CHEMICAL COMPONENTS OF INDIAN TULIP (*THESPASIA POPULNEA*) FLOWERS

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Thespasia populnea, the Indian Tulip or the Portia tree is a fair-sized ever-green tree which flowers throughout the year. The flowers are highly showy and attractive. They are sulphur-yellow in colour with a purple centre and are nearly three inches in diameter.

These flowers have been examined in these laboratories from time to time according to the general procedure adopted for the study of flower-pigments. The dried petals are extracted with alcohol, and the extract concentrated. The pigment that separates out from this alcoholic concentrate is designated as " alcoholic fraction ". After the removal of this fraction, the concentrate is diluted with a large volume of water and again concentrated to a small bulk. Pigment separating out from this aqueous liquor is called " aqueous fraction ". The mother-liquor left is first extracted with ether and the extract evaporated to yield the " ether fraction ". It is then treated with neutral lead acetate and subsequently with basic lead acetate, and the substances produced by the decomposition of these two lead salts are called " the neutral " and " the basic lead acetate fractions " respectively.

A preliminary study of the dried petals of *T. populnea* obtained in October 1933 from Coimbatore was made by Neelakantam and Seshadri.[1] Both the alcoholic and the aqueous concentrates did not yield any pigment. Neutral lead acetate gave only a small amount of a red precipitate, while the basic lead acetate produced a bulky orange-yellow solid. From the last fraction were isolated three compounds : (1) a small quantity of a yellow glycosidic substance which melted at 228–30° (decomp.), (2) a fairly good amount of a non-glycosidic pigment melting at 270–75°, and (3) a very small quantity of a third pigment which was present in the neutral lead acetate fraction also and which could be obtained pure only in the form of its acetyl derivative melting at 182–85°. Substance (1) on hydrolysis with sulphuric acid produced glucose and an aglucone which was found to be

identical with substance (2). The glucoside and its aglucone were named Populnin and Populnetin respectively.

Another sample of the flowers procured from the Trichinopoly district during the summer of 1936 was examined by the same authors[1] and these were shown to contain only the glucoside, populnin which was isolated from the basic lead acetate fraction.

A third sample of the flowers secured from the same place in summer 1939 was studied by Rao and Reddi.[2] This time the pigment was isolated in the form of the aglucone. Since the alcoholic and the aqueous concentrates did not yield any compound, the aqueous solution was boiled with acid and extracted with ether. The aglucone obtained from the ether extract was found to be entirely different from populnetin and was identified as the tetrahydroxy flavonol, herbacetin. It was also shown that the acetyl derivative of the third pigment (substance 3) prepared by Neelakantam and Seshadri from the 1933 sample of flowers was identical with herbacetin acetate.

It is, therefore, clear that populnin, populnetin and herbacetin occur in the flowers of *Thespasia populnea*, but their relative proportions vary considerably depending upon the season. Besides these three yellow pigments, a new colourless compound designated "Populneol" is also present in appreciable amounts.

A large sample of the flowers collected in January 1940 from Trichinopoly has been found to contain all the four entities, and the results of a close and careful examination of this sample are described in detail in this paper. No pigment separated from the alcoholic concentrate. From the aqueous fraction was obtained mainly populnin; and the ether extract of the aqueous mother-liquor gave populneol. From the neutral lead acetate fraction a small quantity of herbacetin was isolated while the basic lead acetate fraction yielded some amount of populnetin.

There was some difficulty in understanding the composition and chemical nature of populnetin and populnin. This was due partly to the accompanying impurities and mainly to the capacity of populnetin and its derivatives to hold water of crystallisation rather tenaciously. The original suggestion of Neelakantam and Seshadri that they are anthraquinone derivatives[1] is not correct. It is now definite that populnetin is a tetrahydroxy flavone and populnin is its monoglucoside.

Populnetin has the formula $C_{15}H_{10}O_6$. With ferric chloride it gives a pale green colour and with basic lead acetate a yellow precipitate. It dissolves in concentrated sulphuric acid with a characteristic green fluorescence.

When reduced with magnesium and hydrochloric acid, it yields a brownish red solution. If, however, the reduction is effected with sodium amalgam and alcohol, and the solution acidified, a deep red colour results. When the Wilson boric-citric acid test[3] is carried out with an acetone solution of the substance, it is positive for the presence of a hydroxyl group in the 5th position. With alkaline buffer solutions it does not exhibit any prominent colour changes. It is, therefore, taken to be a hydroxy flavone. On acetylation it produces a tetra acetyl derivative which like the acetate of kæmpferol exhibits dual melting point; it undergoes vigorous dehydration at about 130° and finally melts at 242–44°.

