

**311. *The Yellow Pigment of Papaver Nudicaule. Part I.***

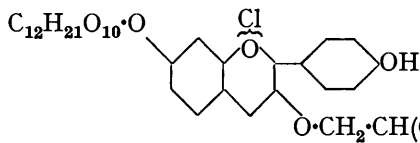
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The pigment of the yellow Iceland poppy is a nitrogenous diglucoside, the ratio of carbon to nitrogen atoms being approximately 30 : 1. It contains a *p*-hydroxyphenyl group and probably an amino-group. The formation of a  $\psi$ -base is the chief reason for suspecting the presence of a flavylum salt structure, but this is not decisive.

THE occurrence in *Papaver alpinum* of a water-soluble yellow pigment resembling the anthocyanins rather than the anthoxanthins in general character but not tallying with known types was noted by Willstätter and Weil (*Annalen*, 1916, **412**, 231). A similar colouring matter is present in yellow flowers of *Meconopsis cambrica* and of *Papaver nudicaule*, and a preliminary examination of the latter was made by Dr. R. Scott-Moncrieff. This clearly indicated the diglucosidic nature of the substance, although the formation of the aglycone by acid hydrolysis was always accompanied by evident decomposition or condensation. We propose the name *nudicaulin* for this particular glucoside and the term flavocyanin for the whole group of naturally occurring yellow colouring matters resembling the anthocyanins.

Miss Scott-Moncrieff isolated nudicaulin chloride as a yellow amorphous powder by repeated precipitation of its solution in methyl-alcoholic hydrogen chloride with ether. In later experiments we adsorbed the pigment on alumina, eluted it with water, and precipitated a highly characteristic, red mercuric salt. This was decomposed by means of acetic acid, and the colouring matter precipitated by addition of ether and finally purified by solution in methyl-alcoholic hydrogen chloride and precipitation with ether. The important observation was then made that the pigment is nitrogenous and analyses indicated a formula of the order  $C_{30}H_{38}O_{15}NCl$  containing 0.3 methoxyl group. Hydrolysis afforded glucose in amount greater than the theoretical for a monoglucoside but considerably less than that required for a diglucoside of this composition. The same phenomenon has frequently been noted among undoubted diglucosidic anthocyanins and is possibly due to condensation of the aglycone with glucose. After methylation and oxidation, anisic

acid could be isolated and this confirms an assumed relationship with the  $C_6-C_3-C_6$  series of natural products. Treatment with nitrous acid gave about half the theoretical volume of nitrogen, but examination of the sample used showed that it had only half of the normal nitrogen content and therefore the presence of



an amino-group is indicated. An aliphatic aldehyde appeared to be produced on oxidation with hydrogen peroxide, but further investigation of this point is necessary. The annexed formula is given only in order to show a structure isomeric with nudicaulin; there is no evidence as to the position of any of the groups other than the *p*-hydroxyl in the phenyl nucleus.

In regard to the methoxyl content, indicating admixture with a methylated component (one-third of the whole), the similar case of bougainvillaeidin (Price and Robinson, *J.*, 1937, 449) is brought to mind. It was suggested that methylation might have been due to esterification of a carboxyl group during the process of isolation. Later experiments have shown that this is not so, because the same methoxyl content has since been observed in bougainvillaeidin isolated without the use of methyl alcohol.

Orange flowers of *Papaver nudicaule* (also pink flowers) and *Meconopsis cambrica* have been found to be coloured by pelargonidin 3-bioside (Robinson and Robinson, *Biochem. J.*, 1931, 25, 1701; 1932, 26, 1659). It is now found that the buds of yellow Iceland poppies, which are red, contain a pelargonidin diglucoside or bioside. This disappears as the flower develops (for similar cases, cf. Chodat, *Compt. rend. Soc. Phys. Hist. nat., Geneva*, 1937, 52). Fresh material has been collected and the investigation is being continued. It is hoped also to study the colouring matter of yellow flowers of *Celosia cristata*, which is apparently another member of the flavocyanin group.

