Application of High-Temperature Gas Chromatography−Mass Spectrometry to the Investigation of Glycosidically Bound Components Related to Cashew Apple (*Anacardium occidentale* L. Var. *nanum*) Volatiles

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Free and bound volatile components of a Brazilian cashew apple variety (*Anacardium occidentale* L. var. *nanum*) were obtained by simultaneous distillation−extraction (SDE) and XAD-2 adsorption. According to gas chromatography−mass spectrometry (GC-MS) analyses and retention indices, 62 free volatile constituents were characterized and quantified. They were esters (40%), terpenes (20%), hydrocarbons (14%), fatty acids (9%), aldehydes (8%), alcohols (3%), lactones (3%), ketones (1%), phenols (1%), and norisoprenoids (1%). The glycosidically bound volatile precursors were analyzed by high-temperature GC-MS, after room temperature silylation. Several conjugated alcohols and cinnamic acids were detected and reported as cashew apple glycosyl constituents for the first time.

Keywords: *Cashew apple; Anacardium occidentale L.; volatile compounds; glycoside; high-temperature gas chromatography*

INTRODUCTION

The cashew tree, *Anacardium occidentale* L., has been important to the Brazilian economy since the 1940s, when it began to be explored as a source of phenolic compounds (cardols and cardanols) for the brake-lining industry (Kosubek and Tyman, 1999; Shoba et al., 1992; Lima, 1988). Nowadays its most significant trade product is the edible cashew nut, accounting for an agribusiness of U.S. ∼$150−170 million/year (Cordeiro and Cardoso, 1998; Pessoa et al., 1995). The national (Brazil) interest for the production of cashew nuts has increased, thus rendering the availability of cashew apple "waste" for several other purposes. Considerable research attention has been devoted to the utilization of cashew crops as well as to the development and/or optimization of technologies for the industrialization of cashew apple derivatives, among which an aromatic beverage called "wine of cashew" has been of particular interest.

Considering the importance of aroma chemistry for the food industry, more data on cashew apple volatiles would be welcome at present, because the literature on this subject (Maciel et al., 1986; MacLeod and Troconis, 1982) is controversial. Furthermore, knowledge of the occurrence of glycosidically bound volatile components, such as monoterpenic, aromatic, and aliphatic alcohols, phenols, and C_{13} norisoprenoids, could also be useful for improvements in cashew apple wine quality, once the relationship of these compounds to grape wine aroma development is well established (Bonnländer et al., 1998; Park et al., 1991; Winterhalter et al., 1990; Strauss et al., 1987; Günata et al., 1985; Williams et al., 1982a,b, 1983). On the other hand, no similar investigation has been done for cashew apple so far.

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In general, glycosidically bound volatiles have been extracted according to the method of Günata et al. (1985), but due to the high complexity of the mixture obtained through XAD-2 adsorption, isolation of individual constituents has been proved not to be practicable (Williams et al., 1983) unless enough material is available for further preparative-scale liquid chromatographic separations (Humpf and Schreier, 1992; Güldner and Winterhalter, 1991; Winterhalter et al., 1990; Schwab and Schreier, 1990; Hasegawa et al., 1989; Bennett et al., 1989; Strauss et al., 1987). However, the heterogeneity of both the glycosyl and aglycon portions often represent an inconvenience from the point of view of partition in all-liquid systems separations; for example, incomplete elution of ionone glycosides by countercurrent chromatography has been reported (Güldner and Winterhalter, 1991).

As another approach, numerous authors have demonstrated that direct analysis of the derivatized conjugate mixture by gas chromatography−mass spectrometry (HRGC-MS) generally enables glycosidically bound compounds to be tentatively identified (Sakho et al., 1997; Chassagne et al., 1996; Voirin et al., 1992a,b; Schwab et al., 1989, 1990; Williams et al., 1983).

As far as we know no conjugates larger than diglycosides have been characterized through the procedures mentioned above. Perhaps it could be a general characteristic of extracts obtained through XAD-2 adsorption or, maybe, an effect of sample discrimination (nonvolatilization of heavier compounds) at the gas chromatograph injector (conventional split/splitless mode).

Since high-temperature gas chromatrography (HT-HRGC) was introduced, allowing HRGC analyses with final temperatures >350 °C, analyses of higher saccharides (molecular masses up to 1400 Da) are no longer a problem for GC (Pereira et al., 1998; Carlsson et al., 1992; Karrer and Herberg, 1992). The technique has been applied for many different types of analyses in

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fields such as food and natural products chemistry, organic geochemistry, and archeology (Pereira and Aquino Neto, 1999) and cold on-column injection has been evaluated and recommended for best performance (Aquino Neto et al., 1994).

In this paper we report the characterization of free cashew apple volatiles and the analysis of the "apple" glycosidic fraction by HT-HRGC and HT-HRGC coupled to mass spectrometry (HT-HRGC-MS).

