OCCURRENCE OF LUTEOLIN IN THE FLOWERS OF *CHRYSANTHEMUM INDICUM*

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THE pigments of the red, scarlet red and the deep red flowers of *Chrysanthemum indicum* L. were examined by Willstatter and Bolton,[1] and were shown to contain, besides carotene and xanthophyll, the anthocyanin chrysanthemin. The latter compound was also shown to be present in the flowers of *C. sinense*,[2] whilst apigenin was isolated in the form of its glucoside by Jacini from the petals of *C. leucanthemum*.[3]

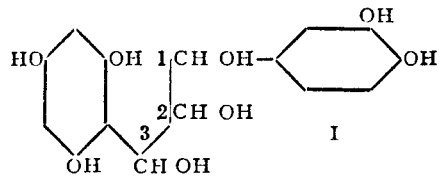
In continuation of our work on the anthoxanthins and their glycosides, the study of the yellow variety of the flowers of *C. indicum* has been undertaken, since there are certain points of interest in them. The flowers were obtained from Ambur in the North Arcot district during the month of November 1939. The colour of the petals was dull yellow and the size of the flowers varied from $1\frac{1}{2}$ to 2 inches in diameter. The petals contained a good amount of carotene, wax and resin which interfered with the separation of the anthoxanthins, and the interfering substances had to be eliminated at every stage during the course of the detailed examination carried out according to the general method described in some of our past publications.[4] On concentrating an alcoholic extract of the petals, carotinoid and some oily and waxy matter separated out. Subsequent dilution with water and extraction with petrol, chloroform and ether removed again the same type of substances. The aqueous solution on concentration yielded an anthoxanthin glucoside which underwent hydrolysis to produce glucose and a crystalline aglucone. The neutral lead acetate fraction was also found to contain a glucoside which gave the same products of hydrolysis. The basic lead acetate fraction was insignificant. Due to the small yields of the anthoxanthins and the presence of interfering impurities in considerable amounts, it was not possible to obtain the glucoside in a pure condition for purposes of identification.

The aglucone had the formula $C_{15}H_{10}O_6$, had all the characteristics of a tetrahydroxy flavone and did not melt below 300°. Its acetyl derivative was found to melt at 222–24°. Hence it was identified as luteolin, and the

identity was confirmed by taking the melting point of a mixture of the acetyl derivative with an authentic sample of tetraacetyl luteolin.

It is thus clear that the petals contain carotinoid material as the major component of the colouring matter, and luteolin is present only to a small extent in the form of its glucoside. The extracts of the flowers were examined for insecticidal properties, and were found to have no such value.

The occurrence of a glucoside of luteolin and chrysanthemin (cyanidin glucoside) in the yellow and the red varieties of *Chrysanthemum indicum* is noteworthy in regard to the biogenetic relationship between anthoxanthins and anthocyanins. The earlier view of Onslow[5] was that anthocyanidins were produced as the result of oxidation of the flavones, and the chief support was the existence of oxidising conditions in the plants having plenty of anthocyanins. When it was subsequently proved by Everest and Willstatter[5] that anthocyanidins were formed by the reduction of flavonols, the ideas had to be changed. In either case a correlation was expected between the constitutions of the anthocyanin and the anthoxanthin pigments. Examination of a number of flowers has yielded conflicting and inconclusive results. Recently Robinson[6] has advocated a theory which embraces all known experimental findings. According to him, flavones, flavonols and anthocyanidins are not formed from one another but arise independently from a common source, and compound (I) has been suggested as the most probable common intermediate. Oxidation yields a flavone, flavonol or anthocyanidin depending upon the point at which it takes place.[7] For example, oxidation at C (1) atom leads to the formation of cyanidin, at C (3) to the flavone luteolin, and at both C (2) and C (3) or at C (1) and C (3) to the flavonol quercetin.



I

Robinson and his collaborators[7] have concluded from a large amount of interesting data that cyanidin is the simplest of the anthocyanidins and is the first to be formed. The formation of others needs more steps in the synthesis. This explains why cyanidin is the most commonly occurring of the three fundamental anthocyanidins. A similar statement could be made regarding quercetin which is the most widely occurring of the flavonols. Though the results relating to the simultaneous occurrence of allied flavonols and anthocyanidins are not very many, still, wherever there is correlation, cyanidin and quercetin are concerned. This has been attributed by Robinson to their

abundant occurrence and might also be due to their fundamental nature in the evolution. In a similar way luteolin the most common of the naturally occurring flavones, and cyanidin should now be recognised as a related pair. More cases of this combination may be expected. In this connection it may be useful to analyse the red variety of the flowers of *C. indicum* for the anthoxanthins.

