inserted between the phenyl and carboxyl group increased, root growth inhibition increased even though the rate of change (slope) was not significantly different among the acids. The root growth inhibition is not attributed to the acidity of the organic acids since the pH did not change for the acids at the concentrations studied. Therefore, the growth inhibition by these compounds can be due to their ability to interfere with enzyme activity. Williams (1963) has shown the effects of simple phenols, chlorogenic acid, and flavonoids against polygalacturonase activity and tissue macerating. Flavonoids like naringenin, found in dormant peach buds, antagonize the action of gibberellins (Phillips, 1962) as well as 5,4'-dihydroxy-7-methoxyflavonone isolated from *Betula verrucosa* (Popravko et al., 1974).

For establishment of the effect of hydroxyl substitution on the phenyl group to root growth inhibition, a series of *p*- and *o*-hydroxyphenyl aliphatic acids were tested (Figure 5). In each case, the ortho-substituted acids were more active than the corresponding para isomers. Blockage of the *o*-hydroxyl group eliminates the phytotoxic properties. The root growth inhibition properties of the coumarin-type compounds are due to the configuration of the molecule.

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Received for review November 2, 1981. Accepted March 19, 1982. MAFES paper no. 5000. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

COMMUNICATIONS

Isolation and Identification of Phenolic Glucosides in Liquid Sugars from Cane Molasses

The major colored components of the orange-yellow liquid sugar from cane molasses have been isolated and fractionated with chromatographic techniques. The main components at low molecular weight have been identified as phenolic glucosides through enzymatic hydrolysis and GC-MS spectrometry.

Liquid sugars obtained from several cane molasses by ion-exchange demineralization and decolorization (Baldassarri, 1972) are always retaining a light yellow-orange color resistant to the usual forms of industrial refining. The purpose of our work was to characterize the minor components of the syrups from cane molasses and obtain more information to improve the industrial decolorization process.

Several colored components from cane and beet juices and sugars have been characterized in the past and classified in four major classes: plant pigments, poliphenolic compounds, caramels, and degradation products of sugars condensed with amino derivatives (Binkley, 1970; Tu, 1974). Methods used to investigate complex mixtures of colorants of cane sugar and juices have already been described; they include colorimetric determination of phenolics, separation by gel filtration for higher molecular weight compounds (Tu, 1974; Muro et al., 1974), precipitation with methanol, and sometimes solvent extraction (Farber et al., 1971). Colored components in raw cane sugar have also been studied by paper electrophoresis and identified as chlorogenic acid, cinnamic acids, and flavones (Farber and Carpenter, 1971; Carpenter et al., 1975). However, with know methods we did not obtain satisfactory recovery of colorants from our can molasses liquid sugars; due to the great sugar affinity they remained in the sugar even after decolorization processes with anionic macroporous resins. Therefore we carried out a new method of recovering highly hydrophilic colored components from sugar syrups, by absorption on nonionic polymers and elution with methanol. We describe here the isolation of the colorants, the fractionation on Sephadex G-10, the purification of the low molecular weight colored fraction by silica gel column chromatography, and their characterization by enzymatic hydrolysis and by GC-MS spectra of the silylated derivatives. In this way a new series of

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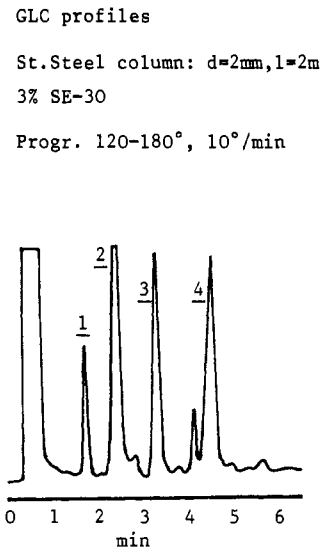
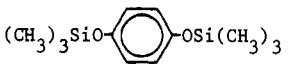
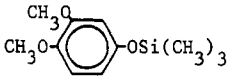
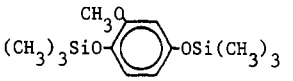
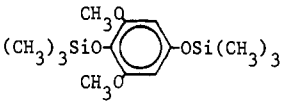
Scheme I. GLC Profile of the Silylated Aglycons Recovered from the B Band Hydrolysate, with Composition and *m/e* Values of the Four Major Peaks



peak N°	structure	m/e (rel. int.)
1		254(M^+44),239(80),223(16),147(9), 133(12),112(41),73(100).
2		226(M^+71),211(100),183(9),167(8), 153(6),105(10),73(69).
3		284(M^+46),269(14),254(100),239(19), 119(8),112(17),73(38).
4		314(M^+51),299(75),283(92),271(44) 135(18),73(100).

phenolic glucosides that contribute to liquid sugar color have been identified.

