192. *Synthetical Experiments on the Nature of Betanin and Related Nitrogenous Anthocyanins. Part I.*

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IN 1918, Willstätter and Schudel (Schudel, Dissertation, Zürich) isolated an anthocyanin of entirely novel type from *Beta vulgaris.* Its unusual instability necessitated the development of a new technique for its purification, the essential feature of which was the extraction of the colouring matter from aqueous solution by means of an organic solvent, for example, a mixture of *iso*amyl alcohol and acetophenone, containing picric acid or, better, dichloropicric acid. Ultimately, the anthocyanin, betanin chloride, was crystallised and by means of alcoholic hydrogen chloride it yielded " betanidin chloride " and a sugar. Although the compositions of these substances were not ascertained, the remarkable feature was clearly established that betanin and betanidin contain nitrogen.

Professor Willstätter has kindly communicated to us that analyses of betanidin chloride made in his laboratory in October, 1919, gave N, 5·42, 5·28, 5·30%. He has also informed us that the beet is not unique and that similar pigments occur in the cockscomb (*Celosia cristata*, L.) and in winter spinach (*Atriplex hortensis atrosanguineus*).

The correct interpretation of the nitrogen content of betanidin is not clear, since the figures quoted are a little too high even for a monohydroxyaminoflavylium chloride (N, 5·1%) and considerably above the theoretical requirements for the more probable di- or tri-hydroxyaminoflavylium salt.

The work described in the sequel supports by analogy the view that betanin is a member of the flavylium salt series, and in that case it must (a) be derived from a very simple aminohydroxyflavylium salt, or (b) contain two nitrogen atoms in the molecule, or (c) be contaminated with nitrogenous impurity such as an ether of chlorostyphnic acid (derived by replacement of one of the reactive chlorine atoms of dichloropicric acid).

In order to determine whether a good *prima facie* case could be made for the aminoflavylium salt hypothesis we proposed to synthesise the various types and examine their properties.

Ridgway and Robinson (J., 1924, **125**, 2240) have already prepared some acylated 3-aminoflavylium chlorides and found that on hydrolysis by means of acids the acylamido-group is replaced by hydroxyl. Attempts to effect the direct synthesis of 3-aminoflavylium salts have not been successful. We now describe some 4′-aminoflavylium salts, and experiments on 5- and 7-aminoflavylium salts are in progress.

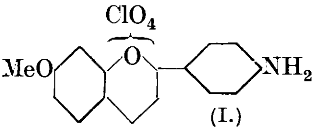
The colour reactions of betanin suggest that the hydroxyl in position 4′ of the ordinary anthocyanins is absent (or modified by substitution) and the properties of the simpler salts bearing an amino-group in that position are not unlike those of the beet pigment. In one respect, however, there was a considerable difference; the bluish-red solutions of the aminoflavylium salts became orange-yellow on the addition of mineral acid in excess, whereas the beet pigment, and also that of *Atriplex hortensis*, remains bluish-red in moderately high acid concentrations and there is a change to a red colour devoid of blue tinge only in concentrated hydrochloric acid solution. By condensing β-resorcylaldehyde with 4-amino-ω-hydroxyacetophenone, however, we have obtained a flavylium salt, probably 4′-amino-3 : 7-dihydroxyflavylium chloride, the properties of which are not unlike those of betanin, especially the colour and the degree of stability in alkaline and in acid solution. The absorption of light in the visible region (Fig. 2) by 0·1% hydrochloric acid extracts of beet and of *atriplex hortensis* is compared with that of an alcoholic solution of the aminodihydroxyflavylium salt. It will be observed that the two natural pigments may be identical, since the only divergence is probably caused by a difference in the concentration of the solution.

A description of 4′-amino-3 : 7-dihydroxyflavylium chloride is reserved, since the analytical data cannot yet be interpreted satisfactorily. We have prepared a large number of salts in this group which have similar general properties but give ambiguous analytical results. Only a few well-characterised members of this group will now be described.

