

betanin and in the readiness with which the crude betanin could be purified.

We have used absorption in the ultra-violet as a criterion for purity and have found that pigment from the variety Green Top Bunching is most easily freed from impurities absorbing in the ultra-violet region. Indeed, pigment prepared from Green Top Bunching by the method of Pucher, Curtis and Vickery⁷ is practically free from ultra-violet absorption.

On the basis of the above considerations it is concluded that betanin is a pyrrole pigment and not a "nitrogenous anthocyanin". This does not preclude the presence of "nitrogenous anthocyanins" in other plant species⁸.

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THE interesting communication of R. G. Peterson and M. A. Joslyn on this subject calls for some comment. In the first place, the term "nitrogenous anthocyanin" is used partly chemically (nitrogenous) and largely botanically (anthocyanin) to denote an obviously related group of water-soluble bluish-red colouring matters, such as those of *Beta*, *Atriplex*, *Celosia*, and certain Cactaceae. The only evidence favouring some form of amino-flavylium salt structure, which was the basis of a working hypothesis, was the colour and some other properties of synthetic amino-flavylium salts containing hydroxyl and methoxyl groups¹. There was a certain resemblance between the absorption spectra in the visible region of betanin and 4'-amino-3:7-dihydroxyflavylium chloride. However, no phenolic degradation products could be obtained from betanidin². We have always recognized that the relation to the flavylium group was unconfirmed and a purely speculative hypothesis. The evidence in favour of a pyrroloid constitution is not much stronger.

The argument from spectra is double-edged and inconclusive in the absence of appropriate models. Thus hydroxyl- and amino-substituents tend to erase fine structure in the visible region and ultra-violet spectra, while it is hard to predict with any confidence the infra-red band positions for likely structures that could explain the colour of these pigments. It is true that $C_{20}H_{23}O_7N_2Cl^2$ could be $C_8H_8N_2Cl$ (dipyrromethene salt) + $C_6H_{10}O_5$ (sugar fragment, perhaps as in barbaloin) + $C_6H_4O_2$ (many possible interpretations), but bougainvillaidin is a member of this group of pigments with about C_{22}/N in the molecule³.

Although it is possible that there are two (or more) types of nitrogenous anthocyanins, and although a pendent C_{20} group cannot be excluded, the more natural view is that the essential content of the chromophore-cum-auxochrome may include only one nitrogen atom. Some years ago my colleague Dr.

J. W. Barltrop suggested an attractive possibility for betanin, but this also demanded N_2 , and bougainvillaidin seemed to be an obstacle at that time.

The solution of the problem awaits further work, especially some revealing degradation.

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Molecular Changes in Exchange Labelling with Tritium

WILZBACH¹ has recently described a convenient method for labelling organic compounds involving their exposure to tritium gas; but both he and others^{2,3} found that appreciable amounts of the exchanged radioactivity appeared in traces of highly tritiated contaminants as well as in labile positions. We have applied this method to the labelling of 1:6-dihydroxyhexoestrol, an oestrogen analogue⁴, and similarly have found that impurities contain the major part of the incorporated non-labile tritium.

These results could be explained by a preferential exchange of tritium for the hydrogen atoms of trace amounts of impurity present in the starting material. Alternatively, highly radioactive by-products might have been formed from chemically pure material by degradation in the course of the exchange reaction.

In order to differentiate between these two possibilities, dihydroxyhexoestrol (10 mgm.) was exposed to tritium gas (200 mc.) for 14 days. The product, after removal of labile tritium, was washed three times with ether; more than 60 per cent of the radioactivity was hereby removed, although dihydroxyhexoestrol itself is virtually insoluble in this solvent. Finally, crystallization of the ether-washed material from aqueous ethanol gave a product which retained its specific radioactivity on further crystallization. On re-exposure of the purified material to tritium gas, comparable amounts of radioactive impurities soluble in ether were again formed. These radioactive contaminants are thus produced by changes in a chemically pure organic compound when it is exposed to tritium gas. Identification of the by-products is now being attempted.

These results further demonstrate the necessity for rigorous purification of radioactive compounds prepared by this useful labelling method.

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