EXPERIMENTAL PROCEDURES

Materials. Pesticide-reagent grade solvents (Grupo Química, Rio de Janeiro, Brazil) were used for all extraction and chromatographic procedures. Pyridine (analytical grade; Grupo Química) was dried over KOH (reflux/24 h). Amberlite XAD-2 resin (20–60 mesh), linear hydrocarbons (C_8–C_{21}), linalool, phenyl-β-D-glucopyranoside, and BSTFA [*N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane] were from Aldrich (Milwaukee, WI). Glucose, sucrose, and maltotriose were from Sigma (St. Louis, MO).

Plant Material. Fresh ripe cashew apples (*A. occidentale* L. var. *nanum*) from Ceará state, Brazil, were purchased from a local market (CEASA-RJ) and were used immediately after acquisition. The nuts were taken off under running tap water just before the extraction procedures. The experiments were made in duplicate.

Simultaneous Distillation–Extraction (SDE). A sample (300 g) from fresh-cut cashew apples was transferred to a 500 mL round-bottom flask to which distilled water (200 mL) was added. In a 50 mL pear-shaped flask dichloromethane (30 mL) was used as the extracting solvent. The SDE head containing a water-cooled condenser (~2 °C) was attached, connecting both the distillation and the extraction flasks. The process was carried out under atmospheric pressure for 4 h. After addition of the internal standard (1 μL of 2.0% linalool in dichloromethane), the extract was dried over anhydrous sodium sulfate, filtered, and concentrated to 200 μL under an N_2 stream.

XAD-2 Extraction. Fresh-cut cashew apples (1 kg) were blended in 300 mL of a saturated solution of KCl (used as a nonspecific enzymatic inhibitor). The slurry was vacuum filtered through Whatman No. 1 filter paper, and the filtrate was passed through a solvent-washed (Günata et al., 1985) Amberlite XAD-2 column (25 × 2 cm) at a flow rate of 2.5–3.0 mL/min. This column was eluted with distilled water (500 mL), ethyl ether (500 mL), and methanol (750 mL). The methanol eluate, called the glycosidic fraction, was concentrated almost to dryness under reduced pressure, redissolved in 2 mL of distilled water, lyophilized, and weighed.

Retention Index (RI) Calculations. Both the cashew apple SDE/4 h extract and a dichloromethane solution of a homologous series of linear hydrocarbons (C_8–C_{21}) were analyzed by HRGC under the same conditions (described below). The GC retention times (t_R) obtained were used for the RI determinations (Grob, 1995).

Quantitation. Cashew apple volatiles (SDE/4 h extract) were quantified by internal standardization (linalool as i.s.) based on the chromatogram peak areas provided by FID response.

Trimethylsilylation. An aliquot of ~3 μg of the glycosidic fraction (lyophilized) was transferred to a 1.0 mL stoppered vial to which 200 μL of BSTFA and 200 μL of anhydrous pyridine were added. The mixture was shaken for 30 s, allowed to stand for 30 min at room temperature (Sweeley et al., 1963), and then used for the chromatographic analyses.

HRGC. A Hewlett-Packard GC model 5890-II (Palo Alto, CA) equipped with a split/splitless injector, a flame ionization detector (FID), and a fused silica capillary column (30 m × 0.25 mm i.d.) coated with HP-5 (5% phenylmethylsilicone, 0.25 μm film) was used. The temperature program began at 40 °C (10 min) and then increased at 3 °C/min to 280 °C (10 min). Hydrogen was used as the carrier gas at a flow rate of 2.5 mL/min; the volume injected was 1 μL in splitless mode; injector and detector temperatures were 250 and 280 °C, respectively. Data were recorded with an HP model 3396-II (Palo Alto, CA) recorder integrator.

HT-HRGC. An on-column injector (Carlo Erba, Milano, Italy) was mounted on a Hewlett-Packard GC model 5890-II. A custom-made high-temperature borosilicate capillary column (Duran-50; 20 m × 0.25 mm i.d.) coated with PS-090 (20%-phenyl–80%-methylpolysiloxane, Petrarch Systems, Inc., 0.25 μm film) was used. The columns were prepared in our laboratory, according to a literature procedure (Blum and Eglinton, 1989).

The temperature program began at 40 °C, increased at 40 °C/min to 250 °C, and then increased at 5 °C/min to 370 °C (5 min). Hydrogen was used as the carrier gas at a flow rate of 3.5 mL/min; the volume injected was 0.4 μL, and the detector temperature was 390 °C. Data were recorded with an HP model 3396-II recorder integrator.

MS. A Hewlett-Packard 5890-II GC coupled to a Hewlett-Packard 5972 MS was used. The same columns and temperature conditions used in the GC were used. The MS conditions were as follows: the interface temperature was 300 °C for HRGC-MS and 390 °C for HT-HRGC-MS, the mode of ionization was electron impact (70 eV), and scanning was performed at 1 s/decade from 40 to 400 Da (for free volatile analyses) and from 50 to 700 Da (for bound volatile analyses).