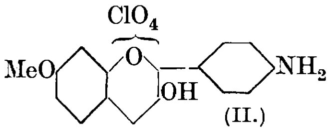
The usual flavylium salt synthesis, consisting in the condensation of an *o*-hydroxybenzaldehyde with an acetophenone derivative with the aid of hydrogen chloride in formic acid, ether, or ethyl acetate solution, may be employed with acylamido-derivatives, but it does not give good results with the free aminoacetophenones. Ultimately we found it best to prepare the Schiff base by condensation of the *o*-hydroxybenzaldehyde derivative with the aminoacetophenone derivative; this was then dissolved in cold concentrated hydrochloric acid, and the solution saturated at 0° with hydrogen chloride. The imines were thus transposed and gradually yielded flavylium salts, one possible view of the course of the change being that hydrolysis of the Schiff base was followed by recondensation of the products.

The most readily crystallisable salts of definite composition were found to be the perchlorates. In this way we obtained 4′-*amino*-7-*methoxyflavylium perchlorate* (I) and 4′-*amino*-8-*methoxyflavylium perchlorate*.

4′-Amino-5 : 7-dimethoxyflavylium perchlorate could, however, be obtained by direct condensation of the appropriate components and treatment with perchloric acid.



(I.)

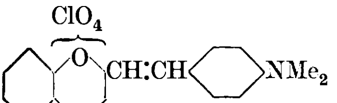


(II.)

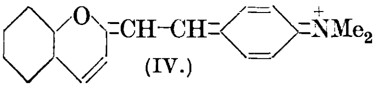
The Schiff-base method was used for the preparation of *4′-amino-3-hydroxy-7-methoxyflavylium perchlorate* (II).

In all these salts the amino-group has a much greater power as an auxochrome than hydroxyl, and comparison of (II) with (I) shows that the strong effect of a hydroxyl in position 3 in deepening the colour of flavylium salts is also observed in the series under discussion.

The rhodamines are a class of substances in which substituted basic amino-groups are associated with the pyrylium nucleus. Furthermore, Buck and Heilbron (J., 1923, **123**, 1395) have prepared 4′-dimethylamino-2-styrylbenzopyrylium salts and made a close study of the behaviour of the substance. It forms a green perchlorate (III) and an orange-yellow diperchlorate as well as a series of complex double salts; on dilution the aqueous solutions become violet and then pure blue as the result of the formation of the kation (IV). Buck and Heilbron reported (J., 1922, **121**, 1198) that solutions of 4′-hydroxy-2-styrylbenzopyrylium chloride also became deep blue on high dilution : this phenomenon they attributed to the formation of the quinonoid colour base (IV, O in place of NMe_2).



(III.)



(IV.)

EXPERIMENTAL.

2-Hydroxy-4-methoxybenzaldehyde.—The semi-methylation of β-resorcylaldehyde as prescribed by Kalle and Co. (D.R.-P. 214153) gave very poor results.

A mixture of β-resorcylaldehyde (10 g.), acetone (150 c.c.), methyl iodide (6 c.c.), and powdered potassium carbonate (10 g.) was refluxed for 80 minutes (optimum), and most of the acetone then removed by distillation. Water was added, and the dimethyl ether collected; the filtrate was acidified and steam-distilled, giving the pure monomethyl ether (5·4 g., m. p. 41°).

4-Amino-ω-acetoxyacetophenone.—To 4-nitro-ω-acetoxyacetophenone (Bradley and Robinson, J., 1928, 2907) (5 g.), suspended in a boiling solution of crystallised ferrous sulphate (50 g.) in water (250 c.c.), aqueous ammonia (30 c.c.; *d* 0·880) was slowly added, and the liquid was boiled for 2—3 minutes and filtered. The *amino-ketone* crystallised on cooling and was recrystallised from hot water, forming pale yellow prisms (2·8 g.), m. p. 129—130° (Found: C, 61·8; H, 5·7; N, 7·4. $C_{10}H_{11}O_3N$ requires C, 62·2; H, 5·7; N, 7·3%). This substance is readily soluble in the simple alcohols, reduces Fehling's solution, slowly in the cold and rapidly on warming, and is readily diazotisable.

4'-Amino-8-methoxyflavylium Perchlorate.—*4-o-Vanillylidene-aminoacetophenone*, obtained by heating a solution of *o*-vanillin (1·5 g.) and *p*-aminoacetophenone (1·35 g.) in alcohol (20 c.c.), crystallised from ethyl alcohol in orange prisms (2·6 g.), m. p. 130° (Found: C, 71·6; H, 5·4; N, 5·3. $C_{16}H_{15}O_3N$ requires C, 71·4; H, 5·6; N, 5·2%).