#### EXPERIMENTAL.

*Preliminary Examination* (by Miss R. Scott-Moncrieff).—The yellow pigment from the Iceland and alpine poppy (prepared as stated above) is quite dissimilar from the yellow sap-soluble flavones, but appears to resemble the anthocyanins in its reactions, solubilities, and general behaviour. The colour and many other characteristics are in keeping with the possibility that it is the glucoside of a simple flavylum salt, the comparative insolubility of the sugar-free pigment in water and in aqueous acid solutions alone being in contradiction to this theory.

The pigment, which is present in the flower as a glucoside, is insoluble in benzene, ether, and ethyl acetate, slightly soluble in amyl alcohol, moderately readily soluble in ethyl alcohol, easily soluble in water, hydrochloric acid, methyl alcohol, and glacial acetic acid (if a trace of water be present) and, on acidification, in acetone. It forms a stable canary-yellow salt with acids, which is easily converted into the colourless  $\psi$ -base on dilution with alcohol or warm water. Addition of hydrochloric acid at once causes regeneration of the yellow colour to its original intensity. With sodium acetate or bicarbonate, a characteristic rose-pink coloration is obtained; with sodium carbonate or sodium hydroxide a darker yellow alkali salt is produced. A buff-coloured precipitate is obtained on addition of lead acetate to an aqueous solution of the pigment, and an amorphous picrate separates on treatment with saturated aqueous picric acid. Acid alcoholic solutions show no fluorescence, and ferric chloride gives no colour reaction. The distribution number is similar to that of a diglucosidic anthocyanin.

Hydrolysis of the glucosidic pigment by boiling with either aqueous or alcoholic hydrochloric acid yields the sugar-free pigment in amorphous form. All attempts to crystallise it have failed up to the present. A monoglucosidic form can be isolated after partial hydrolysis, as is shown by distribution experiments.

The sugar-free pigment is slightly soluble in water and aqueous acid solution, and easily soluble in methyl and ethyl alcohols, no colourless  $\psi$ -base being formed on dilution. An amorphous picrate is obtained on addition of saturated aqueous picric acid solution to a methyl-alcoholic extract of the pigment. As in the case of the normal anthocyanidins, this picrate is soluble in ether. Similarly, the non-glucosidic hydrochloride is readily soluble in amyl alcohol, from which it can be precipitated by means of benzene.

The sugar-free hydrochloride is yellow in alcoholic solutions; a yellowish-pink colour base

is obtained on addition of sodium acetate or sodium bicarbonate, and a transient pink, followed by the formation of a deep yellow alkali salt, is obtained with sodium carbonate.

The chlorine content of the glucosidic pigment was found to be 4.08, 4.1, 3.7%, and the methoxyl content was 1.5%. The chlorine content of the aglycone was found to be 6.3, 6.0, 5.7, and 4.6% in different specimens.

*Isolation of Nudicaulin Chloride.*—After much material had been used in unsuccessful exploratory experiments with lead salts, picrates, etc., the following process was adopted. Disintegrated petals, dried in a stream of warm air at about 70°, were extracted with 1% methyl-alcoholic hydrogen chloride during 2—3 days. The solution was separated, neutralised with ammonia, and shaken with active alumina until no more adsorption took place. The alumina was then collected and well washed with methyl alcohol.

Preliminary experiments, carried out in the usual manner with an alumina column, showed that the extract contained a mixture of at least three and possibly more colouring matters. The major constituent, which is the required substance, was removed from the adsorbent by washing with water, the others remaining behind. These could be removed by means of acids and gave colour reactions similar to those of the main product, but they were darker in colour and were probably more complex products. In the experiments on a larger scale the pigment was removed by washing with warm water. To the filtered solutions, usually 2—3 l. per 80 g. of dried petals, solid sodium acetate was added and then saturated aqueous mercuric chloride. A bright red precipitate was soon formed, and the solutions were kept overnight to ensure as complete precipitation as possible. If they were filtered immediately, more mercury salt came down within 24 hours.