EXPERIMENTAL SECTION

Colorant Recovery from Liquid Sugars. Several absorbing materials (granular carbon, ion-exchange resins, nonionic polymers) have been tested. The best result has been obtained on an experimental resin ES-861 (Dia-Prosim, Vitry, France), a nonionic acrylate polymer having about 500 m^2 of surface area/g. In two steps about 90% of the colored matter is recovered [primary step: 80%, valuation at 420 nm (Payne, 1968)]. Liquid sugar samples obtained by clarification, demineralization, and decolorization of cane molasses had the following average composition: dry substance, 70%; total sugars, 69.3%; ash, 0.05%. The samples were clear and showed an absorbance between 2 and 3 at 420 nm. These samples (200 g), diluted with distilled water (1:1 w/w), were passed through 200 mL of ES-861 resin in a glass column (70-cm bed height), with a flow rate of 1.5 bed volumes/h. Sugars were then displaced with distilled water, and absorbed colored matter was completely recovered by elution with 300 mL aqueous methanol (1:1 v/v). The amounts of the colorants recovered varied from 0.1 to 0.5% by weight of the total sugars of the samples, mainly depending on the source of the cane molasse utilized (16 different samples were examined, coming from several tropical countries).

Fractionation by Gel Filtration and by Silica Gel Column Chromatography. Gel filtration on Sephadex G-10 was carried out. A sample of colored methanolic solution from ES-861 was evaporated under vacuum; the solid residue (1.0 g) was dissolved in 4 mL of distilled water, charged on a glass column filled with the Sephadex (1.5 × 70 cm), and eluted with distilled water at the flow rate of 40 mL/h. Three major colored fractions were recovered, a brown fraction (I, 100–300 mg), a pale yellow fraction (II, 500–800 mg), and an orange-yellow fraction (III, 100–200 mg). The components of fraction III (200 mg), representing the 40% of the color recovered (420 nm), were placed on a silica gel column (1.0 × 30 cm; Kieselgel 60, Merck) and eluted at the rate of 1.0 mL/min with 30% methanol in ethyl acetate (v/v). The low molecular weight colored fraction collected (band B, 120 mg; R_f 00.65–0.70 on silica TLC plates in MeOH–ethyl acetate, 20:80 v/v) represented more than the 10% by weight of the starting material and has been characterized later.

Analytical Tests and Enzymatic Hydrolysis. The main colored fraction (band B) recovered by the silica column showed no reducing power with Fehling reagents, did not contain nitrogen, but gave a positive strong reaction with specific reagents for phenolics. The UV spectrum of its aqueous solution agreed with the phenolic nature of the components (Figure 1). Several attempts at the enzymatic hydrolysis were carried out; only almond β-glucosidase (Feinbiochemica, Heidelberg) was successful: 60 mg of the orange B band was dissolved in 1 mL of distilled water and treated with 10 mg of the enzyme at 25 °C for 10 h (enzyme activity = 5 units/mg). The brown hydrolysate was purified by column chromatography (column, 1 × 20 cm; Kieselgel 60, Merck), eluting with ethyl acetate; the main colored fraction recovered contained the aglycon mixture. The dried residue from this fraction (20 mg) was used for GC–MS characterization.

Gas Chromatography and Mass Spectrometry. Samples of the raw enzymatic hydrolysate (10 mg) and samples of the aglycon mixture (5 mg) were dried, dissolved in dry pyridine (0.2 mL), and treated with a mixture of trimethylchlorosilane and hexamethyldisilazane (30 μL, 1:2 v/v). After being warmed, the silylated samples were analyzed with a Varian Aerograph Series 2700 gas chromatograph connected to a single-focus Varian Mat CH5 mass spectrometer. A 2 m × 2 mm i.d. stainless steel column with a 3% SE-30 phase on 80–100-mesh Chromosorb W packing were used, with helium as carrier gas. The column temperature was programmed from 120 to 180 °C at 10 °C/min. As reference materials silanized sugars and phenols were used. Samples of the B band and enzyme have also been silanized and used as reference standards.

