Nitrile	CH_3-CN	CF_3-CN	NC-CN
S.T.Y., moles/hr./100 cc. $\times 10^4$	4[a]	98	170

[a] G. J. Janz and S. C. Wait, Jr., THIS JOURNAL, **76**, 6377 (1954).

Inspection of the space–time yields accordingly shows that the relative reactivities for acetonitrile, trifluoroacetonitrile and cyanogen are in the ratio of 1:25:43, respectively. The high space–time yield for cyanogen may be attributed in part to the electronic polarizability of the (CN) group and to the fact that there are two potential reaction centers present in this molecule.[11] The enhanced reactivity of trifluoroacetonitrile undoubtedly is due to the influence of the strongly electrophilic (CF_3) group in this molecule. If the higher space–time yield of cyanogen is indeed partly due to the fact that there are two potential reaction centers in each molecule, one may predict that the reactivity of the (C≡N) in trifluoroacetonitrile is the same order of magnitude as in cyanogen. A kinetic study of the trifluoroacetonitrile–butadiene reaction is in progress in this Laboratory.

(11) G. J. Janz and associates, THIS JOURNAL, **74**, 1790 (1952); **75**, 1910 (1953).

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The Anthocyanin Pigments of Sour Cherries[1]

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This paper reports the isolation and identification of antirrhinin and mecocyanin from sour cherries. Crude pigments are obtained through their lead salts, and separation into two individual crystalline pigments is achieved by using silicic acid chromatographic columns. Identities of pigments have been established by means of paper chromatography, color reactions and absorption spectroscopy, including infrared, and by comparison with synthetic specimens.

In connection with the problems of scald formation and discoloration of sour cherries, an investigation of the anthocyanin pigments was made. The chemistry of the anthocyanins of sour cherries has had little attention since the early researches of Rochleder.[2] Willstätter and Zollinger[3] isolated keracyanin from sweet cherries. Robinson and Robinson[4] indicated that the skins of small black cherries (*Prunus avium*) contained cyanidin-3-monoside.

In the present investigation a modified partition chromatographic method involving silicic acid has been devised for the separation and purification of two closely related anthocyanin pigments of sour cherries and, incidentally, the separation also of a diglucoside, a monoglucoside and the aglycone. This method may be extended to handle other types of anthocyanin pigments. The use of infrared spectra for the identification of crystalline anthocyanins represents a simple and discriminating method which had not been employed previously with such highly colored compounds. These analyses can be run on samples as small as 1 mg.

Experimental

Preparation of the Crude Pigments.—The procedure for the preparation of the crude pigments was essentially that of Willstätter and Zollinger[3] in handling sweet cherries. The skins of ripe, fresh sour cherries (*Prunus cerasus* L., var. Montmorency) were extracted with methyl alcohol containing 1% hydrochloric acid. The extracted pigments were precipitated as lead salts, and regenerated by dissolving in methyl alcoholic hydrochloric acid. From the regenerated solution, crude pigments were obtained as a dry red powder by the addition of anhydrous ether.

Separation of the Components in the Crude Anthocyanin Preparation.—A preliminary analysis of the anthocyanin pigments in the skins by paper chromatography has revealed two components of nearly equal concentration, the relative proportions of which remained unchanged in the crude pigment preparation.

Attempts to separate the crude pigments into their individual components by the alumina absorption methods of Karrer and Strong,[5] and of Price and Robinson[6] were unsuccessful. Separation, however, was achieved by a modification of the Spaeth and Rosenblatt[7] method, which had been used for the resolution of microgram quantities of mixtures of synthetic anthocyanidins. In the present modified method, the coarser particles of silicic acid were used instead of the finer particles, and the solvent system was changed from the originally described phenol–toluene–phosphoric acid to *n*-butyl alcohol–acetic acid–water (4:1:5 v./v.).

A typical column 3.5 × 54 cm., prepared from 250 g. of silicic acid, could thus handle 200 mg. of the crude pigments. The chromatogram was developed with the upper layer of the solvent system into two distinct zones (upper zone fraction A, lower fraction B), and elution was complete in about 3 hours at 6.5 ml. per minute.

For ease of handling, each of the anthocyanin fractions in the alcohol-rich eluate was transferred to aqueous solution by shaking with an equal volume of light petroleum ether together with a few ml. of 1% hydrochloric acid. This transfer effected a reduction of about 70% in volume. Crude individual components were obtained dry by evaporation of the aqueous solution under vacuum at room temperature.

Purification and Characterization of Fraction B.—Fraction B was further purified by repeated passage through the

(1) Journal Paper No. 1011, New York State Agricultural Experiment Station.

(2) F. Rochleder, *Ber.*, **3**, 238 (1870).

(3) R. Willstätter and E. H. Zollinger, *Ann.*, **412**, 164 (1916).

(4) G. M. Robinson and R. Robinson, *Biochem. J.*, **25**, 1687 (1931).

(5) P. Karrer and F. M. Strong, *Helv. Chim. Acta*, **19**, 25 (1936).

(6) J. R. Price and R. Robinson, *J. Chem. Soc.*, 449 (1937).

(7) E. C. Spaeth and D. H. Rosenblatt, *Anal. Chem.*, **22**, 1321 (1950).

silicic acid column, which freed it from the brownish admixtures. The anthocyanin obtained dry from the final aqueous solution was crystallized from methyl alcohol containing 2% hydrochloric acid, in clusters of fan-shaped brown-orange needles, when left to evaporate slowly in a loosely covered dish at room temperature.