The separated solid was dried on a porous tile and extracted with glacial acetic acid at 20—25°, the extract filtered, and a yellow solid precipitated by means of ether. This was taken up in 1% methyl-alcoholic hydrogen chloride and reprecipitated by ether, this process being repeated two or three times. The final product was centrifuged and dried in a vacuum over phosphoric anhydride. It was a clean, yellow to orange-yellow, amorphous powder.

After the first extraction, the petals were again treated with 1% methyl-alcoholic hydrogen chloride, and this solution used to extract a fresh batch. The yield of mercury salt obtained was 4.5 g. from 80 g. of the dried petals. The mercury salt was extracted several times with acetic acid, but there remained a considerable amount of residue. This was extracted with 1% methyl-alcoholic hydrogen chloride, giving a further supply of a less pure pigment.

The glucoside is easily soluble in water, and in methyl or ethyl alcohol, but is insoluble in ether, acetone, benzene and other neutral organic solvents. An aqueous acid solution is pure yellow and gives the reactions described by Miss Scott-Moncrieff.

The salt is not extracted from aqueous solution by isoamyl or butyl alcohol even in the presence of sodium chloride, but if the liquid is neutralised there is partial extraction of the colourless  $\psi$ -base.

The substance gives an orange-yellow lead salt and a bright red mercury salt.

Attempts to crystallise the salt under a variety of conditions were unsuccessful, though slow dilution of a methyl-alcoholic hydrogen chloride solution with acetone caused deposition of a small amount of a semi-crystalline solid after 3 weeks.

*Analyses of Nudicaulin Chloride.*—The first sample obtained after adsorption with alumina was not isolated through the mercury salt, but by concentration of the aqueous solution under reduced pressure and precipitation with acetone (Found : N, 2.1%).

Specimens purified through the mercury salt :

(A) Dried in a vacuum over phosphoric anhydride at room temperature (Found : C, 47.15; H, 6.2; Cl, 4.7; loss on drying at 105° in a vacuum, 10.4%. Found in material dried at 105° in a vacuum : C, 53.5; H, 6.1; N, 2.1; Cl, 4.4; OMe, 1.5%).

(B) Dried at 105° in a vacuum (Found : C, 53.0; H, 5.5; N, 2.0; Cl, 3.8; S, 0.0%).

The mean values for the dried material are : C, 53.25; H, 5.8; N, 2.1; Cl, 4.1.

$C_{30}H_{38}O_{15}NCl_0.3CH_2, 4H_2O$  requires C, 47.6; H, 6.1; Cl, 4.7; loss of  $0.2HCl, 4H_2O$ , 10.4%.  $C_{30}H_{37}O_{15}N, 0.8HCl, 0.3CH_2$  requires C, 53.1; H, 5.6; N, 2.0; Cl, 4.15; OMe, 1.4%.

*Hydrolysis of Nudicaulin Chloride.*—Miss Scott-Moncrieff found that the aglycone produced on acid hydrolysis behaved differently according to the conditions under which it was prepared. If the time of contact with concentrated acid was prolonged, the aglycone was almost insoluble in water or dilute acids and was not completely extracted from its suspensions by amyl or butyl alcohol. We have confirmed this and find that, when hydrolysis is effected by boiling with 10—

15% hydrochloric acid for a minute, complete extraction by *isoamyl* alcohol is possible. Nevertheless the product, which separates as a reddish-brown solid if the solution is sufficiently concentrated, is evidently, at least in part, a condensation product. However, it still gives the characteristic rose-pink colour on neutralisation. A specimen which separated from 16% hydrochloric acid was dried in a vacuum at 105° over phosphoric anhydride (Found: C, 65.3; H, 4.3; N, 1.9; Cl, 3.6%). For the reasons stated, these values cannot be used to check the proposed formula, but it is significant that the N : Cl ratio is still 1 : 0.75 as in the glucoside.

