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Characterization of the Main Secondary Components of the Liquid Sugars from Cane Molasses

Gerardo Palla

The main nonsugar components of liquid sugars from cane molasses have been identified as phenolic and phenylpropanetriolic glucosides coming from the lignin material of cane during juice extraction and processing. The glucosides have been recovered by column absorption using nonionic polymers, have been fractionated through a silica gel column, and have been characterized by GC–MS, ^{1}H NMR, and ^{13}C NMR techniques.

In a previous work (Palla, 1982) we reported an efficient method to recover minor nonsugar organic colored components of sugar juices and syrups obtained by cane molasses demineralization. We reported also the structure of some phenolic compounds that we considered the main components of the sugar color. We have continued the work on the characterization of the nonsugar components, and we can now give an exhaustive description of the minor components of this type of sugar syrup. To this purpose we examined the silica gel chromatographic fractions of the raw organic material recovered from a wide series of liquid sugars; we found the greatest amount of nonsugars was composed by phenyl glucosides (10–20%), phenylpropanetriol glucosides (40–60%) and minor amounts of other phenolic derivatives. A fraction with high molecular weight has also been detected (10–30%). The characterization of the compounds has been made by MS, GC–MS, ^{1}H NMR, and ^{13}C NMR and by reference to synthetic standards such as phenols, phenyl glucosides, and phenylpropanetriols.

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EXPERIMENTAL SECTION

Recovery and Fractionation. The recovery of the colored nonsugar material has been made following the absorption method described for nonionic polymers (Palla, 1982); in this way 1.2 g of colored material (80% of the total nonsugars) has been recovered from 300 g of liquid sugar sample (70% of dry matter, 99.3% of total sugars). The material recovered was dissolved in methanol and filtered. The amount dissolved (1.05 g) was chromatographed through a silica gel column (1.5 × 70 cm, Kieselgel 60 Merk), eluted with ethyl acetate and methanol (7/3 v/v). A control on the column effluent showed eight main chromatographic bands: A (9 mg, R_f 0.78, pale yellow), B

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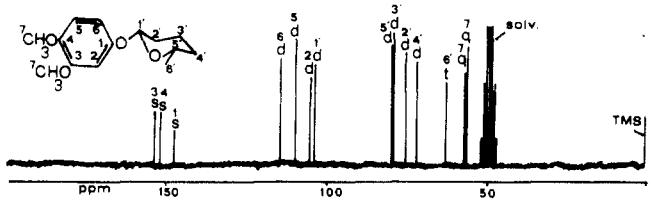

Figure 1. ^{13}C NMR spectrum of the 3,4-dimethoxyphenyl β-D-glucopyranoside, with the probable assignment of the lines.

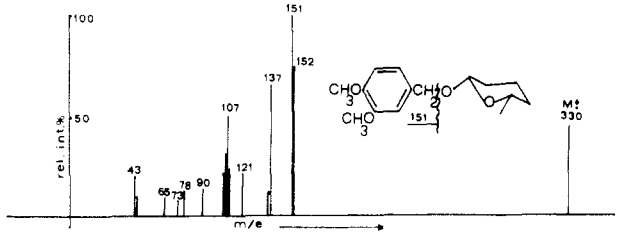

Figure 2. Mass spectrum of the 3,4-dimethoxybenzyl β-D-glucopyranoside.

(182 mg, R_f 0.63, orange), C (48 mg, R_f 0.50, pale yellow), D (32 mg, R_f 0.42, pale yellow), E (14 mg, R_f 0.35, pale yellow), F (512 mg, R_f 0.30, yellow), G (22 mg, R_f 0.18, pale yellow), H (71 mg, R_f 0.08, pale yellow). The strongest UV absorption was given by the bands B and F, corresponding to the fractions III (B) and II (F) recovered with Sephadex G-10 (Palla, 1982). Another brown fraction (110 mg) corresponding to the fraction I from Sephadex has been recovered by washing the silica column with methanol after completion of the chromatographic elution. This brown fraction was 68% retained in dialysis experiments by membranes with retention limits of 10 000.