RESULTS AND DISCUSSION

Free Volatile Compounds. In an attempt to clarify the question of whether the chemical differences between the cashew apple volatiles of a Brazilian/headspace extract (Maciel et al., 1986) and a Venezuelan/SDE/4 h extract (MacLeod and Troconis, 1982) were due to methods of extraction or due to species variations, a SDE/4 h extract of Brazilian material was analyzed to provide comparative data.

Four hours of simultaneous distillation–extraction (SDE/4 h) yielded ~1 mg of cashew apple volatiles per kilogram of fresh material, an amount considerably higher than the ~3.6 μg/kg obtained from a fresh ripe Venezuelan sample (MacLeod and Troconis, 1982). Because similar procedures of extraction were used, the *A. occidentale* varieties studied were either not the same or, possibly, exposed to quite different environmental pressures (Gottlieb et al., 1996). About 90 constituents were detected by both HRGC and HRGC-MS. On the basis of the MS data plus RI (Adams, 1995), 69 volatiles were characterized, among which 62 were quantified: esters (40%), terpenes (20%), hydrocarbons (14%), fatty acids (9%), aldehydes (8%), alcohols (3%), lactones (3%), ketones (1%), phenols (1%), and C_{13} norisoprenoids (1%) (Table 1).

A quite uncommon hydroxy ester, the compound ethyl 2-hydroxy-4-methylpentanoate, accounted for ~70% (702 μg/kg) of the total SDE/4 h volatile mass, being by far the dominant volatile in the extract (Figure 1). Hydroxy esters are known to occur among fruit volatiles (Mathieu et al., 1998; Peppard, 1992; Baek et al., 1997; Pino, 1997; Moshonas and Shaw, 1997; Adedeji et al., 1992), especially in pineapple varieties (Umano et al., 1992; Wu et al., 1991); however, the 2-hydroxy-4-methyl-substituted esters have been rarely reported (Fisher et al., 1995). The extraction of ethyl 2-hydroxy-4-methylpentanoate from cashew apples was dependent on heat because the compound was not obtained either by a static cryogenic headspace or by an SDE lasting 20 min (Bicalho, 1999). Its structural resemblance to the 2-hydroxy-4-methylpentanoic acid, an L-leucine metabolite (Yu and Spencer, 1969), suggested a high concentration of leucine in the matrix, as occurs in some *A. occidentale* varieties (Xavier Filho et al., 1990; Nagajara and Nampoothiri, 1986). Other leucine derivatives such as

Table 1. Free Volatile Compounds Characterized in the Cashew Apple SDE/4 h Extract

compound	μg/kg[a]	RI[b] (HP-5)	compound	μg/kg	RI (HP-5)
		Esters			
methyl 3-methylbutanoate	nd[c]	861	ethyl benzoate[d]	2.1	1170
ethyl 2-butenoate	nd	863	ethyl octanoate[d,e]	5.8	1199
ethyl 2-methylbutanoate	nd	864	ethyl benzoacetate	0.6	1243
ethyl 3-methylbutanoate[d]	nd	867	ethyl 2-octenoate-(*E*)	0.3	1246
methyl 2-methyl-2-butenoate[d]	nd	873	hexyl 2-methyl-2-butenoate	<0.1	1330
methyl hexanoate[d]	0.2	927	ethyl cinnamate-(*Z*)	1.5	1373
ethyl 2-methyl-2-butenoate[e]	11.7	948	methyl cinnamate-(*E*)	1.0	1378
ethyl hexanoate[d]	34.5	1002	ethyl decanoate	0.1	1396
ethyl 3-hexenoate-(*E*)	0.1	1012	ethyl cinnamate-(*E*)	13.9	1463
ethyl 2-hexenoate[e]	2.1	1046	hexyl benzoate	0.9	1577
ethyl 2-hydroxy-4-methylpentanoate	702.0	1078	ethyl dodecanoate	<0.1	1595
methyl benzoate[d]	1.1	1095	ethyl tetradecanoate	<0.1	1794
pentyl isopentanoate	1.3	1106	ethyl hexadecanoate	<0.1	1995
butyl 2-methyl-2-butenoate[e]	<0.1	1136			
		Aldehydes and Ketones			
hexanal[e]	nd	799	phenylacetaldehyde[e]	3.3	1037
furfural[d,e]	nd	nd	nonanal[e]	0.4	1101
benzaldehyde[d,e]	1.1	956	6-methyl-5-hepten-2-one	<0.1	988
		Alcohols			
hexadecanol	1.4	1880	octadecanol	3.8	2084
Phenols					
nonylphenol	0.2	1720			
		Carboxylic Acids			
octanoic acid	0.3	1186	dodecanoic acid	1.1	1569
nonanoic acid	1.0	1282	tetradecanoic acid	<0.1	1776
decanoic acid	<0.1	1379	hexadecanoic acid	22.3	1986
		Lactones			
γ-nonalactone	0.5	1359	γ-dodecalactone	50.8	1684
		Terpenes			
o-cymene	<0.1	1022	β-bisabolene	1.2	1507
limonene[d,e]	0.4	1027	γ-cadinene	<0.1	1513
α-cubebene	<0.1	1348	δ-cadinene	0.5	1523
α-*cis*-bergamotene	0.6	1414	α-calacorene	<0.1	1540
β-caryophyllene[d,e]	0.6	1417	*epi*-α-muurulol	<0.1	1643
α-*trans*-bergamotene	1.6	1435	α-cadinol	1.1	1656
γ-muurulene	<0.1	1474			
		Norisoprenoids (C_{13})			
geranylcetone	<0.1	1452			
		Hydrocarbons			
naphthalene	<0.1	1175	*n*-heptadecane	0.7	1700
n-tridecane	0.3	1299	*n*-octadecane	0.2	1800
n-tetradecane	0.3	1399	*n*-nonadecane	0.5	1900
n-pentadecane	1.4	1500	*n*-eicosane	0.7	2000
n-hexadecane	0.6	1600			

