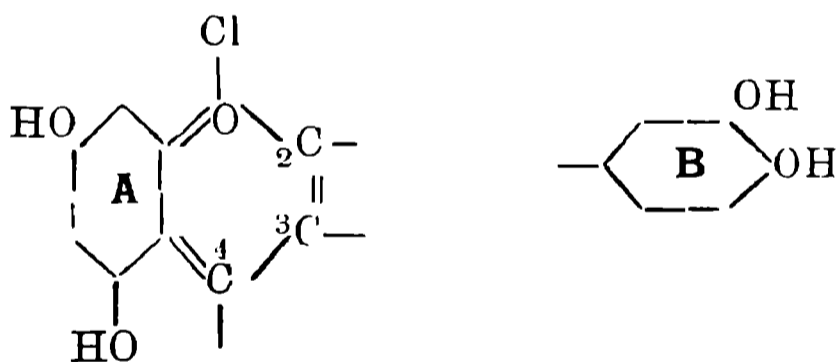


On the Constitution of Cyanidin Chloride.*

BY M. NIERENSTEIN.

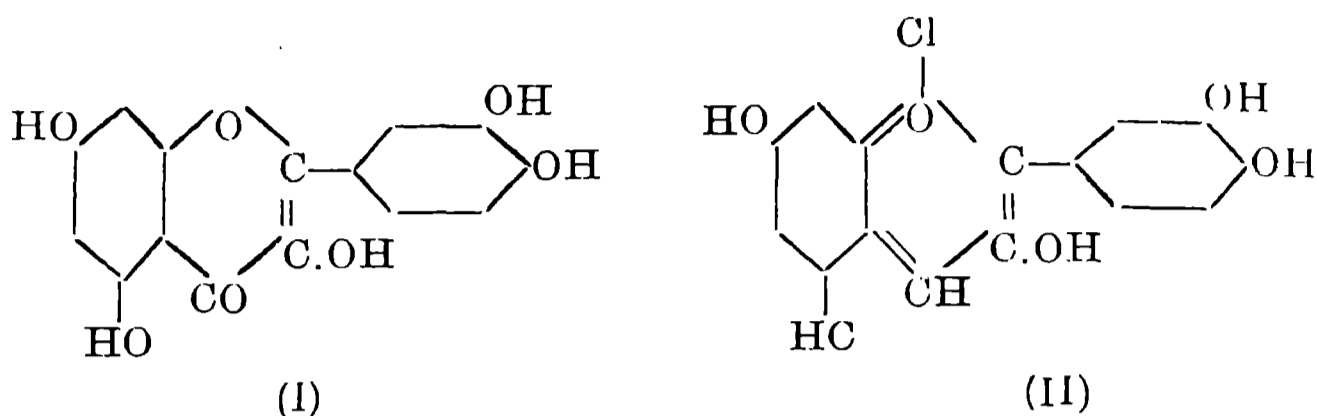
The classical researches of Willstätter have established beyond doubt the presence of the 5:7-dihydroxypyrylium-ring in the anthocyanidins, the red and blue pigments of a large number of flowers. In this ring the positions 2, 3 and 4 are substituted by H, OH and a hydroxylated benzene nucleus. Thus cyanidin, the colouring matter of the corn-flower, the rose and the poppy, which Willstätter has shown to give a beautifully crystallising chloride, $C_{15}H_{11}O_6Cl$, yields phloroglucinol and protocatechuic acid on hydrolysis.



As will be realised, any of the *six* possible formulae of cyanidin would yield phloroglucinol and protocatechuic acid, derived from the nucleus **A** and **B** respectively on hydrolysis, and until more is known as to the fate of the three carbon atoms 2, 3 and 4 during hydrolysis any formula suggested for cyanidin is of a highly speculative character. In spite of this, Formula (II) was assigned by Willstätter to cyanidin (*Sitzungsber. K. Akad. Wiss. Berlin*, 1914, 769) since quercetin (I) is supposed to yield cyanidin chloride (II) on reduction

* From a paper entitled "Rise and Fall of a Biochemical Hypothesis", communicated to the Indian Science Congress at Nagpur, January, 1931.

in hydrochloric acid solution, magnesium, zinc dust or sodium amalgam being used as a reducing agent.