RESULTS AND DISCUSSION

Glucose was the only sugar revealed by GLC on the silylated hydrolysate of the B band; corresponding analyses of the B band and enzyme did not show interference peaks, so we assumed that our derivatives were glucosides; the kind of enzymatic activity, R_f values from TLC, and the lack of hydrolysis intermediates indicated only 1 mol of β-glucose released/mol of aglycon. A gas chromatogram of the silylated aglycons, recovered from the B fraction hydrolysate, is presented in the Scheme I, together with the principal *m/e* values of the four major peaks. The combination of these data and GLC retention times of

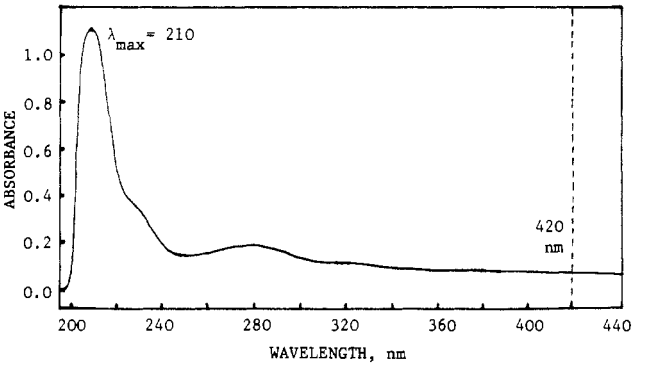


Figure 1. UV spectrum of the colored band (B band) recovered from fraction III by silica gel column chromatography (19 mg/L in distilled water).

some phenolic standards permitted us to recognize their structure. Peak 1 has been identified as *p*-hydroxyphenol (hydroquinone), whose β-glucoside, the arbutine, is a well-known derivative, already detected in plants (Pigman and Horton, 1970). Peak 2 has only one hydroxy group: the mass spectra and GLC retention time agree with 3,4-dimethoxyphenol. This compound is the major component of the aglycon mixture. We explain its high concentration due to the poor retention by the anionic-exchange reins used in processing of molasses, in consideration of the lack of free phenolic OHs; this structural feature increases also the chemical stability against oxidation. Peak 3 has a methoxy and two hydroxy groups asymmetrically disposed; peak 4 has two hydroxy and two methoxy groups; the structures proposed for these two compounds have been 3-methoxy-4-hydroxyphenol (peak 3) and 3,5-dimethoxy-4-hydroxy (peak 4).

Natural substances with similar ring substitution have already been isolated from cane and cane sugars: vanillic, veratric, *p*-hydroxybenzoic, and syringic acids have been recovered by solvent extraction (Farber and Carpenter, 1971). These products probably originate from microbial and chemical degradation of lignins during processing of cane and cane juice, even if some are already present in natural cane. The coloring characteristics of the phenolic compounds isolated was considered. We found the color in liquid sugar from cane molasses increased with time owing to the autoxidation of the phenolic glucosides. We could verify their oxidation on silica gel TLC chromatograms of the raw colored material: when exposed to air, the color intensity of the B band region increased in a few hours, changing from yellow-orange to orange-brown. We may conclude that phenolic glucosides and their oxidation products greatly contribute to the coloring of the liquid sugars examined, even if they represent only the 10–15% by weight of the isolated nonsugar components.

Other minor aglycons have been detected in the GLC profile (Scheme I), but their low concentration did not allow their identification. Our studies are now aimed at the identification of the compounds of the fraction II and at the characterization of other noncolored components, to obtain in this way a detailed and exhaustive composition of the nonsugars of this kind of syrup.

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Deuteration of Mutagenic Aromatic Nitrogen Heterocycles Derived from Protein and Amino Acid Pyrolyzates

The results are described of applications of two methods (platinum catalysis and acid catalysis) for deuterium exchange of three polycyclic aromatic amines of the type isolated from amino acid pyrolyzates. The results indicate that both methods lead to comparable total amounts of exchange when reactions are conducted for similar periods. However, the deuterium label is incorporated into several positions by the platinum catalysis method, which are unaffected by the acid catalysis method.

Several highly mutagenic polycyclic aromatic amines have been isolated from pyrolyzates of proteins and amino acids (Yamamoto et al., 1978; Yoshida et al., 1978; Kosuge et al., 1978; Wakabayashi et al., 1978). Preparation of radiolabeled derivatives of these compounds is desirable to facilitate the many chemical and biological studies anticipated for these substances. A method for deuterium and tritium labeling of a product of tryptophan pyrolysis has been described (Hashimoto et al., 1978). As models for their tritiation reactions we report here the application of two simple methods of deuteration of a soybean globulin pyrolyzate product (Yoshida et al., 1978), 9*H*-1,9-diazafluoren-2-amine (compound **2**), the corresponding parent compound, 9*H*-1,9-diazafluorene (compound **1**), and a

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