[a] Mass per kilogram of fresh matrix. [b] Retention indices on HP-5 (see Experimental Procedures). [c] Not determined. [d] MacLeod and Troconis (1982). [e] Maciel et al. (1986).

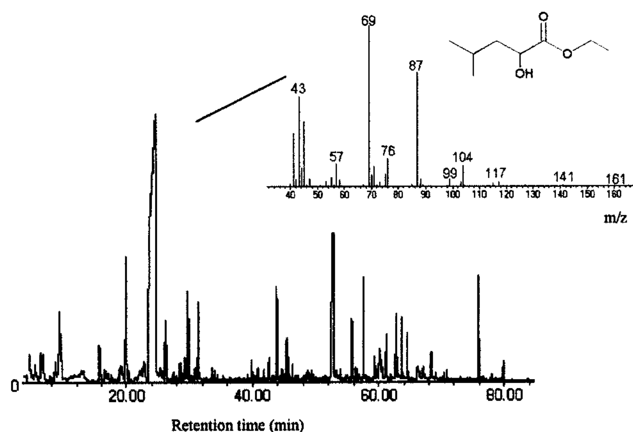

Figure 1. Chromatogram (TIC) of the cashew apple SDE/4 h extract. The mass spectral detection of the dominant constituent ethyl 2-hydroxy-4-methylpentanoate is shown. For HRGC conditions, see Experimental Procedures.

the 3-methylbutanoate esters (Schreier, 1984; Schwimmer, 1981) were also detected (Table 1).

The remaining esters were mainly ethyl derivatives of alkoyl (saturated; unsaturated; methyl branched), cinnamoyl, and benzoyl moieties, ethyl hexanoate, ethyl 2-methyl-2-butenoate, ethyl cinnamate, and ethyl benzoate being the most concentrated. Interestingly, although similar esters were previously characterized in a headspace sample of fresh cashew apples from Brazil (Maciel et al., 1986), in an SDE/4 h extract of Venezuelan cashew apple material, ethyl acetate and ethyl octanoate were found as well (MacLeod and Troconis, 1982).

As Table 1 shows, apart from limonene and β-caryophyllene, the other terpenes characterized have not been previously reported from cashew apple. The analyses resulted in no 3-carene characterization, supporting a previously reported difference between Brazilian and Venezuelan cashew apples (Maciel et al., 1986). This monoterpene was the most abundant Venezuelan cashew apple SDE/4 h extract volatile (~24% of total volatile mass; MacLeod and Troconis, 1982).

Compounds such as benzaldehyde and phenylacetaldehyde have been reported as tropical fruit volatiles

Table 2. Mass Spectral (70 eV) Data of Tentatively Characterized Cashew Apple Glycosidically Bound Components

glycosylated aglycons	t_R[a] (min)	EI-MS, main characteristic fragment ions, m/z (%)
		Alkyl Alcohols
nonyl	11.35	57 (33), 71 (22), 73 (61), 85 (21), 204 (100), 217 (20), 245 (1)
decyl	12.29	57 (40), 71 (25), 73 (70), 85 (23), 204 (100), 217 (20), 259 (1)
undecyl	13.28	57 (87), 71 (69), 73 (36), 85 (45), 204 (100), 217 (22), 273 (0.5)
dodecyl	14.28	57 (52), 71 (38), 73 (47), 85 (37), 204 (100), 217 (17), 287 (20)
tridecyl	15.32	57 (40), 71 (33), 73 (50), 85 (28), 204 (100), 217 (19), 301 (1)
tetracecyl	16.34	57 (13), 71 (9), 73 (39), 85 (14), 204 (100), 217 (13), 314 (14)
		Aromatic Alcohols
benzyl (isomer)	8.40	73 (63), 91 (64), 204 (100), 209 (11), 217 (20)
benzyl (isomer)	8.52	73 (52), 91 (55), 204 (100), 209 (0.5), 217 (28)
2-phenylethyl (isomer)	8.72	73 (55), 105 (100), 204 (59), 217 (96), 223 (1)
2-phenylethyl (isomer)	9.12	73 (60), 105 (59), 204 (100), 217 (16), 223 (9)
benzyl (isomer)	14.92	73 (76), 91 (51), 204 (100), 209 (0.5), 217 (28)
2-phenylethyl (isomer)	15.53	73 (57), 105 (58), 204 (100), 217 (35), 223 (1)
benzyl (isomer)	16.15	73 (40), 91 (22), 204 (100), 209 (1), 217 (20)
		Aromatic Acids
cinnamic (isomer)	10.36	73 (89), 131 (100), 204 (42), 217 (66)
cinnamic (isomer)	10.72	73 (62), 131 (100), 204 (37), 217 (21)
cinnamic (isomer)	11.04	73 (74), 131 (100), 204 (9), 217 (51)
cinnamic (isomer)	11.12	73 (86), 131 (100), 204 (16), 217 (23)
cinnamic (isomer)	11.17	73 (70), 131 (100), 204 (20), 217 (25)
cinnamic (isomer)	11.81	73 (37), 131 (64), 204 (100), 217 (14)

