

THE COLOURING MATTER OF THE FLOWERS OF *TAGETES PATULA*: ISOLATION OF A NEW FLAVONOL, PATULETIN AND ITS CONSTITUTION

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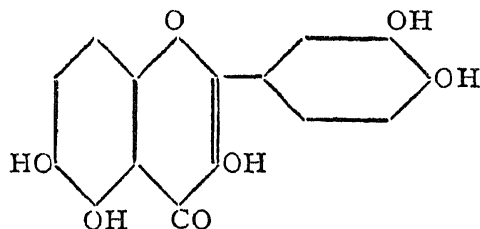
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Tagetes patula, the French Marigold (called *Seemabanthi* in Telugu) is similar to *Tagetes erecta*, except that it has a bushy and spreading habit. The flowers are much smaller in size, but borne in larger numbers. In colour they are red tinted and variegated. They do not seem to have been investigated before. It has been recently pointed out by us¹ how De La Source and Perkin on the one hand and Mahal on the other, thought that they were dealing with the two different species, but actually examined one and the same species *Tagetes erecta*. The confusion arose from a wrong combination of the botanical and the common names.

A genuine sample of the flowers of the *patula* was collected and examined according to the general method already outlined in some of our past publications.² The concentrated alcoholic extract of the petals did not deposit any solid even after several days but on dilution with water, a pale yellow crystalline substance separated out in good yield. After the removal of this solid, the mother-liquor was successively treated with neutral and basic lead acetate solutions, when an orange-red precipitate was produced in each case. The aqueous solution obtained by the decomposition of the neutral lead acetate precipitate did not yield any solid even after six months. It, however, contained a glucoside and on hydrolysis produced glucose and an aglucone which was found to be identical with the yellow substance obtained by the dilution of the original alcoholic concentrate. The glucoside itself could not be obtained and experiments are being carried out to effect its isolation. The basic lead acetate fraction was not much and was therefore, discarded.

The pale yellow crystalline solid isolated as given above is found to be a new pigment, and is named "Patuletin", as it has been obtained from the flowers of the *patula*. It has the elementary formula $C_{15}H_{10}O_7$ and melts at 262–64°. On treatment with lead acetate it gives a deep red precipitate, and in 50% alkali it undergoes ready oxidation in the cold, thereby indicating that it is a flavonol. The substance yields a pentaacetyl derivative and a pentamethyl ether, and hence it should be a tetrahydroxy flavonol. It is, therefore, isomeric with quercetin and herbacetin. As a result of oxidation in alkaline solution, it yields protocatechuic acid, which could be isolated

after methylation, in the form of veratric acid. So the flavonol contains two hydroxyl groups in the 3' and 4' positions. Since the naturally occurring hydroxy flavones and flavonols with the exception of one or two invariably contain a hydroxyl group in position 5, it is very likely that the new substance does not add yet another member to the exceptions to the general rule, and hence it may be presumed that one of the two hydroxyl groups in the benzopyrone ring is in position 5. For the other hydroxyl therefore, one of the remaining three positions 6, 7 and 8 is available. It does not seem to be present in position 8, as the substance is not oxidised by *p*-benzoquinone to give the "gossypetone" reaction (*cf.* cannabiscetin³). Nor can it be in position 7, as the compound is not identical with quercetin. Hence patuletin is tentatively represented as 3:5:6:3':4'-pentahydroxy flavone.



In further support of the above structure, it may be pointed out that the flavonol present in the closely allied species *T. erecta* has two hydroxyl groups in 5 and 6 positions (quercetagetin). The resemblance between patuletin and quercetagetin is very close in regard to many of their reactions. Like the latter the new flavonol immediately dissolves in alkali forming yellow solutions. When treated with a drop of ferric chloride, an alcoholic solution of the substance produces a greenish brown colour. With alkaline buffer solutions, as in the case of quercetagetin, no prominent colour changes are produced. When treated with sodium amalgam (Bargellini's test), patuletin immediately yields bluish green flocks; but the colour changes to almost pale yellow in about ten minutes and remains so even after 48 hours. By the action of diazomethane, the pigment undergoes complete methylation. This may be counted as evidence against the existence of a hydroxyl group in the 5th position. But the objection cannot be valid since quercetagetin itself is completely methylated under the same conditions.⁴ The resistance of the hydroxyl group in position 5 to methylation seems to be dependent on the disposition of other hydroxyls in the ring and to be greatest when they are present in the 5-7-8 combination as in herbacetin⁵ and gossypetin.⁶

Support for the suggested structure of patuletin is expected to be obtained from synthesis which is in progress.

