may be used for giving a quantitative estimate of ketohexoses and their derivatives. Optical density at 625 mμ is proportional to ketose concentration in the range 0·001–0·003 *M*. The method is less sensitive than the estimation of total carbohydrate by heating, but in the system investigated (elution of sugars from charcoal columns) the concentration of sugars in the fractions was sufficient to give satisfactory results. Fig. 1 shows the application of the method to the incompletely resolved peaks of sucrose and lactose eluted from a charcoal column.

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Enzymatic Identification of the Anthocyanin Pigment of Blackberry

Karrer and Pieper reported in 1930 that the principal pigment of blackberry, *Rubus fructicosus* L., was either identical or isomeric with chrysanthemin or cyanidin-3-β-monoglucoside[1]. They obtained compact polyhedral rhombs of a chloride which was indistinguishable in colour properties from an authentic sample of chrysanthemin chloride, and differed from it only in respect of crystal form. Natural chrysanthemin chloride from *Chrysanthemum indicum* L.[2], and the chloride of synthetic cyanidin-3-β-monoglucoside[3] were known to crystallize in diamond-shaped leaflets. It was suggested that the observed difference might be due to traces of impurity in the product from blackberry[1]. Afterwards, blackberry was shown to contain a second unidentified anthocyanin by paper chromatography[4]. More recently, I have isolated from blackberry[5] a crystalline anthocyanin chloride, essentially free from the second minor component. The product was obtained in leaflets, and appeared to bear a reasonable resemblance to the crystals of chrysanthemin chloride shown in a published microphotograph[2]. However, an air-dried sample of this material was analysed correctly as a monohydrate[5], while chrysanthemin chloride crystals had previously been found to contain 1½ moles of water of crystallization[2,3]. No analysis for an air-dried sample was provided by Karrer and Pieper[1].

The data available[1,5] indicate conclusively that the anthocyanin from blackberry is a cyanidin-3-monoglucoside. Its identity with chrysanthemin would require that it be a β-glucoside. Confirmation on this point, however, appeared desirable in the light of results acquired in a study on the decolorization of berry fruit anthocyanins by fungal enzymes. It was found that the process involved, first, an enzymatic hydrolysis of the anthocyanin to anthocyanidin and sugar, and secondly, a spontaneous conversion of the aglucone into colourless derivatives[5]. Thus, if the blackberry pigment were actually a β-glucoside, the classical β-glucosidase of almond emulsin, which is capable of hydrolysing a variety of phenolic β-glucosides[6], would be expected to be an effective decolorizing agent. Emulsin, nevertheless, was found

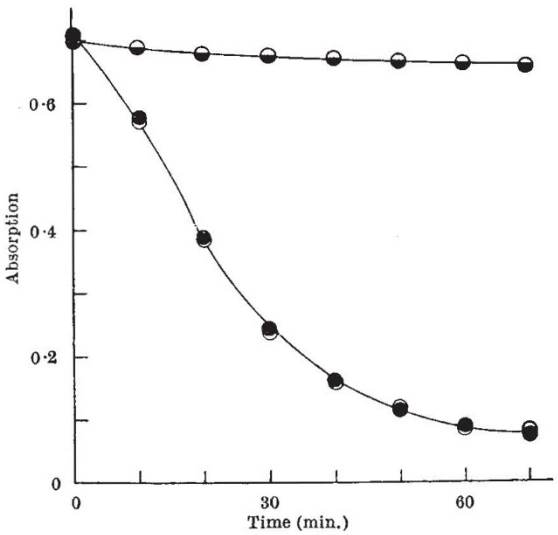

Fig. 1. Effect of anthocyanase *CN* 558 and emulsin β-glucosidase on blackberry anthocyanin and synthetic chrysanthemin. Each optically calibrated tube contained 1·10 μmole pigment and enzyme in 5 ml. of 0·045 *M* sodium lactate buffer at *p*H 3·95. The tubes were incubated at 30° C. and readings of the absorption at 510 mμ made at intervals as indicated. ⊙, Synthetic chrysanthemin and 1 mgm. of *CN* 558; ●, blackberry anthocyanin and 1 mgm. of *CN* 558; ◒, either pigment and 1 mgm. emulsin. Under the same conditions of enzyme, substrate and buffer concentration, hydrolysis of salicin by emulsin was 42 per cent in 60 min.

to have no measurable decolorizing action on blackberry anthocyanin.

It was possible to resolve the nature of this apparent discrepancy when a specimen of synthetic cyanidin-3-β-monoglucoside chloride became available. Equimolar quantities of the synthetic pigment and the blackberry product were each subjected to the action of anthocyanase *CN* 558 and of almond emulsin respectively. Both samples were dried at 50° C. *in vacuo* for 24 hr., and used in the anhydrous state. The enzyme preparations and experimental conditions were the same as those described previously[5]. The results are summarized in Fig. 1. The curves for the decolorization of the natural and synthetic pigments by *CN* 558 are evidently identical. They demonstrate conclusively, therefore, that the blackberry anthocyanin is chrysanthemin. Differences in crystal form and amount of water of crystallization noted earlier are consequently of minor importance. Furthermore, Fig. 1 shows that neither sample is attacked by emulsin. Lack of hydrolysis is accordingly a manifestation of the inherent specificity of emulsin β-glucosidase, and need not be taken as indicating the possibility that the glucosidic linkages in the substrates might be in the α-configuration.

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