[a] See Experimental Procedures for HT-HRGC-MS conditions used.

(Peppard, 1992; MacLeod and Pieris, 1983; Buttery et al., 1982) often related to the oxidation of benzyl and phenylethyl alcohols, which, although not presently detected, were previously reported as Brazilian cashew apple headspace constituents (Maciel et al., 1986). Because benzyl and phenylethyl alcohols are found in fruits as glycosides (Ollé et al., 1998; Pérez et al., 1997; Young and Paterson, 1995; Marlatt et al., 1992; Buttery et al., 1990; Schwab and Schreier, 1988), these aldehydes could have also been generated due to oxidation of aglycons released in the distillation medium during the extensive SDE process (matrix pH 4.0−4.5). The presence of nonanal, decanal, and 2-decenal strongly suggested the oxidation of oleic acid [(*Z*)-9-octadecenoic acid] (Shipe, 1980; Tressl et al., 1980), whereas the compounds γ-nonalactone and γ-dodecalactone pointed to linolenic acid [(*Z,Z*)-9,12-octadecadienoic acid] oxidation (Albrecht et al., 1992). Several saturated fatty acids were characterized, the hexadecanoic (palmitic acid) being by far the most concentrated. Although similar aldehydes (benzaldehyde, phenylacetaldehyde, and nonanal) were reported as Venezuelan cashew apple SDE/4 h extract constituents, lactones and saturated fatty acids were not (MacLeod and Troconis, 1982).

Bound Volatile Compounds. The XAD-2 methanolic fraction obtained according to Günata et al. (1985) was concentrated under reduced pressure, lyophilized, and weighed (29.7 mg/kg of fresh matrix). To investigate the presence of glycosides, an aliquot of the fraction was derivatized with BSTFA/pyridine and analyzed by HT-HRGC and HT-HRGC-MS. A commercial standard of phenyl-β-D-glucopyranoside, glucose, sucrose, and maltotriose were treated in the same way and used as retention time references.

Several constituents of the XAD-2 fraction analyzed presented a mass spectrum containing ions typically related to the electron impact (70 eV) mass fragmentation of trimethylsilyl (TMS) derivatives of carbohydrates (ions at m/z 204 and 217). Most of them were reducing sugar units (mono-, di-, and trisaccharides), whereas GC t_R revealed a dominance of monosaccharides in the fraction analyzed (t_R = 5.0−7.0 min; Figure 2). Several minor constituents presented a carbohydrate-containing

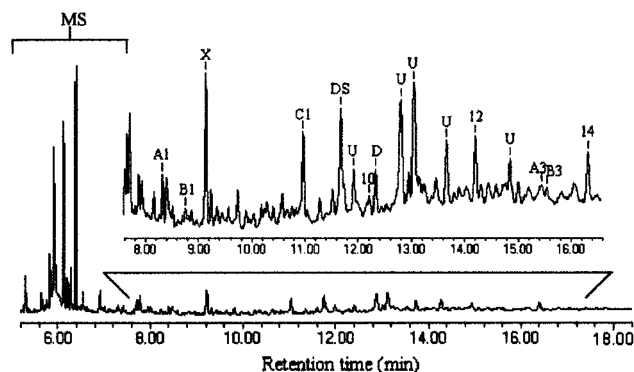

Figure 2. Chromatogram (TIC) of TMS derivatives of the cashew apple XAD-2 glycosidic fraction. The relative dominance of monosaccharides (MS) is shown along with the expanded region of the glycoside detection. Peaks: A1, benzyl monoglycosyl ether; B1, 2-phenylethyl monoglycosyl ether; X, contaminant phthalate; C1, monoglycosylcinnamate; DS, disaccharide; U, unknown glycosides; 10, decanyl monoglycosyl ether; D, an alditol; 12, dodecanyl monoglycosyl ether; A3, benzyl diglycosyl ether; B3, 2-phenylethyl diglycosyl ether; 14, tetradecanyl monoglycosyl ether. For HT-HRGC conditions, see Experimental Procedures.