Characterization of the Chromatographic Bands. Band A (9 mg) gave a color reaction with resorcinol and sulfuric acid but not with $FeCl_3$; the UV spectrum showed a large absorption band between 250 and 300 nm; the identification was unsuccessful. Band B (182 mg), as previously reported, is composed of several phenolic glucosides, the most abundant being the 3,4-dimethoxyphenyl β-D-glucopyranoside, of which we can now give the ^{13}C NMR spectrum, recorded on a Varian XL-100 instrument (FT 25.15 MHz, in CD_3OD) (Figure 1).

Bands C–E (96 mg) have been considered together. They seemed composed of small amounts of several compounds; free glucose and fructose were also detected in this fraction. The mass spectra performed on a Varian Mat CH-5 spectrometer, at 70 eV, suggested the presence of glucosides of ketols such as 1-(3,5-dimethoxy-4-hydroxyphenyl)-3-hydroxy-2-propanone (M^+ = 388) and 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-2-propanone (M^+ = 358) or their isomers. Traces of 3,4-dimethoxybenzyl glucoside have also been detected (M^+ = 330, identical with the synthetic sample, see Figure 2). The enzymatic hydrolysis of these fractions by α- and β-glucosidase failed; the treatment with methanolic acid solution (HCl, 8%, for 2 h, at 60 °C) provided products whose GC–MS analyses, performed on a Varian gas chromatograph connected with a single-focus Varian Mat CH-5 mass spectrometer, using a 1.5% SE-30 glass column and programmed conditions (100–250 °C, 20 °C/min), gave a series of peaks apparently confirming the presence of the ketols (M^+ = 226 and M^+ = 196) and simple polyphenols such as dimethoxyphenols (M^+ = 154), dimethoxyhydroxyphenols (M^+ = 170), and trimethoxyphenols (M^+ = 184).

Band F (512 mg, brown-green reaction with $FeCl_3$) was the most abundant fraction recovered; the enzymatic hydrolysis, carried out as previously reported for the B fraction, gave glucose and a series of aglucons that were silanized and characterized by GC–MS as follows: the raw hydrolysate (100 mg) was passed through a silica gel column (1 × 20 cm) eluting with ethyl acetate and methanol (8/2 v/v); the UV-absorbing fraction was collected, dried, and treated with 1 mL of silanizing reagent. After being warmed, the mixture was analyzed by GC–MS (Figure 3A). The phenolic nature of the aglucons was confirmed by GC–MS spectra of the peracetylated derivatives that were obtained by treating 100 mg of the raw hydrolysate with 2 mL of acetic anhydride and 1 mL of acetic acid under reflux for 2 h (Figure 3B).

Bands G–H (93 mg) had UV characteristics similar to the ones of F fraction and gave the same green reaction with $FeCl_3$. Enzymatic and acid hydrolyses did not give enough material for further analyses. The ^{1}H NMR spectrum showed signals in the aromatic region, as well as the products of the F fraction.

For the brown final methanolic fraction (110 mg), both enzymatic and acid hydrolyses released UV-absorbing products with slightly increased R_f compared with that of the starting material. Silanized products, however, did not show volatility enough for GC–MS analysis.

Synthesis of the Phenolic Standards. Phenolic glucosides, benzylic glucosides, and phenylpropanetriols have been synthesized by known methods to facilitate the identification of the compounds isolated. Glucosides have been obtained by reaction of phenol, 3,4-dimethoxyphenol, benzylic alcohol, and 3,4-dimethoxybenzylic alcohol with acetobromoglucose, in presence of silver oxide (Helferich et al., 1935). The acetobromoglucose has been synthesized from peracetylated glucose and hydrobromic acid (Horning, 1955). Phenylpropanetriols have been synthesized from the corresponding cinnamic acids (Adler and Gustafsson, 1963).