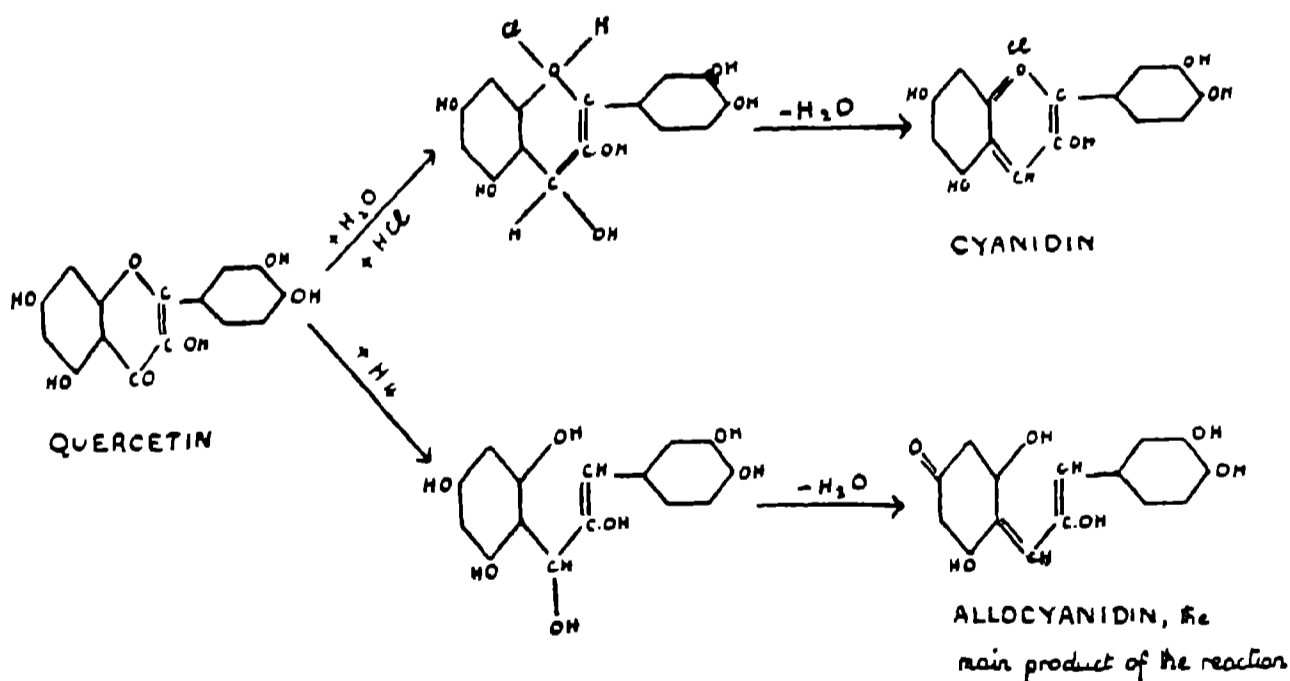
The supposed reduction of an anthoxanthone (quercetin) to an anthocyanidin (cyanidin) is of particular interest not only to chemists but also to botanists, as it offers a chemical explanation of the inter-relationship between these two groups of plant pigments so well known to all who follow the colour changes in plants, where starting from a very insignificant yellow coloured stock some of the most beautiful reds, pinks and blues have been produced. Stein (*J. pr. Chem.*, 1862, **85**, 351; 1863, **88**, 280; 1863, **89**, 4491), already noticed that yellow plant extracts turn deep red on reduction with zinc dust and hydrochloric acid. His observations were confirmed by Hlasiwetz and Pfaundler (*Sitzungsber. Wiener. Akad.*, 1864, **50**, 6), who further observed that these red coloured solutions turn blue-green on the addition of alkali, the specific reaction of the anthocyanidins already known to Robert Boyle and fully described by him in 1664 in his "Experiments and Considerations touching Colour." It was therefore not surprising that Hlasiwetz and Pfaundler should have claimed to have reduced the anthoxanthones to the anthocyanidins.