spectra incremented by fragment ions derived from aglycon moieties (Figure 2); the molecular ions ($M^{\bullet+}$) were never detected, and the fragment ions resulting from the sugar portion were assignable only to monosaccharide units, predominantly hexapyranoses (ions at m/z 361, 331, 271, and 249). Even accounting for known characteristic ions, the identification of carbohydrate derivatives by mass spectral analyses alone is rather difficult because isomers present quite similar mass spectra. Hence, a generic nomenclature was adopted for the carbohydrate portions because the aglycons were the main target of the present survey. Thus, by essentially regarding the aglycon mass fragmentation plus the occurrence of the fragment ion aglycon−O−CH$^+$−OTMS (Voirin et al., 1992a,b; Creaser et al., 1991), formed via an −OTMS rearrangement (DeJongh et al., 1969), the following bound compounds were tentatively characterized (Table 2):

Alkyl Alcohols. By selecting the fragment ion at m/z 85 a group of glycosides (t_R = 11.35−16.34 min) with

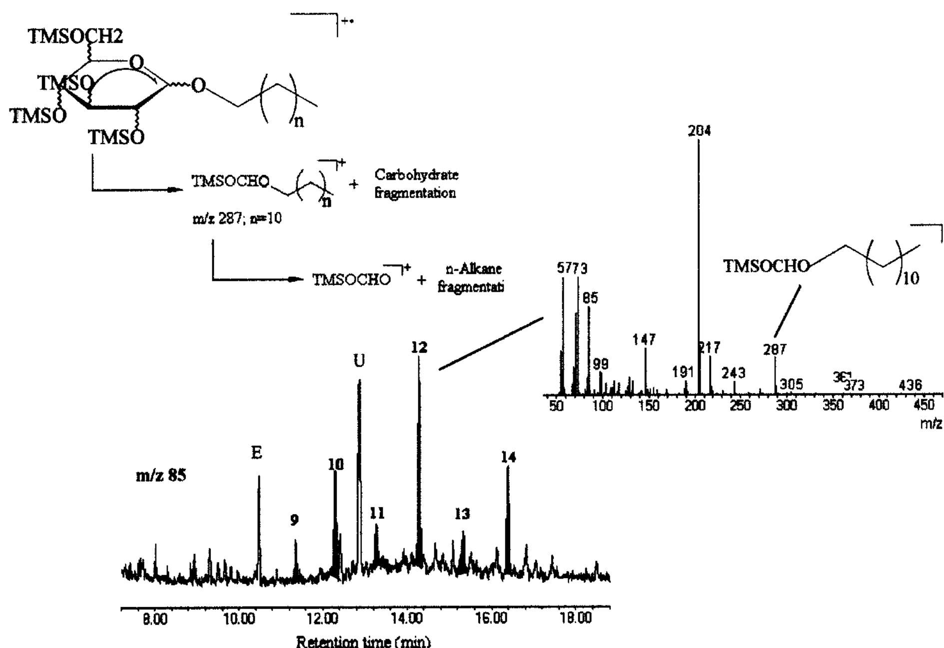

Figure 3. Mass fragmentogram (*m*/*z* 85) of the cashew apple XAD-2 glycosidic fraction. Peaks: 9–14, homologous series of bound *n*-alkyl alcohols between nonanol and tetradecanol; E, hydrocarbon; U, unknown glycoside. The mass spectrum of the bound dodecanol is shown to illustrate the typical pattern of fragmentation of the conjugates detected. For HT-HRGC conditions, see Experimental Procedures.