Experimental

Isolation of Patuletin.—The dry petals (1,000 g.) of the flowers of *Tagetes patula* were extracted twice with boiling alcohol, each extraction

lasting over 12 hours, and the extract concentrated to about 300 c.c. After filtering through fluted filters the resins and the waxes that separated out, the clear concentrate was allowed to stand for a month; but no solid was deposited. It was, therefore, diluted with a large volume of water (1,500 c.c.), when a yellow crystalline solid began to precipitate out. The next day it was filtered and washed with water. As it was still impure and sticky, it was dissolved in a little pyridine and water was added to the solution till the impurities separated out as a suspension. They were coagulated by the addition of calcium chloride and filtered off. The clear filtrate which did not develop any more turbidity on further dilution was concentrated till the pigment separated out as yellow needles. Further purification of the substance was effected by crystallisation twice from alcohol, when it came out as clusters of dull yellow needles sintering at 260° and melting at 262–64°. It was sparingly soluble in water but easily dissolved in pyridine, alcohol and acetic acid. (Found in air-dried sample: C, 53·5; H, 4·4; $C_{15}H_{10}O_7$, $2H_2O$ requires C, 53·5; H, 4·1%. Found in the sample dried at 120° *in vacuo*: C, 59·4; H, 3·5; $C_{15}H_{10}O_7$ requires C, 59·6; H, 3·3%.) On acetylation with acetic anhydride and anhydrous sodium acetate, the pigment yielded a pentaacetyl derivative melting at 170–72°. It crystallised from dilute acetic acid as clusters of colourless needles. [Found: C, 58·1; H, 3·9; $C_{15}H_5O_2(OCOCH_3)_5$ requires C, 58·6; H, 3·9%.]

The Neutral Lead Acetate Fraction.—The aqueous solution left after the removal of the new flavonol was treated with excess of neutral lead acetate. The orange red precipitate produced was filtered, washed, suspended in water and decomposed with hydrogen sulphide. The aqueous solution thus obtained was concentrated and left aside after the addition of a few drops of toluene. Even after six months no pigment separated out but a small amount of resin settled down. The clear liquor was extracted with ether and the extract yielded a small amount of patuletin on evaporation. To see if the aqueous solution contained any glucosides, it was made 7% acid by the addition of the calculated amount of concentrated sulphuric acid and boiled under reflux. In about fifteen minutes some brown solid separated giving rise to bumping, and the subsequent heating had to be carried out on a boiling water-bath. After two hours, the contents were cooled and filtered. A portion of the filtrate was neutralised with barium carbonate, concentrated to a small bulk and then treated with phenyl hydrazine in acetic acid solution, when an osazone was produced. This was identified as glucosazone melting at 204–06°. The solid residue was brown in colour and it was contaminated with a considerable amount of resin, which could not be removed by direct crystallisation. So the mixture was macerated with cold alcohol when all the brown resin went into solution leaving most of the pigment

behind. By this treatment and subsequent crystallisation from fresh alcohol, it was obtained as clusters of yellow needles melting at 260–62°. It was identified as the new flavonol, patuletin, from a study of its properties and the comparison of the acetyl derivatives.