By the method of Robinson and Robinson,[8] fraction B was shown to be a pentoseglycoside as distinguished from other diglycosides. In order to identify the aglycone and the sugar residue, an acid hydrolysis of fraction B was carried out.

(a) **Identification of the Anthocyanidin.**—The anthocyanidin from the hydrolysis of fraction B has a crystalline form[9] and melting behavior[10] suggestive of cyanidin. On paper chromatograms, it has R_f values of 0.68–0.69 in *n*-butyl alcohol–2 *N* HCl (1:1), agreeing with those given by Bate-Smith and Westall[11] for cyanidin. It also showed the cyanidin color tests of Robinson and Robinson,[4] and Robertson and Robinson.[12] Such properties were compared with crystalline cyanidin chloride, prepared by hydrolyzing synthetic chrysanthemin (cyanidin-3-monoglucoside), and shown to be identical. The absorption spectra of these two anthocyanidins in 95% ethanol containing 0.1% HCl were identical, with maximum absorptions at 550 and 278 mμ. These spectra closely resemble those of cyanidin as published by other authors.[9,13]

(b) **Identification of the Sugars.**—From the sugar solution after the acid hydrolysis of fraction B, osazone derivatives were prepared with phenylhydrazine. Two different forms of osazone crystals with different melting points were observed under the hot-stage microscope. However, the mixed osazones after recrystallization from 70% ethanol, gave but one form of fine yellow needle crystals, which melted at 182° (cor.). Osazone derivatives of an equimolecular mixture of glucose and rhamnose showed the same behavior as above.

The sugar solution gave two spots on paper chromatograms, corresponding to those of rhamnose and glucose. Authentic samples of a mixture of glucose and rhamnose run alongside the unknown sugars showed no significant differences in their corresponding R_f values in several solvents.

(c) **Identification of the Anthocyanin.**—It having been concluded that fraction B was a rhamnoglucoside of cyanidin, a study was made of its properties by comparison with a synthetic sample of antirrhinin, cyanidin-3-rhamnoglucoside.

On paper chromatograms, fraction B and antirrhinin, individually and when co-chromatographed, have the same R_f values, 0.34 in *n*-butyl alcohol–acetic acid–water (4:1:5 v./v.), and 0.54 in phenol–water (73:27 w./w.).

The absorption spectra of fraction B and antirrhinin, in both the ultraviolet and visible regions were determined in 60% ethanol containing 0.1% HCl and shown to be identical, having the same absorption peaks at 532, 333 and 282 mμ. Hayashi, *et al.*,[14] have reported almost the same three maxima for keracyanin which, according to Robinson and Robinson,[8] is identical with antirrhinin. In addition the infrared absorption spectra of fraction B and antirrhinin were determined with the Perkin–Elmer model 21 recording infrared spectrophotometer, employing the KBr pellet technique.[15] The results of this analysis showed that the two compounds were identical.

The color reactions of fraction B and antirrhinin in graded *p*H solutions recommended by Robertson and Robinson,[12] were identical, and in agreement with those recorded by Scott-Moncrieff[9] for antirrhinin isolated from the flowers of *Antirrhinum majus*.

Purification and Characterization of Fraction A.—By the solvent partition method of Robinson and Robinson,[8] fraction A was shown to be a diglycoside, which, however, was not extracted by the amyl alcohol–acetophenone–picric acid reagent[16] for diglycosides.

When fraction A was further purified by passing through the silicic acid column, it gave three distinct red bands, the upper one being the unchanged diglycoside, the two lower ones were, in minor proportions, the monoglycoside and the aglycone. This partial decomposition which occurred during the time when fraction A in aqueous solution was being dried very slowly under vacuum at room temperature, rendered crystallization of the anthocyanin difficult. However, fraction A was finally obtained as microcrystals by lyophilizing the aqueous pigment solution, and was hydrolyzed in 20% HCl for the identification of the aglycone and the sugar components.

(a) **Identification of the Anthocyanidin.**—The anthocyanidin from the hydrolysis of fraction A was shown to be cyanidin by the tests previously described for fraction B.

(b) **Identification of the Sugar.**—From the sugar solution after the acid hydrolysis of fraction A, an osazone derivative was prepared with phenylhydrazine, and this proved to be identical in appearance and melting point with the osazone prepared from pure glucose. That the sugar was glucose was confirmed by paper chromatography. The unknown sugar showed only single spots with several developing solvents, and their R_f values were the same as glucose, which had been used alongside also with fructose and galactose for the control.

(c) **Identification of the Anthocyanin.**—Willstätter and Zollinger[3] have distinguished three types of diglycosides of cyanidin, represented by cyanin, mecocyanin and keracyanin by the delicate sodium carbonate test. Fraction A gave the mecocyanin reaction with sodium carbonate. On paper chromatograms, fraction A gave spots of R_f value 0.28 in *n*-butyl alcohol–acetic acid–water (4:1:5), corresponding to that of mecocyanin as reported by Bate-Smith and Westall.[11] The absorption spectra of fraction A and fraction B were almost identical in the ultraviolet and visible regions, but showed some marked differences in the infrared.

Willstätter and Weil[17] have reported that mecocyanin easily gives chrysanthemin under mild hydrolysis. A controlled partial hydrolysis of fraction A according to the method of the above authors yielded a monoglucoside, which was identical in properties with a synthetic specimen of chrysanthemin.

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