RESULTS AND DISCUSSION

The analytical studies on the chromatographic fractions A–H permitted us to recognize the nature of about 70–80% of the nonsugar material recovered from the liquid sugars examined. The β-glucosides of polyphenols and phenylpropanetriols represented 18 and 50%, respectively, of the total raw material extracted in the sample studied.

Phenyl glucosides, whose structure has been previously described (Palla, 1982), greatly contribute to the color of the syrup, as well as the phenylpropanetriols, which generate yellow compounds under oxidative conditions. The series of silylated and peracetylated phenylpropanetriols with GLC profiles and the main m/e peaks is reported in Figure 3.

The structure of the most abundant aglucons of the F band has been confirmed by their ^{13}C NMR spectra being in good agreement with the ones of similar compounds coming from lignin degradation (Lüdeman und Nimz, 1974) and by comparison with the synthetic products obtained from cynnamic acids. The ^{13}C patterns of the (4-hydroxy-3-methoxyphenyl)-, the (3,5-dimethoxy-4-hydroxyphenyl)-, and the (3,4,5-trimethoxyphenyl)-propanetriols are reported in the Figure 4.

The line assignments to the carbons have been made by considering similar products and models; in many cases, however, especially for substituted aromatic carbons, the assignments are probable but not certain. A problem is also the assignments of the configuration to the α- and β-carbons of the side chain (carbons a and b in Figure 4): we think the more abundant isomers have the threo form, as this one seems to be favored in the enzymatic synthesis (Higuchi et al., 1974). But in this way we assign the lower GLC retention time to the threo form, in contrast with the

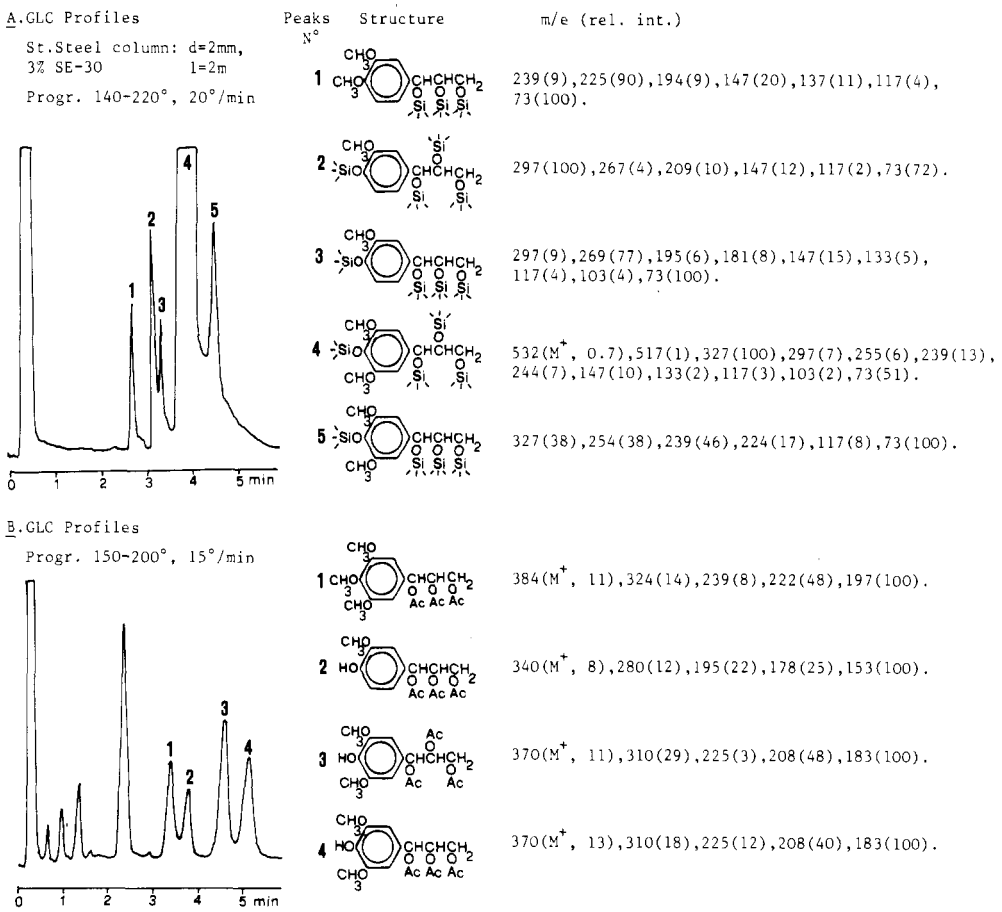

Figure 3. GLC Profiles and m/e values of the silylated aglycons (A) and of the acetylated aglycons (B) recovered from the F band hydrolysate.

