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Terminal Apiose: A New Sugar Constituent of Grape Juice Glycosides

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Apiose [3-(hydroxymethyl)-D-erythrofuranose] has been identified as a component of the carbohydrate moiety of glycosides from the juice of Muscat grapes by acid hydrolysis, alditol acetate derivatization of liberated sugars, and GC–MS. Permethylation of the bulk of glycosides and subsequent GC–MS analysis of the partially methylated alditol acetates showed that apiose was under terminal nonreducing position.

Grapes from the group of Muscat cultivars (Frontignan, Alexandria, etc.) contain glycosides (e.g., arabinosyl and rhamnosyl glucosides) (Williams et al., 1982; Gunata et al., 1988; Strauss et al., 1988) of monoterpenols (mainly geraniol, nerol, and linalool) that might liberate by enzymic hydrolysis their strongly aromatic aglycon moieties. During the purification of the bulk of monoterpenyl disaccharide glycosides from a Muscat juice, other unidentified glycosides were detected, behaving in most separative techniques as the aromatic precursors that rendered them impossible to separate and yielded additional unknown peaks in GC (Gunata et al., 1988) and HPLC (Bitteur et al., 1989).

After acid hydrolysis of the carbohydrate moieties of the bulk of glycosides, a new compound appeared in addition to expected arabinose, rhamnose, and glucose (Gunata et al., 1988). The purpose of the present work was to identify this unknown sugar for a better knowledge of the constitution of the glycoside mixture with the aim of fully elucidating the GC and HPLC chromatograms.

RESULTS AND DISCUSSION

When the mixture of grape glycosides was submitted to strong-acid hydrolysis, TLC of released monosaccharides revealed rhamnose, arabinose (R_{rha} 0.52), glucose (R_{rha} 0.42), and an unknown component (R_{rha} 0.93) stained green or gray by naphthoresorcinol depending on its concentration (Gunata et al., 1988). Alditol acetate derivatives of liberated sugars were separated on a fused silica capillary column bonded with OV-225, and an unknown peak emerged hardly separated from xylose (RRt/xyl 0.98). The relative proportions were Rha:Ara:unknown:Xyl:Glc = 5.4:10.3:13.0:trace:71.3 (Gunata et al., 1988).

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Methylation analysis of carbohydrate moieties of glycosides in admixture using sodium (methylsulfinyl)-methanide and methyl iodide and subsequent alditol acetate derivatization of partially methylated sugars revealed (GC) an unknown peak hardly separated from 2,3,4-Me_3-rha (RRt 0.99) on an OV-1 column but well isolated from all other ethers on an OV-225 column (Gunata et al., 1988); its relative proportion was similar to that observed by direct sugar analysis.

According to the retention times of both the alditol acetate and the partially methylated alditol acetate derivatives of this unknown component, it was suspected to be a pentose under terminal nonreducing position. The alditol peracetate gave a chemical ionization spectrum showing intense ions at m/e 380 $(M + NH_4)^+$ and 303 $(M - OAc)^+$ corresponding to a molecular weight of 362 for a pentitol peracetate. The electron impact fragmentation pattern of the alditol acetate (Figure 1) was identical with the spectrum of apiitol peracetate obtained by acid hydrolysis of apiin and alditol acetate derivatization of liberated apiose; furthermore, it coeluted on the OV-225 column with apiitol peracetate from apiin.

The electron impact mass spectrum of the partially methylated alditol acetate (PMAA), shown in Figure 2, exhibited all the characteristic ions expected from 2,3,3[1]-tri-*O*-methyl-1,4-di-*O*-acetylapiitol and was identical with the spectrum of the PMAA from terminal apiose obtained by permethylation of apiin, acid hydrolysis, and alditol acetate derivatization. The primary and secondary fragmentation patterns of this last component are shown in Figure 3. It coeluted with the PMAA from apiin on both OV-1 and OV-225 capillary columns.

Finally, our unknown compound migrated similarly to apiose (R_{rha} 0.93) obtained by acid hydrolysis of apiin and gave the same staining with naphthoresorcinol.

Thus, it can be concluded from the above results that apiose [3-(hydroxymethyl)-D-erythrofuranose] is present in our mixture of grape glycosides under terminal nonreducing position. Although apiose has been detected in a great number of plants (Watson and Orenstein, 1975), it is the first report of its presence in grape juice, most likely linked to a glucopyranosyl moiety, since a large amount of 6-linked glucose was also detected in grape glycosides

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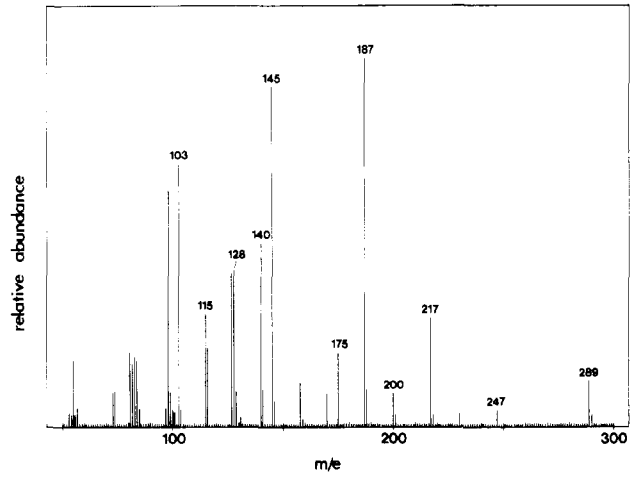

Figure 1. Electron impact mass spectrum of 1,2,3,3[1],4-penta-*O*-acetylapiitol.