Experimental

Isolation of Luteolin.—The dried petals (6 kg.) of the flowers of *Chrysanthemum indicum* were extracted in batches with methylated spirit, and the combined extract was concentrated to about 800 c.c. The yellow waxy substances which separated during the course of the concentration was filtered through fluted filters, and the clear concentrate was allowed to stand. The waxy solid did not appreciably dissolve even in boiling alcohol, nor the solution did develop any prominent colour when treated with a drop of dilute alkali. It was evidently carotinoid in character and was, therefore, discarded. From the alcoholic concentrate no solid was deposited; but an yellow oil began to appear at the bottom of the flask. After six months, the mixture was diluted with a large volume of water, and extracted with petroleum ether which dissolved all the yellow viscous oil. Subsequent extraction with chloroform and ether removed some waxes and resins. The nature of the oil isolated is still under investigation. The aqueous solution was concentrated to a small bulk (500 c.c.) and set aside. After three months some brownish yellow solid (A) began to be deposited. It was filtered at the end of the third month and was found to contain a good amount of resin. The initial purification was effected by boiling it with water in which one part dissolved, while the other carotinoid part stuck to the sides of the test-tube. The clear solution on cooling deposited a pale yellow crystalline substance. Its further purification was not attempted for fear of losses. When boiled with 7% sulphuric acid, the compound underwent hydrolysis, and the aglucone separated out as a crisp yellow solid. After filtration of the products of hydrolysis, the filtrate was neutralised with barium carbonate, concentrated to a small bulk and treated with phenyl hydrazine in acetic acid solution. On heating the mixture for some time on a boiling water-bath, an osazone separated out, and it was identified as glucosazone from a study of its melting point and the crystal structure. The aglucone obtained crystallised from dilute alcohol as pale yellow needles, and the yield was 0·5 g. It did not melt below 300°. [Found: C, 59·3; H, 4·2; $C_{15}H_{10}O_6$, H_2O requires C, 59·2; H, 3·9%.] An alcoholic solution of the substance gave a brownish green colour with ferric chloride, a reddish orange

solution with a little dilute alkali and a yellow precipitate with lead acetate. With pH solutions the pigment did not produce any prominent colour changes. It dissolved merely to form yellow solutions. When boiled with acetic anhydride and anhydrous sodium acetate, it formed the tetraacetyl derivative which crystallised from dilute acetic acid as fine colourless needles and melted at 222–24°. [Found: C, 60·5; H, 4·5; $C_{15}H_6O_2(OCOCH_3)_4$ requires: C, 60·8; H, 4·0%.] All these properties indicated that the flavone was luteolin, and this was confirmed by taking the mixed melting point of the acetyl derivative with an authentic synthetic sample of acetyl luteolin.

Neutral Lead Acetate Fraction : Luteolin.—The mother-liquor left after the removal of solid (A) was treated with neutral lead acetate, when a yellow bulky precipitate was produced. After filtration the precipitate was suspended in water and decomposed in the usual way with hydrogen sulphide. The aqueous liquor produced thereby was concentrated and allowed to stand. Even after three months no solid came out. So the requisite amount of concentrated sulphuric acid was added so as to make the solution 7% in its acid content, and the mixture boiled under reflux. After half an hour a good amount of dark resin began to separate out and stick to the walls of the flask. The clear liquid was carefully decanted into another flask and the boiling continued when a brown resinous mass (solid B) came out after an hour. When the hydrolysis was over (two hours) the products were filtered while still hot. The clear filtrate did not yield any solid on cooling. It was, therefore, ether-extracted, when only a small amount of yellow crystalline substance was obtained. The acid solution was then neutralised with barium carbonate, and worked up for the isolation of the osazone as already described. The amount of the glucosazone formed was disproportionately high, when compared with the small amount of the pigment obtained by the ether-extraction of the filtrate from the products of the hydrolysis. Solid B should, therefore, have contained a considerable amount of the aglucone. It was brown in colour and highly resinous in character. After dissolving the substance in a small amount of pyridine, the solution was gradually diluted with water till the impurities began to separate as a suspension. They were then precipitated by the addition of calcium chloride and filtered off. The clear filtrate which did not develop any more turbidity on further dilution was concentrated and left overnight, when a yellow solid crystallised out. It was filtered and further purified by crystallisation from dilute alcohol, when it appeared as pale yellow needles not melting below 300°. It was also identified as luteolin, since the melting point of its acetyl derivative (222–24°) was not depressed by admixture with acetyl luteolin. The yield of the pure substance was 3 g.

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Summary

Luteolin is present in the form of its glucoside in the yellow variety of the flowers of *Chrysanthemum indicum*, whereas the red, scarlet red and the deep red variety contains the anthocyanin, chrysanthemin. Just as the simultaneous occurrence of quercetin and cyanidin is frequent, a similar correlation between the corresponding flavone, luteolin and cyanidin may also be common.

REFERENCES

1. Willstatter and Bolton .. *Ann.*, 1916, **412**, 136.
2. Hattori and Hayashi .. *Br. Chem. Abs.*, 1937, A, ii, 464.
3. Jacini .. *Helv. Chim. Acta*, 1935, **18**, 276.
4. Neelakantam, Rao and Seshadri *Proc. Ind. Acad. Sci* (A), 1935, **2**, 490.
5. Onslow .. "The Anthocyanin Pigments of Plants," *Cambridge University Press*, 1925.
6. Robinson .. *Nature*, 1936, **137**, 172.
7. Lawrence, Price, Robinson and Robinson *Phil. Trans. of Roy. Soc.*, B, 1939, **230**, 149.