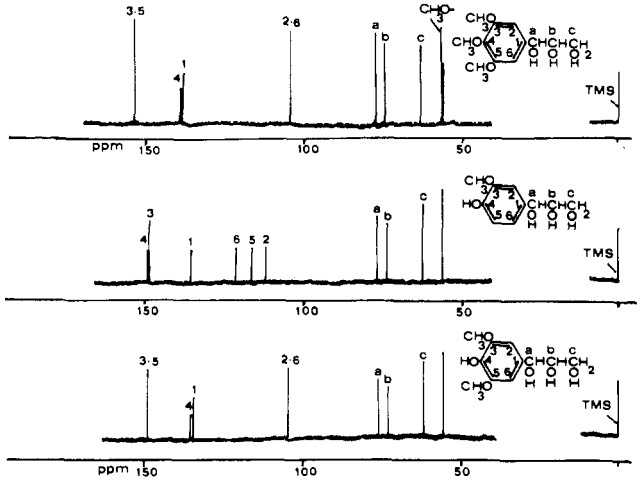

Figure 4. ^{13}C NMR spectra and probable assignments for natural phenylglycerols.

results of Nurok et al. (1968) that proposed lower GLC retention times for a series of erythro derivatives of butanediols.

We studied also the bonding site of glucose in the phenylpropanetriols. Examples are reported of bonding to the α-, the β-, and the γ-carbons (Theander, 1965). We could reasonably state through a mass fragmentation study made on natural and synthetic glucosides that the glucose, in our phenylpropanetriols, is bound to the α-carbon. In Figure 5 we report the typical fragmentation of a phenyl glucoside (path A), a benzyl glucoside (path B), a phenylpropanetriol glucose (path C), and a phenylpropanetriol (path D).

We can see that if glucose is bound to the aromatic ring or if the α-carbon brings a free hydroxy group, then the main mass peak retains the glycosidic oxygen or the hydroxy group (A, m/e = 154; D, m/e = 183); the glycosidic oxygen is instead missing when the glucose is bound to the α-carbon (B, m/e = 151), and this happens also in the

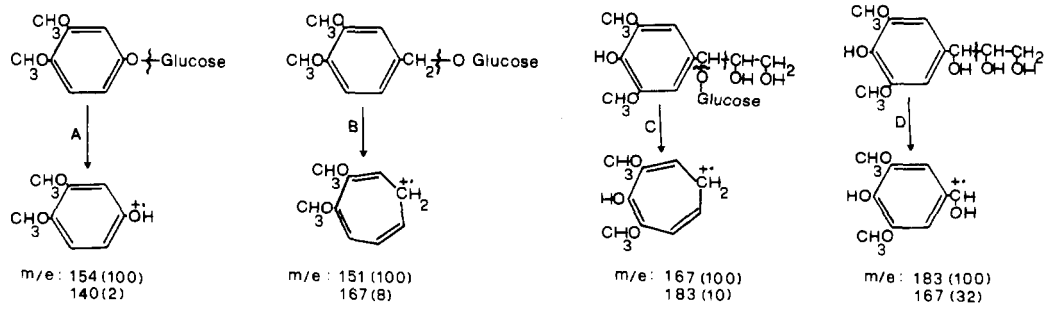

Figure 5. Typical mass fragmentation of phenolic glucosides, benzyl glucosides, and phenylpropanetriols.

fragmentation of our phenylpropanetriol glucosides (C, m/e = 167).