Estimation of the amount of reducing sugars produced gave the following results: (a) 23.2 Mg. of glucoside dried at 105° in a vacuum over phosphoric anhydride gave 9.3 mg. of glucose, and 12.0 of aglycone dried over phosphoric anhydride in a vacuum, whence: glucose, 40.1; aglycone, 51.7%. (b) 23.0 Mg. of glucoside gave 8.8 mg. of glucose and 11.2 mg. of the aglycone, whence: glucose, 38.3; aglycone, 48.7%. The theoretical requirement for the diglucoside is glucose, 52.8%.

In an experiment on a larger scale the sugar produced on hydrolysis was converted into its osazone, which was identified as glucosazone, m. p. and mixed m. p. with an authentic specimen, 204.5—205.5° after crystallisation from aqueous alcoholic pyridine.

*Methylation and Oxidation of the Aglycone.*—The aglycone (0.23 g.) was methylated by means of methyl sulphate (15 c.c.) and sodium hydroxide (18 g.) in aqueous solution. The reagents were added in two portions, and the solution shaken vigorously after each addition. A solid separated from the strongly alkaline solution. After being kept overnight, the mixture was thrice extracted with ether and the aqueous solution was acidified, more solid then separating, and again thoroughly extracted with ether. The solid in suspension, which appeared to represent the greater part of the starting material, was insoluble in ether and was collected. It was suspended in aqueous soda, and potassium permanganate added, with occasional gentle heating on the steam-bath until oxidation was complete. The excess of permanganate was destroyed by means of sodium sulphite and the solution was filtered and acidified; the white crystalline precipitate obtained crystallised from water in long, colourless needles, m. p. 180—182°, alone or mixed with anisic acid (m. p. 181—183°).

*Potash Fusion of the Aglycone.*—The air-dried aglycone (0.8 g.), produced by hydrolysis with boiling 15% hydrochloric acid for 2 minutes, was heated at 200—220° with potassium hydroxide (10 g.) and water (2 c.c.) for 15 minutes. Working up in the usual way gave dark tarry phenolic and acid fractions. The phenolic fraction gave no coloration with ferric chloride and the only indication that a phenol may have been present was that the addition of benzenediazonium chloride to an alkaline solution gave a slight yellow precipitate. There was considerably more of the acid fraction, but it was very dark, yielded a tar on evaporation, and could not be crystallised. It was therefore methylated with methyl sulphate and alkali, and the product oxidised with alkaline permanganate. On acidification and extraction with ether a colourless solid was obtained which after crystallisation from water had m. p. 179—181°, mixed m. p. with anisic acid, 181—183°.

*Action of Nitrous Acid on Nudicaulin.*—Quantitative determinations of the amount of nitrogen liberated on treatment of the pure pigment with nitrous acid have not yet been carried out, but two estimations were done with a specimen obtained from the mercury salt residues. The nitrogen evolved corresponded to 50% of the theoretical on the basis of the C<sub>30</sub> formula, but on analysis of the specimen the nitrogen content was found to be very low (1.0%). Allowing for this, the amount of nitrogen approximated to the requirement for an amino-group.

During treatment with nitrous acid there was little or no change in colour, but afterwards the characteristic rose-pink colour was not developed on neutralisation. When the solution after nitrous acid treatment was hydrolysed by boiling with 15% hydrochloric acid, a marked colour change from deep yellow to very pale yellow took place and, on cooling, a pale yellow solid separated. This has not been obtained in sufficient amount for a detailed examination, but it is readily soluble in ether to a yellow solution, and can be extracted from ether with alkalis, giving deep yellow solutions, from which it is precipitated on acidification.

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