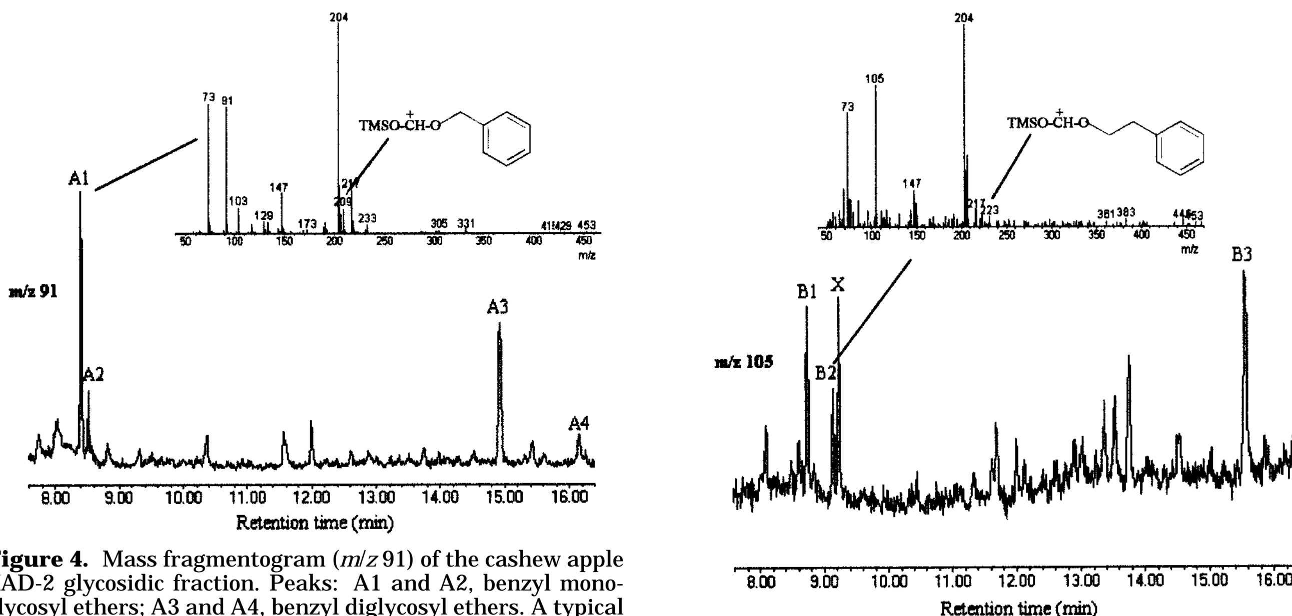

Figure 4. Mass fragmentogram (*m*/*z* 91) of the cashew apple XAD-2 glycosidic fraction. Peaks: A1 and A2, benzyl monoglycosyl ethers; A3 and A4, benzyl diglycosyl ethers. A typical mass spectrum is shown for illustration. For HT-HRGC conditions, see Experimental Procedures.

Figure 5. Mass fragmentogram (*m*/*z* 105) of the cashew apple XAD-2 glycosidic fraction. Peaks: B1 and B2, 2-phenylethyl monoglycosyl ethers; B3, 2-phenylethyl diglycosyl ether; X, contaminant phthalate. A typical mass spectrum is shown for illustration. For HT-HRGC conditions, see Experimental Procedures.

typical *n*-alkyl aglycons (*m*/*z* 57, 71, 85, etc.) was evident (Figure 3). From both the retention times and the fragment ions aglycon–O–CH$^+$–OTMS, a homologous series of monoglycosyl saturated alkyl ethers between C_9 and C_{14}, with C_{max} (highest peak) at C_{12}, was characterized.

Aromatic Alcohols. By selecting the fragment ions at *m*/*z* 91 and 105 two groups of glycosides were evident. The first group comprised four conjugates (t_R = 8.40, 8.52, 14.92, and 16.16 min; Figure 4) with the same pattern of aglycon fragmentation, consisting of ions at *m*/*z* 91 (tropylium) and 209 (aglycon–O–CH$^+$–OTMS). Such mass fragments supported the characterization of benzyl aglycons (Voirin et al., 1992a), which, due to the retention time differences between the conjugates around t_R = 8 and 16 min, were bound to monosaccharides and disaccharides, respectively.

Similarly, the second group comprised three conjugates (t_R = 8.72, 9.12, and 15.53 min; Figure 5) with the same pattern of aglycon fragmentation, consisting of ions at *m*/*z* 77 ($C_6H_5^+$), 105 ($C_8H_9^+$), and 223 (aglycon–O–CH$^+$–OTMS), which enabled the characterization of 2-phenylethyl aglycons (Voirin et al., 1992a). The retention time differences between the conjugates around t_R = 9 and 15 min indicated these aglycons were also bound to a monosaccharide and a disaccharide unit.

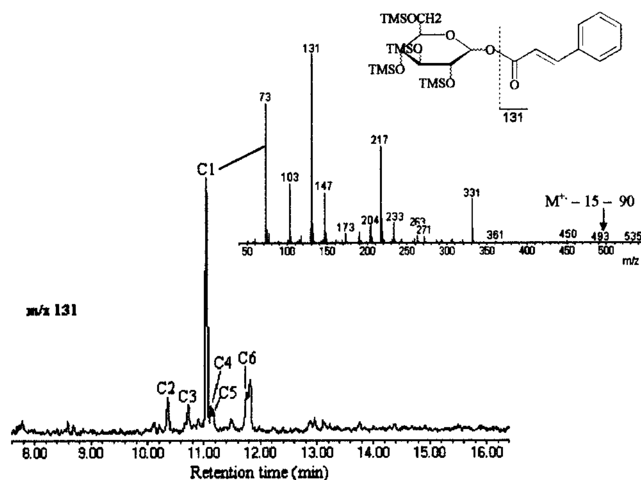

Figure 6. Mass fragmentogram (*m*/*z* 131) of the cashew apple XAD-2 glycosidic fraction. Peak C, monoglycosylcinnamate; peaks A, B, and D−F, possible monoglycosylcinnamates (low abundance hampered the characterization). A typical mass spectrum is shown for illustration. For HT-HRGC conditions, see Experimental Procedures.

