediately a brownish red colour developed. With ferric chloride a brownish red colour and with lead acetate a deep red precipitate were produced. On heating, the substance shrank and became dark at about  $300^{\circ}$  but did not melt even at  $360^{\circ}$ . When boiled with acetic anhydride and anhydrous sodium acetate, it formed the acetyl derivative which could not, however, be easily crystallised. On boiling with the ordinary solvents like alcohol and acetic acid, it seemed to undergo some decomposition and assumed a sticky resinous form. It was, therefore, dissolved in a small amount of acetic anhydride and the clear solution was treated with some absolute alcohol. From this mixture the substance crystallised during the course of two days as colourless long narrow rectangular plates which sintered at about  $160^{\circ}$  and melted at  $198-200^{\circ}$  (decomp.).

*Colour Reactions of Sabdaritrin and Sabdaretin with Alkaline Buffer Solutions*

pH	Sabdaritrin	Sabdaretin
8·6	Slowly dissolved to form pale yellow solution	Readily dissolved to give deep golden yellow solution, which became brown in 3 minutes and faded gradually within 15 minutes
9·2	More ready dissolution, and the solution was deep yellow	Same changes as above, the brown appearing within a minute
10·4	Same behaviour as the above	Deep golden yellow solution with a brownish tinge ( $\frac{1}{2}$ minute)
11·0	Do.	Immediate yellow solution which became yellowish brown on shaking
11·6	Do.	The yellow was visible only for a moment and then changed to brown
12·2	Deep yellow solution which became crimson red within 3 minutes	Quick succession of changes :— Yellow solution, yellowish brown (shaking), dull green ( $\frac{1}{2}$ min.), emerald green (1 min.), brownish yellow ( $1\frac{1}{2}$ min.), yellowish brown (2 min.)
12·8	Yellow solution, brownish yellow ( $\frac{1}{2}$ min.), brownish red (1 min.), crimson red (2 min.)	Same changes as above but very rapid :— Golden yellow, brown, green ( $\frac{1}{2}$ min.), brown (1 min.). The green and brown were alternating for some time on shaking with air. The solution became completely brown in an hour
13·4	Same changes as the above but more quickly	Same changes as the above but more rapidly, the green following the yellow almost immediately

After 24 hours the colour was yellowish brown in the case of sabdaritrin and light brown with sabdaretin solutions.

### Summary

The flower petals of *Hibiscus sabdariffa* contain hibiscitrin as the main component. Gossypitrin is present to a smaller extent. Besides these two, a small amount of a new compound named sabdaritrin has also been isolated. On boiling with dilute sulphuric acid it yields a new hydroxyflavone called sabdaretin.

### REFERENCES

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