

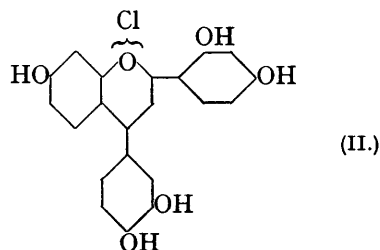
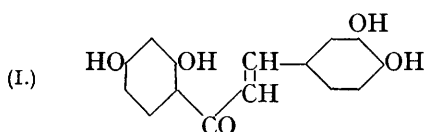
218. The Yellow Colouring Matter of *Dahlia variabilis*.

By J. R. PRICE.

The yellow colouring matter of the flowers of *Dahlia variabilis* has been identified as butein. The bearing of this identification upon the problem of the biogenesis of the anthocyanins and related substances is discussed. The pigment readily undergoes the Robinson-Walker chromylum salt synthesis (J., 1934, 1435).

SCHMID and WASCHKAU (*Monatsh.*, 1928, **49**, 83) isolated apigenin (m. p. 347°) from flowers of the yellow *Dahlia variabilis*, and noted that it was accompanied by a second substance which was evidently responsible for the intense colour. An attempt was made by Schmid and Seebald (*ibid.*, 1932, **60**, 32) to purify this second constituent. Their product had the molecular formula $C_{15}H_{10}O_5$, melted with decomposition at 324°, and contained three hydroxyl groups, one of which was present in a *p*-hydroxybenzene nucleus. They were unable to obtain a pure acetyl derivative, or to identify the phenolic fraction produced by fusion with potash. Further work by Schmid and Haschek (*ibid.*, 1933, **62**, 317) revealed the surprising fact that this substance, whose colour with alkalis is quite distinct from that of apigenin, gave triacetyl apigenin on acetylation and apigenin dimethyl ether on methylation. Schmid and Haschek were unable to account for the conversion of their pigments into apigenin and suggested that it was a case of dimorphism. There can be no doubt that they were dealing with impure apigenin.

The identification of the yellow pigment was undertaken to facilitate a study of the inheritance of flower colour in *Dahlia* (see Lawrence and Scott-Moncrieff, *J. Genet.*, 1935, **30**, 155). When genetically analysed plants whose flowers were deep yellow and contained little or no flavone were used, the substance was readily obtained pure. It has the formula $C_{16}H_{12}O_5$, contains four hydroxyl groups, and can be degraded to resacetophenone and protocatechuic acid. Mixed melting points of the substance and its acetyl derivative with synthetic butein (I) and butein tetra-acetate showed no depression. As expected, condensation with resorcinol took place smoothly under the conditions employed by Robinson and Walker (J., 1934, 1435; 1935, 941) with the formation of 7 : 2' : 4'-trihydroxy-4-(3'' : 4''-dihydroxyphenyl)flavylium chloride (II).



Perkin and Hummel (J., 1904, **85**, 1459) obtained a small amount of butein as a by-product in the isolation of butin from flowers of *Butea frondosa*. Lal and Dutt (*J. Indian Chem. Soc.*, 1935, **12**, 262) also identified butein in mother-liquors from the isolation of butrin, the butin diglucoside of *Butea frondosa*. However, dyeing experiments (Perkin and Everest, "The Natural Organic Colouring Matters," London, 1918) suggest that butein does not occur as such in *Butea frondosa*, but is formed from butin during the extraction or drying of the flowers. *Dahlia* seems therefore to be the first example of the occurrence of the substance in nature.

The yellow pigment is accompanied by another substance, which shows similar behaviour to alkalis but is much more soluble in water. This is possibly glycosidic and, since the method of extraction involved heating with dilute acid, the butein may be present as an easily hydrolysable glycoside. The isolation of this more soluble fraction will be undertaken when fresh material is available.

The identification of butein in *Dahlia* flowers is of interest in connection with the scheme put forward by Robinson ("The Molecular Architecture of Some Plant Products," 9th

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Int. Congr. Chem., Madrid, 1934; see also *Nature*, 1936, **137**, 172) for the formation in the plant of the anthocyanins and other members of the flavan group. Genetical data obtained by Lawrence and Scott-Moncrieff (*loc. cit.*) show that in *Dahlia* the anthocyanins, flavones and butein are produced from a common intermediate, and that there is competition between the three lines of synthesis for this intermediate. The existence of such competition in anthocyanin and flavone (or flavonol) synthesis has been realised for some time, and the recognition of a third competing process is of value as a step towards the determination of the structure of all substances in whose synthesis this intermediate is involved. When this has been accomplished, it may be possible to deduce the structure of the intermediate and to trace the stages in the formation of a large group of plant products.