The reduction theory has since been generally favoured by chemists. It is, however, noteworthy that botanists were strongly of the opinion that if there is any inter-relationship between the anthoxanthones and the anthocyanidins, it was due to oxidation and not to reduction (compare, for example, Gertz, "Studier öfver Anthocyan", Lund, 1906; Onslow, "The Anthocyanin Pigments of Plants," Cambridge, 1925). It will be realised that botanical evidence was thus contrary to the chemical interpretation put forward by the supporters of the reduction theory which has in recent years been upheld particularly by Everest and Willstätter.

Everest (*Proc. Roy. Soc.*, 1914, B, **87**, 444), in a series of qualitative experiments which were identical with those described by Hlasiwetz and Pfaundler, claimed to have synthesised several anthocyanidins,

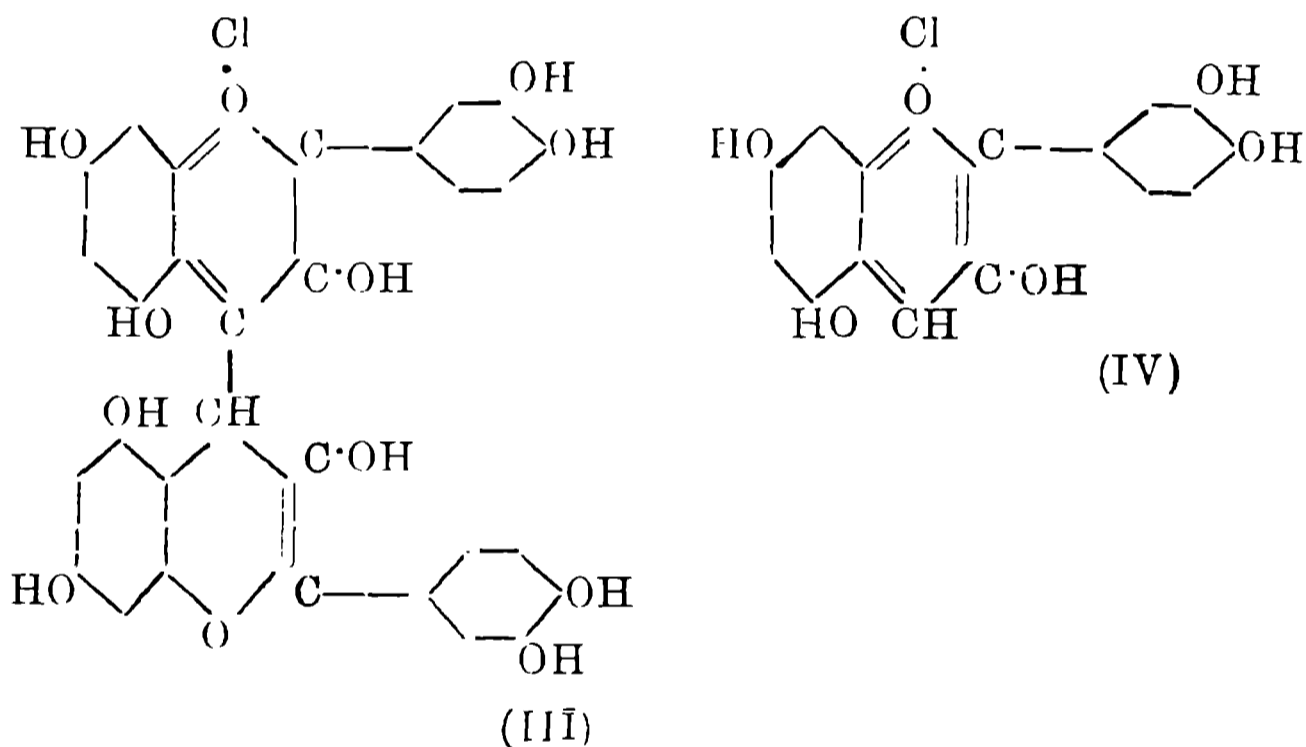
although in no case were the products analysed or even isolated. Encouraged by these qualitative experiments, Everest put forward a general scheme for the reduction of the anthocyanidins as illustrated in the reduction of quercetin to cyanidin chloride.

