Anthocyanins of Blood Oranges

THE fleshy endocarp of the blood orange, a variety of sweet orange, *Citrus sinensis*, contains a water-soluble red pigment which Matlack[1] showed to be anthocyanin in nature.

Factors influencing the production of anthocyanin in Sicilian varieties have been discussed by Carrante[2]; estimates of the amounts found in the juice have been made by Ajon[3]; and Patanè has published a method for its partial isolation[4]. However, no formal identification has apparently yet been made. Evidence is presented here to show that cyanidin-3-glucoside is the major pigment of the Moro variety of the blood orange, with a second pigment, probably delphinidin-3-glucoside, as a minor component.

Extraction of the pulp (2 kgm.) with methanol containing 1 per cent hydrochloric acid was followed by precipitation of the pigment as the lead complex. The precipitate was decomposed with hydrochloric acid, and the recovered pigments were taken up in butanol to which 4 volumes of benzene were then added. The solution was extracted with one volume of 1 per cent hydrochloric acid. The difficultly soluble hesperidin remained in the organic layer from which it was slowly deposited on standing. Purification was effected by cellulose column chromatography with the organic phase of butanol/2*N* hydrochloric acid as solvent, used for separation of the black currant anthocyanins[5]. Three bands appeared on the column as elution proceeded: a rapidly moving brown band, a scarlet band, and a small slowly moving mauve band. The eluate was collected in 50 ml. fractions and a graph of the optical density at 535 mμ against fraction number indicated that the third pigment represented only about 1 per cent of the total.

The pigment in the first band was concentrated by precipitation as the lead complex and the other two by absorption on a second cellulose column as already described[5]. Paper chromatography of these concentrates and their hydrolysis products gave the following results, confirmed where possible by absorption spectra and colour tests.

(*a*) The pigment in band 1 consisted largely of red-brown material running at all times with the solvent front. Also present were traces of an anthocyanidin, chromatographically identical with cyanidin, obtained by hydrolysis of the second strawberry pigment, cyanidin-3-glucoside[6]: R_F in butanol/2*N* hydrochloric acid, 0·75; in Forrestal solvent, 0·49.

(*b*) The pigment in band 2, amounting to about 95 per cent of the total, was a cyanidin derivative, the aglycone obtained on hydrolysis by treatment with 5 per cent perchloric acid for 30 min. at 50° C. being chromatographically identical with cyanidin. The anthocyanin behaved as a 3-glycoside, co-chromatographing with the second strawberry pigment in four solvent systems: R_F in butanol/2*N* hydrochloric acid, 0·26; in butanol/acetic acid/water, 0·38; in *m*-cresol/acetic acid/water, 0·34; in Forrestal solvent, 0·74.

(*c*) The pigment in band 3 was a delphinidin derivative, and chromatographed as a 3-glycoside in the four solvent systems given above, with R_F values of 0·16, 0·21, 0·16 and 0·65, respectively. The aglycone obtained on acid hydrolysis was chromatographically identical with delphinidin, the hydrolysis product of delphin, obtained from blue delphiniums and purified by column chromatography: R_F in butanol/2*N* hydrochloric acid, 0·45; in Forrestal solvent, 0·28.

(*d*) Glucose was the only sugar present in the hydrolysates from the two glycosides, co-chromatography with glucose producing no separation in three solvent systems: R_F in *n*-collidine, 0·38; in ethyl acetate/water/pyridine (2 : 2 : 1), 0·30. R_x in water-saturated *n*-amyl alcohol, 0·42 (R_x of xylose, 1·00). Galactose in these solvent systems gave values of 0·35, 0·25 and 0·33, respectively.

From the hydrolysate of the main pigment an osazone was prepared, melting point 205° C.; no depression when mixed with authentic glucosazone, melting point 207° C.; but depressing the melting point of authentic galactosazone (197° C.) by 15–20 deg.

These results identify the main pigment as cyanidin-3-glucoside, confirmed by the determination of the aglycone : glucose ratio, using the method and the molecular extinction coefficient given by Nordstrom[7].

Identification of the second glycoside as delphinidin-3-glucoside is based on its behaviour on the paper chromatogram, partition between solvents, colour tests, and chromatographic behaviour of the sugar moiety.

These observations apply specifically to the Moro variety. The main pigment of three other varieties, Tarocco, Florida and Ruby Red, is indistinguishable chromatographically from that of the Moro, and no major varietal difference seems likely in anthocyanin pigmentation.

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Incorporation of Radioactive Sulphur by *Thiobacillus thioparus*

IN the course of our investigations on the metabolism of sulphur in the autotrophic bacteria *Thiobacillus thioparus* we have found that this micro-organism metabolizes only the outer sulphur atom of thiosulphate. The inner sulphur atom probably does not enter the bacterial cell and is left in the medium as a sulphate ion[1]. We carried this investigation further with thiosulphate labelled in the outer position with sulphur-35 ($Na_2O_3S^{35}S$), tracing the pathway of the radioactive sulphur in the bacterial cells.

The pool of free amino-acids and related compounds were investigated in the following manner: the ethanol extract from a culture of *Th. thioparus* which had been incubated for 24 hours with radioactive thiosulphate was separated by paper electrophoresis and chromatography, after which autoradiograms were made. We proved that radioactive sulphur is incorporated into cysteine, cystine and methionine as well as in cysteic and cysteinesulphinic acids. A few other radioactive spots were also