Populnin resembles its aglucone very closely in its reactions. Ferric chloride imparts a dull green colour to an alcoholic solution of the substance and basic lead acetate produces a yellow precipitate. Like populnetin the glucoside also gives a green fluorescence in concentrated sulphuric acid solutions. With alkaline buffer solutions it does not exhibit any display of colours.

Populneol is a colourless crystalline substance melting at 116–18° and it has the molecular formula $C_{15}H_{12}O_3$. It is phenolic in nature since it dissolves in aqueous alkali, and gives a brownish violet colour with ferric chloride. It forms a yellow solution in concentrated sulphuric acid without any fluorescence.

The final constitutions of populnetin, populnin and populneol are under investigation.

Experimental

Isolation of Populnin.—The dry petals of the flowers (3 kg.) were extracted with boiling methylated spirit in batches and the combined extract was concentrated to a small bulk. After filtering off through fluted filters the tarry impurities that separated out, the clear concentrate was allowed to stand. As no pigment was deposited from this solution even after a long time, it was diluted with a large volume of water, alcohol was evaporated and the solution concentrated to a small bulk (500 c.c.) on a water-bath. During this operation large amounts of a crisp pink coloured resin separated out and it was removed. From the clear aqueous liquor a yellow solid began to crystallise out after four months and the deposition was almost complete after six months. The solid was filtered and purified by crystallisation first from aqueous pyridine and then from alcohol. It was finally crystallised from dilute acetic acid, when it appeared as short yellow needles melting at 228–30° (decomp.). The pure compound was obtained in an yield of 10 g. (0·3%). (Found: C, 54·4 ; H, 4·6 ; $C_{21}H_{20}O_{11}$, H_2O requires C, 54·1, H·4·7%.)

Isolation and properties of Populneol.—The mother-liquor left after the filtration of populnin was repeatedly extracted with ether. After distilling off the solvent the extract left behind a brownish oily liquid which became a solid on the addition of a little water. It was filtered but was found to be still pasty and brownish. It was, therefore, pressed on a porous plate when almost all the brownish oily impurity was absorbed by the plate, leaving behind a light brown dry solid. When the latter was crystallised from dilute alcohol, colourless rectangular plates melting at 116-18° were obtained. The yield was 5 g. (Found: C, 74·7 ; H, 5·4% ; m.wt. by Rast's method 228, 230; $C_{15}H_{12}O_3$ requires C, 75·0; H, 5·0% and molecular weight, 240.)

The compound was sparingly soluble in water but freely dissolved in alcohol and acetic acid. It was soluble in dilute sodium hydroxide but not in carbonate or bicarbonate solution. With ferric chloride it gave a brownish violet colour, and with concentrated sulphuric acid a yellow solution without any fluorescence.

Isolation of Herbacetin:—The aqueous solution left after the extraction of populneol was treated with excess of neutral lead acetate solution. Immediately a brownish orange precipitate was formed. It was separated, suspended in water and decomposed with hydrogen sulphide in the usual manner. The aqueous solution obtained thereby contained a great deal of impurities and no pigment separated out even after four months. It was, therefore, made 7% acid by the addition of the requisite amount of concentrated sulphuric acid, and boiled under reflux for two hours. During this hydrolysis the resins began to float up and stick to the walls of the flask. After the boiling was over, the clear liquid was carefully decanted and after cooling ether-extracted. A small amount (1 g.) of a yellow pigment was obtained. After recrystallisation from alcohol it melted at 282–85°. It was identified as herbacetin from a study of its colour reactions with alkaline buffer solutions and a comparison of its acetyl derivative with a pure sample of acetyl herbacetin melting at 193–95°.

Isolation of Populnetin : (*a*) *From basic lead acetate fraction.*—The mother-liquor left after the removal of the neutral lead acetate precipitate was treated with basic lead acetate when a bulky yellow precipitate was obtained. It was decomposed and worked up as in the case of the neutral lead acetate fraction. About 2 grams of populnetin melting at 274–76° were obtained.

(*b*) *By the hydrolysis of populnin.*—When the glucoside was boiled with 7% sulphuric acid under reflux it went into solution during the course of half an hour. Within an hour yellow populnetin separated out, but

the boiling was continued for another hour to complete the hydrolysis. After cooling the contents were filtered, and the solid recrystallised from alcohol to obtain a pure sample.

