VIII. NOTE ON THE USE OF BUTYL ALCOHOL AS A SOLVENT FOR ANTHOCYANINS.

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In the purification and preparation of anthocyanins from flowers or fruits, there is need for a solvent, not miscible with water, which will extract the pigments from their solution in dilute acids. Amyl alcohol, the only solvent available so far, has the disadvantage that only mono- and rhamnoglucosides are soluble in it to any extent and even in their case the solubility is so small that as many as twenty extractions have been found necessary. The large bulk of solvent required to work up even small quantities of the colouring matter makes its use impracticable for the purification of the pigment in quantity. Willstätter and Zollinger in their work on the pigment of sloes, have therefore attempted to replace amyl alcohol by other solvents, without, however, finding a suitable one. (Phenol and cyclohexanol were the solvents tried.)

In the course of an investigation into the formation of the pigments in the grape vine, I observed that butyl alcohol removes all the anthocyanins (mono- and rhamnoglucosides as well as normal diglucosides) from their solution in dilute acids. The sugar-free pigments (anthocyanidins) are still more soluble in this solvent. On repeatedly washing the butyl alcoholic layer with fresh dilute acid, the non-glucoside pigments remain in the butyl alcohol, whilst the glucosidic anthocyanins pass again into the acid aqueous layer as oxonium salts. Butyl alcohol behaves therefore in this respect exactly like amyl alcohol.

As this observation promised to be useful for the purification of anthocyanins, I made some experiments with typical representatives of each type and compared quantitatively their solubility in butyl alcohol, taking their solution in amyl alcohol as a standard. In order to make the conditions comparable to those obtaining during their purification, the anthocyanin solutions were prepared by extracting the petals of flowers or skins of fruits with ethyl alcohol containing 1 per cent. hydrochloric acid and precipitating the filtered extracts with ether. After washing the pigments with ether and removing the latter with a current of air, they were dissolved in $N/2$ sulphuric acid and the solutions diluted to a uniform arbitrary tint. They were then divided into two parts, of which one was shaken out with an equal volume of butyl alcohol and the other with amyl alcohol under identical conditions. The alcohols were previously saturated with the dilute acid employed, in order to keep the volume of the solutions constant. The relative strength of the first extract with each solvent was determined colorimetrically in a Hellige-Autenrieth's Colorimeter. The extractions were then continued until all but traces of the pigment was removed. The figures given under (1) in the following table refer to butyl alcohol only.

	Mono-glucosides			Rhamnoglucosides	
	Chrysanthemin	Oenin	Idaein	Prunicyanin	Violanin
1. Number of extractions	2–3	3–4	3–4	4–5	4–5
2. Solubility (amyl alcohol = 1)	4	4	10	16	25

The diglucosides examined were pelargonin, cyanin, and delphinin. They required from 7–8 extractions for the complete removal of the pigment. No comparative figures for their solubility in butyl alcohol can be given, as they are practically insoluble in amyl alcohol. It will be seen that even the less soluble anthocyanins possess a solubility in butyl alcohol four times as great as that in amyl alcohol, whilst that of the most soluble examined is twenty-five times as great. The unidentified anthocyanins of numerous flowers and fruits were, without exception, found to be soluble in butyl alcohol[1].

It would appear from these results that butyl alcohol, which is now a commercial product, would be of advantage in the preparation of mono- and rhamnoglucosides especially. The solubility of the diglucosides in the solvent, whilst not sufficient for purposes of preparation, is of such an order as to make it useful in those cases where their removal is essential, e.g. for the study of the colourless substances accompanying them in the plant.

I have successfully used butyl alcohol in the preparation, on a relatively small scale, of oenin (from grapes) and of idaein (from cranberries), the well-crystallised picrates of which may be easily obtained from the butyl alcohol extracts by the usual methods. Butyl alcohol may also replace ethyl alcohol and glacial acetic acid for the direct extraction of anthocyanins, especially from dried petals or leaves. In the latter case the pigment is only soluble in butyl alcohol when present as the oxonium salt of a mineral acid. This has the advantage that by a preliminary treatment of the dry powder with the solvent, such substances as chlorophyll, flavones, etc., are removed. The powder is then treated with butyl alcohol containing 2–3 per cent. hydrochloric acid, which extracts the anthocyanin as oxonium salt. From this solution the pigments are precipitated by light petroleum and purified by appropriate methods.

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[1] It is interesting to note that the pigment of beetroot, which differs in many respects from anthocyanins, is insoluble in butyl alcohol.