Cinnamic Acids. By selecting the fragment ion at *m*/*z* 131 a third group of glycosides (t_R = 10.36, 10.72, 11.04, 11.12, 11.17, and 11.81 min; Figure 6) having the fragmentation pattern of a bound cinnamoyl aglycon (*m*/*z* 77, 103, and 131) was evident. In these spectra the fragment ion of the aglycon−O−CH^+−OTMS (*m*/*z* 249) was small, if present at all. However, these conjugates had retention times of monoglycosides and showed hexapyranose fragment ions (*m*/*z* 361, 331, and 271) in their mass spectra. Thus, the detection of fragment ions resulting from expected losses of $^{\bullet}CH_3$ and HOTMS from $M^{\bullet+}$ (DeJongh et al., 1969) was important for the aglycon characterization. Possibly for reasons of concentration, only the most abundant conjugate (t_R = 11.04 min) presented a fragment ion at *m*/*z* 493 to support the proposed cinnamoyl aglycon (*m*/*z* 598, $M^{\bullet+} \rightarrow$ *m*/*z* 583 + $^{\bullet}CH_3 \rightarrow$ *m*/*z 493* + HOTMS; Figure 6).

Previous mass spectral data from TMS ethers of several glycoside standards showed that the relative intensity between the ions at *m*/*z* 204 and 217 is dependent on the aglycon structure and not on the carbohydrate ring geometry, in contrast to the mass fragmentation behavior of TMS derivatives of reducing sugars (DeJongh et al., 1969). For instance, whereas TMS ethers of (*R*,*S*)-terpenyl-β-D-glucopyranoside presented mass spectra with the fragment ion at *m*/*z* 217 as the base peak, benzyl- and 2-phenylethyl-β-D-glucopyranosides presented the ion at *m*/*z* 204 (Voirin et al., 1992a). All of the above characterized cashew apple glycosides, except a monoglycosyl 2-phenylethyl ether isomer (t_R = 8.72 min) and most of the monoglycosylcinnamate isomers (t_R = 10.36, 11.04, 11.12, and 11.17 min), presented the ion at *m*/*z* 204 ≫ 217.

As demonstrated by deuterated experiments (DeJongh et al., 1969), the fragment ions at *m*/*z* 117, 204, and 217 do not retain the anomeric carbon of pertrimethylsilylhexoses. The presence of these ions in the mass spectra of all monoglycosides detected suggested the aglycons bound to the anomeric carbons, as they are most usually found (Stahl-Biskup et al., 1993 and references cited therein). Because diglycosides already have an anomeric carbon involved in the glycosidic bound, the aglycon site in the carbohydrate structure could not be suggested from the presence of the fragment ions just mentioned.

There were also other glycosides detected in the XAD-2 fraction, but the tentative characterization of their aglycon structures was not possible due to the unavailability of published reference mass spectra.

In addition to the above compounds, two nonglycosylated homologous series were characterized, one of TMS derivatives of even *n*-alkanols ranging from C_{16} to C_{30} with C_{max} of C_{18} and the other of TMS derivatives of fatty acids ranging from C_{14} to C_{28} with a strong even-carbon-number predominance and C_{max} of C_{16}. Thus, the direct analysis of TMS derivatives of glycosides by HRGC-MS presents advantages in relation to the traditional methods of acid or enzymatic hydrolysis used for the characterization of bound aglycons (Günata et al., 1985), reducing incorrect assignments.

Even though conjugates larger than diglycosides were not found, HT-HRGC and HT-HRGC-MS are being proposed as simple and quick methods for the determination and characterization of glycosidic compounds and other constituents in XAD-2 extracts. These methods, which are used for the first time for the analysis of this kind of sample, present an advantage, when compared with HRGC, which is the possibility of analyses of high molecular weight compounds, including saccharides up to seven carbohydrate units (Carlsson et al., 1992). Higher saccharides, not identified, were previously reported as cashew apple constituents (Nagajara and Nampoothiri, 1986).

CONCLUSION

The volatile composition of a Brazilian cashew apple SDE/4 h extract has been presented for the first time. The results indicated that the differences found in the literature regarding Brazilian and Venezuelan cashew apple volatiles are not dependent on methods of extraction but, rather, due to distinct *A. occidentale* varieties.

Glycosidically bound *n*-alkyls (homologous series), benzyl, 2-phenylethyl, and cinnamoyl aglycons were reported for the first time as cashew apple constituents. These aglycons were also found as free volatile esters or aldehydes in the SDE/4 h extract. The homologous series of even *n*-alkanols (C_{16}−C_{30}) and the fatty acids up to C_{20} found are also reported as cashew apple constituents for the first time.

The HT-HRGC and HT-HRGC-MS proved to be powerful tools for the analysis and characterization of glycosides and other fruit constituents without a need for isolation or extensive cleanup. The technique proposed allowed the rapid qualitative analysis (e.g., profiling) of bound aglycons as an advantageous alternative in comparison with traditional flavor chemistry methodology, notably for the analysis of trace compounds. In addition, mass spectrometry was shown to be capable of handling crude extracts, being the ideal technique for the systematic study of glycosides as a screening method.

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