The phenylpropanetriols contribute to the color of the liquid sugars examined, probably as their oxidation or dehydration products. We found these products to behave like the phenyl glucosides, generating a bright yellow color when exposed to air on silica plates. The glucosides isolated most probably come from the lignin fraction of stalks of cane. It is known that acid media and high temperatures accelerate the depolymerization of the lignin with formation of phenolic material (Lundquist and Lundgren, 1972). It is not surprising that sugar syrups from beet molasses, produced without grinding and without acid treatment with phosphoric and sulfurous acid in the clarification steps, do not contain appreciable amounts of these phenolic derivatives. In the syrups from beet molasses we never detected more than 0.04% of phenolics (percent based on the total sugar content).

The knowledge of the chemical structure of the colorants suggests that anionic macroporous resins would decolorize these syrups. The resins can interact with the free phenolic groups and adsorb the organic polar compounds. Experiments until now gave satisfactory absorption only by passing the diluted and well-demineralized syrup through the resins, after regeneration with ammonia. Work is in progress to improve the decolorizing process.

The chemical and the biological significance of the presence of phenolic glucosides in liquid sugars has also been considered; trace amounts of these phenolics are very widespread in nature (Harborne, 1964), and toxic effects for human nutrition have never been reported in literature. The polyphenol derivatives can exert some antioxidant and antimicrobial effect (Harborne, 1964); we found that diluted liquid sugars from cane molasses were more microbiologically stable than the corresponding syrups from beet molasses, due, perhaps, to the higher content of phenolic compounds.

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Registry No. Lignin, 9005-53-2; 3,4-dimethoxyphenyl β-D-glucopyranoside, 84812-00-0; 3,4-dimethoxybenzyl β-D-glucopyranoside, 81381-73-9; 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-2-propanone, 4899-74-5; 1-(3,5-dimethoxy-4-hydroxyphenyl)-3-hydroxy-2-propanone, 35263-53-7; glucose, 50-99-7; fructose, 57-48-7; (3,4,5-trimethoxyphenyl)propanetriol, 76774-03-3; (4-hydroxy-3-methoxyphenyl)propanetriol, 1208-42-0; (3,5-dimethoxy-4-hydroxyphenyl)propanetriol, 4204-29-9.

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Gas-Liquid Chromatography-Chemical Ionization Selected Ion Monitoring Assay for Glycerol Formal in Animal Tissues

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Glycerol formal, a 60/40 mixture of the two cyclic condensation products [5-hydroxy-1,3-dioxane and 4-(hydroxymethyl)-1,3-dioxolane] of glycerol and formaldehyde, is used as a nonaqueous solvent in parenterally administered animal health products. Recently this substance was reported to be a teratogen when administered to rats in large doses. We have developed an assay for glycerol formal in the edible tissues (fat, kidney, liver, muscle) and plasma of food-producing animals (cattle, swine, horses). The assay utilizes packed column GLC–chemical ionization (isobutane) selected ion monitoring (glycerol formal-d_2 serving as the internal standard) and possesses an overall lower limit of sensitivity of 0.05 ppm. The maximum glycerol formal residue found in an edible steer tissue (injection-site muscle) at 5 days postdose (14.6 mg/kg of body weight) is ~0.1 ppm. Our teratolgy studies demonstrate that doses of glycerol formal less than 150 mg/kg do not elicit a teratogenic response in rats. Thus, the pressure of the negligible residue (~0.1 ppm) in steer injection-site tissue does not appear to represent a significant hazard to the consumer.

Although numerous studies aimed at detecting submicrogram quantities of drugs in the tissues of food-producing animals are carried out routinely in the development of new animal health drugs, little attention is usually given to the tissue concentration of the components of the dosing vehicle. Glycerol formal, a mixture of the cyclic condensation products of glycerol and formaldehyde, has been used as a nonaqueous solvent in parenterally administered products (Spiegel and Noseworthy, 1963) such as trivetrin ("ABPI Compendium of Data Sheets for Veterinary Products", 1978) and oxytetracycline (Green-

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