Willstätter and Mallison (*Sitzungsber. Preuss. Akad. Wiss.*, 1914, 769), who had previously (*ibid*, p. 402) expressed themselves against the view that the anthoxanthones may be reduced to anthocyanidins, soon after the publication of Everest's paper announced the *synthesis* of the cyanidin, which they claimed to have obtained on reduction of quercetin. They pointed out, however, that Everest's product was not true cyanidin, but a substance to which they assigned the name *allocyanidin*, and that true cyanidin is only formed in minute quantities from quercetin. The true cyanidin, which they obtained in a crystalline form and which they subjected to analysis, they found to be in every respect identical with natural cyanidin from corn-flowers and roses. The mechanism of the reaction leading to the production of cyanidin and *allocyanidin* was shown by Willstätter and Mallison as follows:—



The chemistry underlying the production of Willstätter's *allocyanidin* was queried by Pummerer ("Organische Chemie," 1921, p. 127), which is not surprising considering the abnormality of its supposed formation. In a similar way it was pointed out by Malkin and

Nierenstein (*J. Amer. Chem. Soc.*, 1930, **52**, 2864) that the production of cyanidin chloride from quercetin is quite unexpected since fluorone, xanthone, thioxanthone, acridone, anthraquinone, pyrone and their derivatives lead to *dimolecular* products on reduction in acid solution. Since quercetin is a pyrone-derivative it was hard to see why it should not conform to this general rule established by Baeyer and Piccard (*Annalen*, 1911, **384**, 208 ; 1915, **407**, 346). Malkin and Nierenstein therefore reinvestigated this reduction process and they were able to show that the reaction proceeds *normally* leading to the production of quercetylene chloride (III) and not to 3:5:7:3':4'-pentahydroxyflavylium chloride (IV, cyanidin chloride).



The following quotation from the memoir by Malkin and Nierenstein explains the reason why Willstätter and Mallison were misled in believing that they reduced quercetin to cyanidin chloride:

“The remarkable similarity between quercetylene chloride, $C_{30}H_{21}O_{12}Cl + 2H_2O$. and cyanidin chloride, $C_{15}H_{11}O_6Cl$, which not only resemble each other in almost all respects, but require exactly the same carbon content (C, 55.8), easily accounts for the fact that Willstätter and Mallison should have assumed that they had synthesised cyanidin chloride, since owing to the scarcity of material (0.165 g.) at their disposal, no chlorine analysis, which would have clearly emphasised the difference between these two substances, was possible.”

However, not only quercetylene chloride (III) but also 3:5:7:3':4'-pentahydroxyflavylium chloride (IV) has been shown by Willstätter, Zechmeister and Kindler (*Ber.*, 1924, 57, 1944) and by Pratt and Robinson (*J. Chem. Soc.*, 1925, 127, 172, 1180) to be in every respect identical with natural cyanidin chloride. The position therefore is: two *different* substances A (quercetylene chloride) and B (3:5:7:3':4'-pentahydroxyflavylium chloride) have been proved to be in every respect identical with C (natural cyanidin chloride), which is perturbing.

