

OCCURRENCE OF HERBACETIN IN THE FLOWERS OF THE INDIAN TULIP (*THESPASIA POPULNEA*)

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A FEW years ago¹ the flowers of *Thespasia populnea* were examined in these Laboratories by Neelakantam and Seshadri according to the general method described in connection with the study of the cotton flowers.² They analysed two samples collected in two different seasons and found that the composition of the pigment present in the two was not the same. The flowers secured from Coimbatore in October 1933 yielded as the main portion a non-glycosidic pigment named populnetin ($C_{14}H_8O_6$) melting at $270-75^\circ$ (I), a small amount of its glucoside, populnin ($C_{20}H_{18}O_{11}$) melting at $228-30^\circ$ (II) and a very small quantity of a third pigment which could be isolated only in the form of its acetyl derivative (III) melting at $182-85^\circ$. Another sample procured from Trichinopoly in the summer of 1936 contained populnin only.

During the course of their investigation Neelakantam and Seshadri noticed that the extract of the flowers was giving a small amount of an orange-red precipitate with neutral lead acetate, and that the crude samples of the pigments, before complete purification, showed slight but significant differences from the normal properties of a pure sample of either populnin or populnetin.

Treatment	Crude samples	Populnin	Populnetin
With dilute sodium hydroxide	Bluish-green colour	Deep yellow solution	Deep red solution
With neutral lead acetate	A small quantity of orange-red precipitate	No precipitate	No precipitate

These discrepancies indicated that the flowers contained, besides populnin and populnetin, a third substance which might be the pigment which could not be isolated pure but was obtained only in the form of its acetyl derivative (III).

With a view to obtain more of populnetin for further investigation, flowers secured from the Trichinopoly District in summer 1939 have been examined by us adopting the following procedure. The dried petals were extracted with alcohol and the extract was concentrated by distillation to a small bulk. It was then diluted with a large volume of water and the resulting solution was again concentrated on a water-bath. During this operation considerable amounts of resin separated out. It was filtered off and the clear filtrate was boiled under reflux with sulphuric acid in order to hydrolyse any glucosides present. After the hydrolysis only a small amount of a yellow substance separated out on cooling the aqueous solution. So it was extracted with ether four times and the extract on the removal of the solvent gave a good amount of the pigment. The yield of the pure substance was 8 gm. from 4,000 gm. of the dried petals. This pigment was found to be a tetrahydroxy flavonol giving a penta-acetyl derivative on acetylation, and was noticed to be completely different from populnetin in all its properties as shown in the following table:

Property	Populnetin	The pigment isolated now
1. Molecular formula	$C_{14}H_8O_6, H_2O$	$C_{15}H_{10}O_7, H_2O$
2. Melting point	270-75°	280-82°
3. Reaction with neutral lead acetate ..	No precipitate	Red precipitate
4. Reaction with basic lead acetate ..	Yellow-orange precipitate	Red precipitate
5. Reaction with ferric chloride ..	Pale green	Olive green
6. Alkaline buffer solutions	Does not give prominent colours	Very prominent and rapid colour changes: Yellow-olive green-Violet-blue
7. Treatment with con. H_2SO_4 ..	Yellow solution with a green fluorescence	Dissolves forming a yellow solution
8. M.P. of the acetyl derivative ..	127-29°	190-93°

From a consideration of the melting points, it seemed probable that the acetyl derivative obtained now might be the same as the one (III) isolated before by Neelakantam and Seshadri. The sample of these authors, on repeated recrystallisations, became purer, melting at 188-90°. The identity of these two acetyl derivatives was established by taking the mixed melting point, which remained undepressed. The properties of the aglucone indicated that the substance might be the tetrahydroxy-flavonol, herbacetin originally obtained by Neelakantam, Rao and Seshadri³ from the herbaceum

cotton flowers and later shown to be present in the indicum cotton flowers also^{4, 5}. The identity of the aglucone as herbacetin was confirmed by comparing its acetyl derivative with an authentic sample of acetylherbacetin. To state the results in terms of the aglucones, the first sample of the *Thes-
pasia* flowers yielded both populnetin and herbacetin (very small) and the second one only populnetin. The third sample contained mainly herbacetin and little of populnetin. It is thus quite evident that both populnetin and herbacetin are present in the flowers of *Thes-
pasia populnea* and their relative proportion seems to vary considerably depending on the season of collection of the flowers.

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