A solution of this intermediate (2·6 g.) in the minimum of concentrated hydrochloric acid, cooled in ice-water, was saturated with hydrogen chloride for 6 hours. After some hours the liquid was filtered and mixed with 20% aqueous perchloric acid, and the solid collected. The *perchlorate* crystallised from acetic acid or 20% perchloric acid in dark crimson needles (Found: C, 54·8; H, 4·3; N, 3·5; Cl, 9·2. $C_{16}H_{14}O_6NCl$ requires C, 54·6; H, 4·0; N, 4·0; Cl, 10·1%). The salt is easily soluble in methyl and in ethyl alcohol to wine-red solutions, which become yellow on the addition of sodium carbonate and red again on re-acidification; it is sparingly soluble in acetone or water.

4'-Amino-7-methoxyflavylium Perchlorate (I).—A solution of 2-hydroxy-4-methoxybenzaldehyde (0·5 g.) and 4-aminoacetophenone (0·45 g.) in a little alcohol was boiled for a few minutes; on cooling, 4-(*2'-hydroxy-4'-methoxybenzylideneamino*)*acetophenone* separated. It crystallised from alcohol in yellow plates (0·7 g.), m. p. 159—160° (Found: C, 71·2; H, 5·6; N, 5·3. $C_{16}H_{15}O_3N$ requires C, 71·4; H, 5·6; N, 5·2%).

This Schiff base (6·7 g.) was treated as the previous one (concentrated hydrochloric acid, 400 c.c.; saturation, 18 hours). *4'-Amino-7-methoxyflavylium perchlorate*, collected after several days, crystallised from acetic acid (6 litres) or aqueous perchloric acid in reddish-brown needles (Found in air-dried material: C, 52·2; H, 4·2; N, 3·2; Cl, 10·0. $C_{16}H_{14}O_6NCl,H_2O$ requires C, 52·0; H, 4·3; N, 3·8; Cl, 9·6%).

The salt is readily soluble in the simple alcohols to deep bluish-red solutions exhibiting a fine green fluorescence; a very dilute solution in acetic acid also exhibits this property. It is readily soluble in dilute hydrochloric acid and can be diazotised. Addition of sodium carbonate to an alcoholic solution gives a colourless solution of the pseudo-base; the eosin-red colour of the oxonium salt is quantitatively regenerated on addition of hydrochloric acid. The solution in an excess of hydrochloric acid is orange-yellow. Trituration of the solid salt with aqueous sodium acetate gives the chocolate colour-base, which is readily soluble in organic solvents, *e.g.*, chloroform, to red solutions. The perchlorate itself is slightly soluble in chloroform to a bluish-red solution.

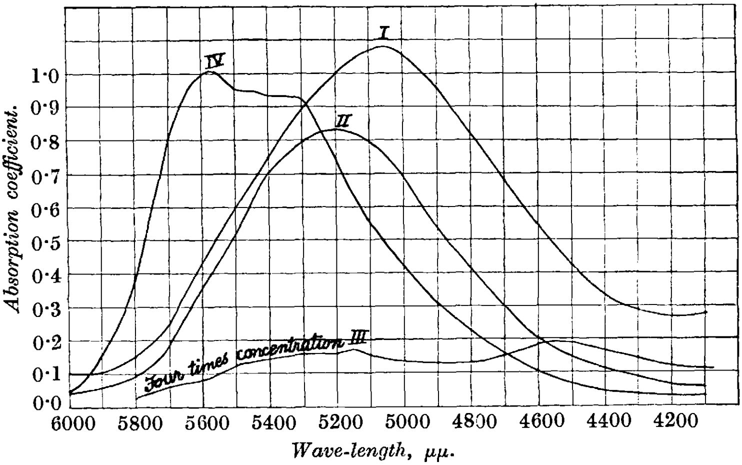
4′-*Amino*-5 : 7-*dimethoxyflavylium Perchlorate.*—A solution of 4 : 6-dimethoxy-2-hydroxybenzaldehyde (0·45 g.) and 4-aminoacetophenone (0·34 g.) in concentrated hydrochloric acid (20 c.c.) was kept for a day, filtered, and mixed with 20% perchloric acid. After a day, the *perchlorate* was collected and crystallised from aqueous perchloric acid, forming bright scarlet prisms (Found in air-dried material: C, 48·6; H, 4·9; N, 3·0; Cl, 8·4. $C_{17}H_{16}O_7NCl,2H_2O$ requires C, 48·9; H, 4·8; N, 3·4; Cl, 8·5%). This salt is freely soluble in the simple alcohols to orange-red solutions, the blue tone being conspicuously absent; on dilution with water a yellow solution with an intense green fluorescence is obtained.