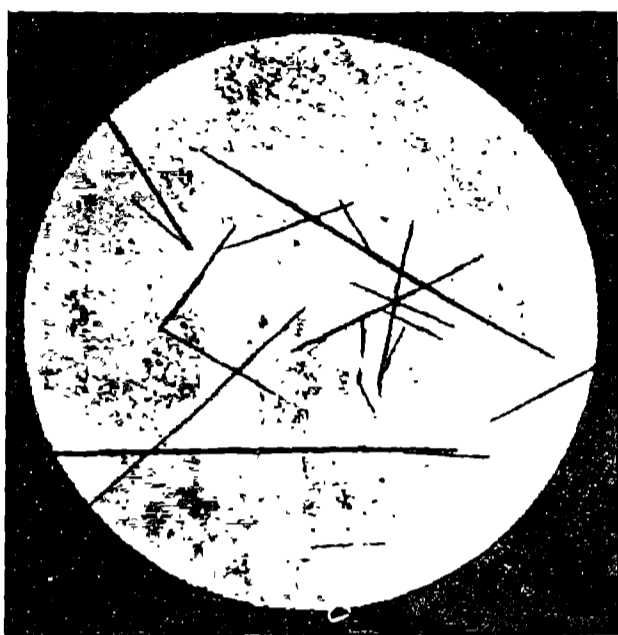
The comparisons between the natural and synthetic products are based (1) on a series of colour reactions, (2) on the absorption spectra and (3) on the general crystallographical appearance of the substances in question, as unfortunately neither the natural nor the synthetic products possess melting points. Such methods do not yield orthodox evidence of identity and are scarcely convincing.

(1) *Colour Reactions.*—Purely *qualitative* colour reactions were originally used in the comparisons between the natural and synthetic products. The application of *quantitative* methods as elaborated by Malkin and Nierenstein (*Ber.*, 1928, 61, 796) and by Fear and Nierenstein (*Biochem. J.*, 1928, 22, 615) revealed some striking differences between natural cyanidin chloride and the synthetic product. Thus, for example, cyanidin chloride turns pure blue at p_{H} 8.04 with alkali, whereas 3:5:7:3':4'-pentahydroxyflavylium chloride (synthetic cyanidin chloride) requires as high a p_{H} as 11.57 to give pure blue. However, in any case nothing can be deduced from the colour reactions as to the constitution of flavylium salts, as pointed out by Buck and Heilbron (*J. Chem. Soc.*, 1923, 123, 2524) who showed that substitution in positions 2 and 4 in the pyrylium-ring leads to compounds which are remarkably similar in their colour reactions.

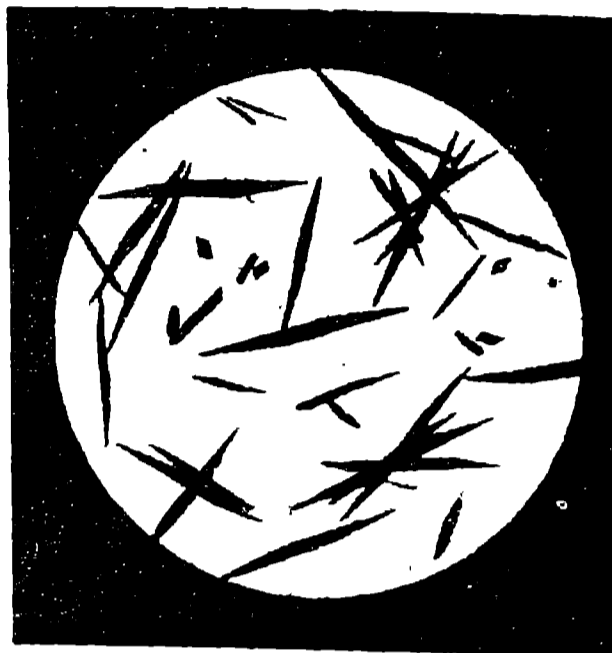
(2) *Absorption Spectra.*—The absorption spectra of the anthocyanidins have been studied by a large number of investigators, and special reference must be made to the very valuable contribution by Schou (*Helv. Chim. Acta*, 1927, 10, 910) who has shown that two such different substances as peonidin chloride and malvidin chloride give the same absorption spectra.