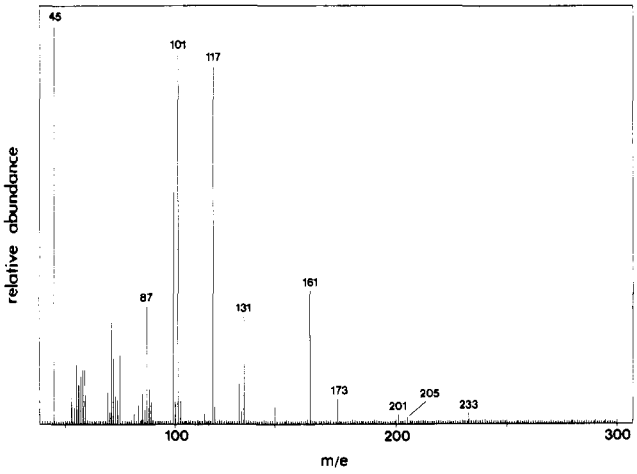

Figure 2. Electron impact mass spectrum of 2,3,3[1]-tri-*O*-methyl-1,4-di-*O*-acetylapiitol.

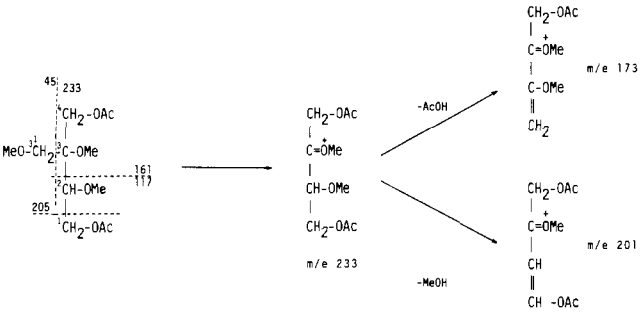

Figure 3. Fragmentation pattern (EI-MS) of 2,3,3[1]-tri-*O*-methyl-1,4-di-*O*-acetylapiitol.

(Gunata et al., 1988). It must be noted that its concentration in grape juice is almost equivalent (on the basis of relative proportions of terminal sugars) to the sum of rhamnosyl and arabinofuranosyl glucosides (Gunata et al., 1988). Plant apiosides are known to carry derivatives of flavone, isoflavone, phenol, and anthraquinone as aglycons (Watson and Orenstein, 1975); however; in our case, more work is needed to isolate and elucidate the structure of this apioside.

EXPERIMENTAL SECTION

Chemicals. Apiin (4',5,7-trihydroxyflavone 7-apiosylglucoside) from parsley was a gift from Profs. A. Sosa and C. Andary.

Isolation of Grape Glycosides. All operations were conducted at 1 °C to prevent any degradation by endogenous grape enzymes. The grape juice was obtained by standard winery procedures from mature sound grape berries of the Muscat of Frontignan cultivar (Gunata et al., 1985, 1988). Glycosides were adsorbed onto activated charcoal and separated from free sugars by washing with water. Glycosides were then extracted from the charcoal with acetone and purified twice on a column of Amberlite XAD-2 (Gunata et al., 1988).

General Methods. TLC of sugars and glycosides was achieved on silica gel using ethyl acetate–1-propanol–water (65:30:10) and detection with naphthoresorcinol (Gunata et al., 1988). Monosaccharides obtained after acid hydrolysis (2 M trifluoroacetic acid; 120 °C; 1.25 h) (Albersheim et al., 1967) were converted into their alditol acetates (Sawardeker et al., 1965; Albersheim et al., 1967) and analyzed by GC on a fused silica capillary column bonded with OV-225 (DB-225; J&W Scientific; 30 m × 0.32 mm (i.d.); 0.25-μm film; 180 °C isothermal; hydrogen as carrier gas at 65 kPa). Permethylation of the carbohydrate moieties of glycosides was performed according to Hakomori (1964) as described by Jansson et al. (1976); after acid hydrolysis, partially methylated sugars were converted to their alditol acetates and separated on two capillary columns (OV-1 and OV-225) as described (Gunata et al., 1988).

Mass Spectrometry. Combined GC–MS was performed on a quadrupole R 1010C Nermag in both electron impact and chemical ionization modes. The mass spectrometer was coupled to either a fused silica capillary column coated with SP 2340 (Chrompack; 25 m × 0.32 mm (i.d.); 160–220 °C at 2 °C/min; helium as carrier gas at 110 kPa) for separation of alditol acetates or to the above OV-1 column (130–220 °C at 5 °C/min; helium at 120 kPa) for PMAAs Operating parameters for both EI-MS and CI-MS: ionization potential, 70 eV; filament emission current, 0.2 mA; ion-source temperatures, 200 °C for EI and 220 °C for CI-MS mode. The CI-MS spectra were obtained with a mixture of ammonia and isobutane (2:1).