EXPERIMENTAL.

Isolation.—Fresh flowers were boiled for 1–2 hours with $\frac{1}{2}\%$ hydrochloric acid, and the filtered extracts saturated with sodium chloride and shaken three times with amyl alcohol. The amyl-alcoholic solution was mixed with an equal volume of benzene and shaken with saturated aqueous sodium acetate. This treatment removed a substance whose colour reactions resemble those of the principal colouring matter, but which has a greater solubility in water and is probably glycosidic. The benzene-amyl-alcohol solution was shaken with 10% sodium hydroxide solution, which was then acidified with hydrochloric acid and the precipitate filtered off, dried, and extracted with ether (Soxhlet). After evaporation of the ether, the substance was crystallised from acetone-chloroform (once) and aqueous acetone (twice) and obtained in golden-yellow needles, m. p. 211–213° after drying at 105° in a vacuum, mixed m. p. with synthetic butein 210–212° (Found for a specimen dried at room temperature: C, 62.4; H, 4.9. Calc. for $C_{16}H_{12}O_5 \cdot H_2O$: C, 62.1; H, 4.8%. Found for material dried at 105° in a vacuum: C, 65.7; H, 4.6; OMe, 0; *M*, Rast, 267. Calc. for $C_{15}H_{12}O_5$: C, 66.2; H, 4.4%; *M*, 272).

The acetyl derivative formed almost colourless needles from methyl alcohol, m. p. 130–131°, mixed m. p. with butein tetra-acetate 129–131° [Found: C, 62.4; H, 4.5; $CH_3 \cdot CO$, 40.5. Calc. for $C_{15}H_8O(O \cdot CO \cdot CH_3)_4$: C, 62.7; H, 4.6; $CH_3 \cdot CO$, 39.1%].

Potassium Hydroxide Fusion.—The pigment (0.9 g.) was heated with potassium hydroxide (5 g.) and water (5 c.c.) until the red colour changed to pale brown. Separation of the acidic and phenolic fractions gave protocatechuic acid (m. p. 191–193° after crystallisation from benzene, mixed m. p. 192–194°) and resacetophenone (m. p. and mixed m. p. 142–144° after crystallisation from water). *Resacetophenone 2:4-dinitrophenylhydrazones* formed maroon plates from alcohol, m. p. 242–245° (Found: N, 17.0. $C_{14}H_{12}O_6N_4$ requires N, 16.9%).

7:2':4'-Trihydroxy-4-(3':4'-dihydroxyphenyl)flavylium Chloride.—Butein (0.5 g.) and resorcinol (0.2 g.) were dissolved in ethyl-alcoholic hydrogen chloride (12%; 7 c.c.) and kept in presence of chloranil (0.45 g.) for 10 days. Much solid had then separated, but as a little unchanged chloranil remained, the mixture was diluted with ether, and the flavylium salt dissolved in 0.5% alcoholic hydrogen chloride. The solution, filtered from chloranil, was treated with a large excess of saturated aqueous picric acid, and the precipitated picrate crystallised from half-saturated alcoholic picric acid. This was converted in the usual manner into the *chloride*, which crystallised from 10% ethyl-alcoholic hydrogen chloride in brownish-orange needles (Found for material dried in a vacuum over phosphoric oxide: C, 60.4; H, 4.5; Cl, 7.9. $C_{21}H_{15}O_6Cl \cdot H_2O$ requires C, 60.5; H, 4.1; Cl, 8.5%).

The salt dissolves in concentrated sulphuric acid to a yellow solution with a green fluorescence. It is almost insoluble in aqueous acids, but an alcoholic solution is orange-red. The quinone base produced by addition of sodium acetate to an aqueous alcoholic solution is crimson and can be extracted by ether; with sodium carbonate the colour changes to magenta, becoming bluer in tone on addition of 10% sodium hydroxide solution.

Synthesis of Butein.—The conditions employed for the condensation of resacetophenone and protocatechualdehyde were those used by Nadkarni and Wheeler (J., 1938, 1320) in the synthesis of 2:4-dihydroxyphenyl 4-hydroxystyryl ketone. After crystallisation from aqueous alcohol (charcoal) the butein had m. p. 210–212°; the acetyl derivative had m. p. 129–131°.

Saiyad, Nadkarni, and Wheeler (J., 1937, 1737) state that their butein gave a dark purple colour with ferric chloride. Neither the synthetic specimen prepared as above, nor that isolated from natural sources, gave such a colour. In each case the ferric reaction was olive-brown.

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