The diazotisable amino-group was recognised in the yellow acid solutions of the salt. Trituration with aqueous sodium acetate gives a brown colour-base which is soluble in chloroform to a deep brown-red solution. Addition of sodium carbonate to an alcoholic solution gives a deep brown-yellow fluorescent solution, and no colourless pseudo-base has been observed in this case.

4′-*Amino*-3-*hydroxy*-7-*methoxyflavylium Perchlorate* (II).—The crude Schiff base obtained by the condensation of 4-amino-ω-acetoxyacetophenone (0·97 g.) and 2-hydroxy-4-methoxybenzaldehyde (0·76 g.) in absolute alcohol had m. p. 150—152° (yield, 1·4 g.) and crystallised from alcohol in yellow plates, m. p. 155—156°.

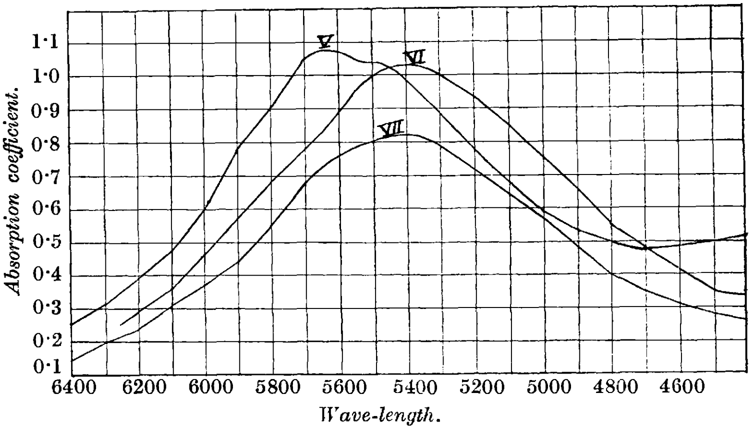
A solution of the base (5·57 g.) in concentrated hydrochloric acid (300 c.c.) was saturated with hydrogen chloride at 0°, condensation to a pyrylium salt occurring more readily than in the analogous case in which the ω-acetoxy-group was not present. The reaction was completed in 6 hours and the filtered solution was mixed with 20% aqueous perchloric acid and kept for several days to allow complete separation of the *perchlorate*, which crystallised from much glacial acetic acid (6 litres afforded 1·2 g. of crystals and did not suffice for the solution of the whole product) in long hair-like needles, almost black in mass (Found in material dried at 110° in a high vacuum over phosphoric oxide : C, 47·6; H, 4·7. $C_{16}H_{14}O_7NCl,2H_2O$ requires C, 47·6; H, 4·5%). Estimation of the nitrogen content in this salt gave a low result and it is possible that a portion of the amino-nitrogen has been replaced by hydroxyl. Direct examination of the salt did not, however, confirm this view, because ordinary anthocyanidin-type flavylium salts appeared to be absent.

FIG. 1.



The salt is freely soluble in the simple alcohols to dark bluish-red solutions which are purple in thin layers. Addition of water to the

FIG. 2.



V. *4′-Amino-3 : 7-dihydroxyflavylium chloride.* VI. *Crude betanin in* 0·1% *aqueous hydrochloric acid.* VII. *Crude pigment of* Atriplex hortensis *in* 0·1% *hydrochloric acid.*

alcoholic solution changes the colour to violet and the violet colour-base is slowly precipitated; the flavylium salt is regenerated on the addition of hydrochloric acid. Sodium acetate causes immediate precipitation of the colour-base, but in the presence of sodium carbonate or sodium hydroxide the colourless pseudo-base is produced, and this is also converted into the red pyrylium salt by the action of mineral acids.

The symbols I, II, III, and IV refer to 8-methoxy-, 7-methoxy-, 5 : 7-dimethoxy-, and 3-hydroxy-7-methoxy-4′-aminoflavylium perchlorate, respectively, and the bands were observed with $0{\cdot}25N/10^4$-solutions in 0·1% methyl-alcoholic hydrogen chloride in a cell of 20 mm., except where stated otherwise.

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