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Partial Characterization of a Protein–Carbohydrate Complex from the Rumen of Steers Fed High-Quality Forages

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Four ruminally and abomasally cannulated steers fed coastal Bermuda grass and alfalfa hay were used to investigate and characterize cell-free rumen fluid. The cell-free rumen fluid contained carbohydrates and uronic acids bound to an unidentified component that interacted with Sephadex and prevented the determination of molecular weight. Because Klason lignin or phenolic acids were not found, the strong 280-nm ultraviolet absorption and the interaction with the Sephadex indicated that proteins were involved. Amino acid analysis verified the presence of proteins; solid-state ^{13}C NMR indicated that proteins were present in the complex, but further indicated very low levels of phenolic components. The data support the conclusion that the material isolated from the rumen was a protein–carbohydrate complex and not a lignin–carbohydrate complex (LCC). This finding suggests that cattle consuming these high-quality forages produce little or no soluble LCC in the rumen.

Rumen contents are composed of stratified layers of particulate matter and solutes suspended in a buffered liquid phase. Particulate matter is composed of feed particles at various stages of digestion and the microflora and microfauna that inhabit the rumen. Solutes originate from ingested feed or liquids, saliva, secretions into the rumen, or metabolites of microorganisms. Solutes of particular nutritional importance are the nitrogenous components, the volatile fatty acids as well as other organic acids, minerals, vitamins, and buffers.

During the investigation of microbiol fermentation of carbohydrate components of feeds, Gaillard and Van't Kooster (1973) reported that polysaccharides and glycoproteins were added to the rumen liquor. In further studies on the digestion of glycoproteins, Gaillard and Richards (1975), using centrifugation, separated the digesta into discrete fractions and discovered that the cell-free rumen fluid (CFRF) contained sizable quantities of carbohydrate- and lignin-derived components. According to these authors, the CFRF contained 20% carbohydrate and 50% Klason lignin and gave UV and IR spectra characteristic of the aromatic components of lignin. In addition, polymers in the CFRF did not pass a dialysis membrane. When purified by gel filtration, an interaction between the polymers and Sephadex occurred, preventing the determination of molecular weight. From these results, Gaillard and Richards (1975) concluded that the CFRF was a lignin–carbohydrate complex.

This investigation was conducted to characterize the CFRF from steers fed high-quality alfalfa and coastal Bermuda grass hay.

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MATERIALS AND METHODS

Feeding Trial. Four Holstein steers (averaging 160 kg) fitted with permanent ruminal and abomasal cannulae were used to obtain rumen fluid. The rumen fluid was obtained in conjunction with a 4 × 4 Latin square arrangement of treatments to evaluate hemicellulose digestibility by steers fed alfalfa and coastal Bermuda grass as hay or drum dehydrated (Windham et al., 1987). However, only the rumen fluid from steers consuming coastal Bermuda grass hay (CBG-H) and alfalfa hay (Alf-H) were used in this study due to the complex and time-consuming laboratory analyses.

Isolation of Cell-Free Rumen Fluid. Ruminal fluid (4 L) for isolation of CFRF was collected prior to the 10:00 a.m. feeding on day 5 of each collection period, which followed a 10-day diet adjustment period. The rumen fluid was filtered through four layers of cheesecloth and transported to the laboratory. The CFRF was collected by centrifuging the fluid (100 mL/min, 27000*g*; Gaillard and Richards, 1975) in a Sorvall Model SS-3 centrifuge equipped with a Szent-Gyorgyi-Blum continous-flow system. The CFRF from each steer was dialyzed (10000 molecular weight cutoff) against running water for 2 days and then freeze-dried (Gaillard and Richards, 1975).

Analyses. Portions of the CFRF were analyzed for moisture by drying at 105 °C for 24 h and for crude protein (AOAC, 1976). Amino acid hydrolysates of the CFRF were prepared by refluxing aliquots of the CFRF in 6 N HCl under N_2 for 24 h (Wilkinson et al., 1968); amino acids were analyzed as described previously (Amos et al., 1976). Neutral carbohydrates were quantitated by hydrolyzing aliquots in 2 N trifluoroacetic acid for 30 min at 121 °C and determined by high-pressure liquid chromatography (HPLC) (Windham et al., 1987). Total carbohydrate was determined on the dialyzed CFRF by the phenol–sulfuric acid method (Dubois et al., 1956) and expressed as a percentage of CFRF. The percentage of carbohydrates obtained from acid hydrolysis and HPLC analysis was used to construct a standard curve for the phenol–sulfuric acid method. For evaluation of phenolic acids, CFRF was hydrolyzed with 50 mL of 1 N NaOH for 2.5 h and analyzed by capillary gas chromatography as described by Akin et al. (1987). Uronic acids were determined as outlined by Blumenkrantz and Asboe-Hansen (1973). Gel permeation chromatography was carried out on a 94 × 2.5 cm column