The filtrate was examined for sugar by first neutralising it with barium carbonate, concentrating the neutral solution to a small quantity and finally treating it with phenyl hydrazine in dilute acetic acid solution. Glucosazone was obtained and was identified from its characteristic crystal structure and melting point.

If produced from impure samples of the glucoside, populnetin was always contaminated with herbacetin. The crude pigment could, however, be purified by dissolving in 50% potash, keeping the solution exposed to air for 24 hours and acidifying. By this treatment the flavonol herbacetin was decomposed leaving behind populnetin. Frequently it was still impure due to admixture with resins. It was dissolved in a mixture of acetone and ether, and when petroleum ether (B.P. 60-80°) was added to this solution the impurities separated out first. After sometime, the clear supernatant liquid was carefully decanted and evaporated, when pure populnetin was obtained. On crystallisation from aqueous alcohol it came out as fine needles with a dull yellow colour and melted at 278–80°. [Found : loss on drying at 110° *in vacuo* (H_2O), 6.2%. $C_{15}H_{10}O_6$, $1\frac{1}{2}H_2O$ requires for loss of 1 H_2O 5·8%. Found in the sample dried at 110° *in vacuo*: C, 61·5; H, 3·4; $C_{15}H_{10}O_6$, $\frac{1}{2}H_2O$ requires C, 61·0; H, 3·7%.]

Properties of Populnin and Populnetin.—Populnin and populnetin resembled very closely in many respects. Both of them gave no precipitates with neutral lead acetate but yielded yellow precipitates with basic lead acetate. In both cases ferric chloride imparted a dull green colour to alcoholic solutions of the substances. They dissolved in concentrated sulphuric acid to produce yellow solutions with a characteristic green fluorescence which became more prominent on adding more acid. Neither of them displayed any colour changes with alkaline buffer solutions. They merely dissolved to form yellow solutions. However, they differed slightly in their reactions towards alkalis. Populnin readily dissolved to produce a deep yellow solution, whereas populnetin gave a bright red colour with strong alkali, and it changed to brownish yellow on dilution. If contaminated even with traces of herbacitrin or herbacetin, the yellow solution rapidly assumed a greenish tinge and gradually faded to brownish yellow.

When reduced with magnesium and hydrochloric acid, populnetin gave a brown-red solution. If, however, sodium amalgam was used for the reduction of the substance dissolved in alcohol, and the solution was then acidified, a deep red colour was produced. On acetylation

with acetic anhydride and anhydrous sodium acetate, tetraacetyl populnetin was formed. The acetyl derivative seemed to undergo some decomposition when boiled with either alcohol or acetic acid. It could best be recrystallised by dissolving in a small amount of acetic anhydride, adding an equal amount of absolute alcohol and allowing the solution to stand. After a few days, it appeared as long narrow rectangular plates. It crystallised as a hydrate, and on rapid heating, it lost the water of hydration at about 128–30°. The dehydration was so rapid and vigorous with swelling and shooting up of the material that it could be easily mistaken for more serious decomposition of the substance. If the heating was, however, slow and controlled, the compound merely shrank from 124–134° losing the water of hydration which could be seen condensing in the melting point tube above the shrunken mass, became a brownish glassy solid at a higher temperature and finally melted into a clear liquid at 242–44°. (Found in the air-dried sample : C, 59·3 ; H, 4·2 ; loss (H_2O) on heating 2·0% ; $C_{15}H_6O_2(OCOCH_3)_4$, $\frac{1}{2}H_2O$ requires C, 59.6 ; H, 4·1 ; loss of H_2O on heating 1·9%.)

Summary

A sample of the flower-petals of *Thespasia populnea* is found to contain populnin (0·33%), populnetin (0·07%) and herbacetin (mostly as its glucoside, 0·03%). The proportions vary with different samples depending on seasonal and other factors. Besides these three yellow pigments, a colourless phenolic compound called populneol is also present (0·16%).

Populnetin seems to be a new tetrahydroxy flavone and populnin is its monoglucoside.

REFERENCES

1. Neelakantam and Seshadri *Curr. Sci.*, 1938, 7, 16.
2. Rao and Reddi .. *Proc. Ind. Acad. Sci.*, (A), 1940, 12, 372.
3. Rangaswami and Seshadri .. *Ibid.*, 1942, 16, 129.