Properties of Patuletin.—The substance was not a glycoside since it remained unaffected, when boiled with 7% sulphuric acid for two hours. It very easily dissolved in alkalis producing orange-yellow solutions. Ferric chloride imparted a brownish green colour to an alcoholic solution of the substance. When a small amount of sodium amalgam was added to a solution of the substance in absolute alcohol, bluish green flocks were immediately produced; but their colour changed to almost pale yellow in about 10 minutes and remained so even after 48 hours. Neutral lead acetate produced a deep red precipitate, when added to an alcoholic solution of the pigment. When a solution of the substance in absolute alcohol was treated with *p*-benzoquinone, the original colour of the solution did not change, nor did any solid separate out even after several days. The colour reactions of the flavonol with alkaline buffer solutions were not prominent. In this and most of the other properties given above, it resembled quercetagenin closely.

Alkaline Oxidation of the Flavonol: Isolation of Veratric Acid.—The flavonol (1 g.) was treated with 50% aqueous potash (20 c.c.) when it dissolved immediately to form an orange-yellow solution. On leaving exposed to air with occasional shaking for 24 hours, the solution became opaque and brown. It was then diluted, and treated with excess of dimethyl sulphate (20 c.c.) in small quantities. After shaking for an hour, the contents were heated on a water-bath for about 30 minutes to complete the methylation and decompose the excess of the dimethyl sulphate. The clear alkaline solution was then acidified, and extracted with ether, when a colourless crystalline acid was obtained. It crystallised from alcohol in the form of needles melting at 183–84° and was found to be identical with veratric acid.

Preparation of Pentamethyl Patuletin.—(a) Pentaacetyl patuletin (0.5 g.) was dissolved in acetone (20 c.c.) and treated in small quantities alternately with dimethyl sulphate (10 c.c.) and 20% sodium hydroxide (10 c.c.). Subsequently further quantities of dimethyl sulphate (5 c.c.) and alkali (5 c.c.) were added, and finally the medium was made definitely alkaline by the gradual addition of more alkali (15 c.c.). After leaving overnight, the mixture was refluxed for half an hour to complete the methylation. When the excess of the solvent was distilled off, a yellowish white substance was produced. It was recrystallised from dilute acetic acid using a little animal charcoal, when it appeared as colourless needles and narrow rectangular plates.

(b) The flavonol (1 g.) was dissolved in anhydrous methyl alcohol (50 c.c.) and then treated in small quantities during two days with excess of diazomethane (3 g.) in ether solution. After each addition, the mixture assumed an orange colour which gradually faded on subsequent shaking. After the completion of the reaction, the ether and the excess of diazomethane were driven off on a water-bath, when a brown solid was left behind. On crystallisation from alcohol using a little animal charcoal, the ether was obtained as colourless narrow rectangular plates.

The two samples of the ether as obtained by the above methods were found to be identical. The pure substance sintered at about 143° and melted completely at $158-59^{\circ}$. [Found: OCH_3 , 39.6; $\text{C}_{15}\text{H}_5\text{O}_2 (\text{OCH}_3)_5$, H_2O requires OCH_3 , 39.7%.]

Alkaline Oxidative Hydrolysis of Pentamethyl Patuletin.—The Pentamethyl ether (1 g.) was refluxed in a silver flask with 50% potash (20 c.c.) for 6 hours, at the end of which it was completely disrupted. The clear alkaline solution on acidification did not precipitate any solid. When extracted with ether, however, and when the ether solution was evaporated, a crystalline solid was obtained and was found to be identical with veratric acid melting at $182-84^{\circ}$.

Summary

A new flavonol, patuletin has been isolated from the petals of the flowers of *Tagetes patula* (French Marigold). It contains five hydroxyl groups and yields a pentaacetate and a pentamethyl ether. It is, therefore, isomeric with quercetin and herbacetin. On oxidation in cold alkali, the substance decomposes into protocatechuic acid which could be isolated after methylation as veratric acid. The completely methylated ether also yields veratric acid on boiling with potash. Assuming that a hydroxyl group is present in position 5, as is the case with most of the naturally occurring flavones and flavonols, the remaining hydroxyl group in the compound is concluded to be in position 6 in view of the properties of the flavonol and its resemblance to quercetagenin. Hence patuletin is represented as 3:5:6:3':4'-pentahydroxy flavone.

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