(3) *Crystallographical Appearance.*—Here special attention must be drawn to a paper by Kuhn and Wagner-Jauregg (*Ber.*, 1928, 61, 2506) who find that natural cyanidin chloride and the synthetic product, if crystallised under *identical* conditions, lead to crystal forms

which differ fundamentally as illustrated in the accompanying photographs.



Cyanidin chloride.



3 : 5 : 7 : 3' : 4'-Pentahydroxy-
flavylium chloride.

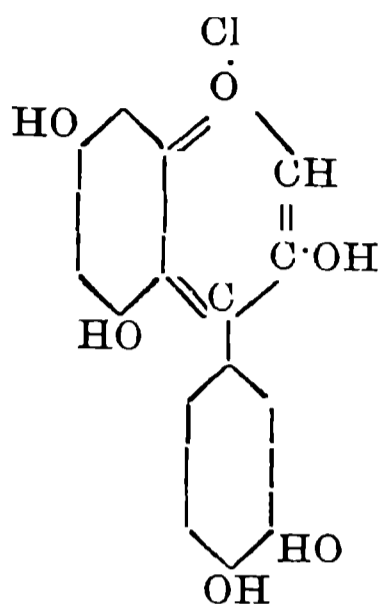
Far more convincing is the fact established by Willstätter and Everest (*Annalen*, 1913, 401, 231,) that natural cyanidin chloride yields a definite, well-crystallising product on oxidation, whereas it was shown by Pratt and Robinson (*J. Chem. Soc.*, 1925, 127, 172) that no such oxidation product can be obtained from synthetic 3 : 5 : 7 : 3' : 4'-pentahydroxyflavylium chloride. Malkin and Nierenstein have confirmed the observations of Willstätter and Everest and of Pratt and Robinson with the natural and synthetic products respectively. This in itself suffices to establish the fact that cyanidin chloride is *not* 3 : 5 : 7 : 3' : 4'-pentahydroxyflavylium chloride. They also showed that the oxidation product from natural cyanidin chloride is not quercetin, as believed by Combes (*Compt. rend.*, 1913, 157, 1002, 1454) who assumed that quercetin and cyanidin are interconvertible in the living plant.

In conclusion, attention may also be drawn to the following:—

(1) That X-ray analyses of cyanidin chloride and 3:5:7:3':4'-pentahydroxyflavylium chloride (synthetic cyanidin chloride) show *not a single* crystal spacing which is common to both substances, as already published by Malkin and Nierenstein (*Ber.*, 1928, 61, 794).

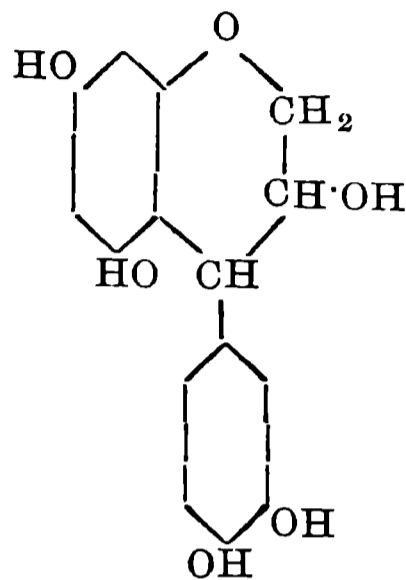
(2) That Freudenberg (*Annalen*, 1925, 444, 143) has succeeded in reducing cyanidin chloride to epi-catechin. From a series of investigations published by the author it is evident that catechin is

represented by formula (VI), which would suggest formula (V) for cyanidin chloride.



(V)

Cyanidin chloride [?]



(VI)

Catechin

It is not, however, suggested with any degree of certainty that cyanidin chloride is represented by this formula, since up till now neither the epimerisation of catechin to Freudenberg's epicatechin (Nierenstein, *J. Indian Chem. Soc.*, 1930, 7, 279) nor its production from cyanidin chloride on reduction has been confirmed.

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