African Mango Glycosidically Bound Volatile Compounds

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Carbohydrate and aglycon moieties released, respectively, by acid and enzymatic hydrolysis of African mango pulp extracts containing glycosidically bound compounds were identified by TLC, GC, and GC/MS. Glucose was found to be the most important sugar constituent of the glycoside saccharidic moiety, while significant amounts of arabinose and trace amounts of rhamnose were detected. Several aglycons [(*Z*)-hexen-3-ol, hexanol, hexanoic acid, 2,5-dimethyl-4-hydroxy-3-(2*H*)furanone (furaneol), linalool oxides, α-terpineol, carvacrol, vanillin, *cis*- and *trans*-6-*p*-menthen-2,8-diol, 1,8-*p*-menthadien-7-ol, 1-*p*-menthen-7,8-diol, and 9-hydroxymegastima-4-en-3-one] were identified as additional mango bound volatile compounds. Fatty acids (myristic and stearic acids) were also found in glycosidically bound form. Ten glycosides (benzyl, 2-phenylethyl, and α-terpineyl glucosides and rutinosides, eugenyl, vanillyl and furaneyl glucosides and α-terpineyl arabinoglucoside] were identified for the first time in mango by GC/MS of trifluoroacetylated derivatives and GC using reference compounds. Linalyl oxide glucosides (four isomers) and C_{13} norisoprenoid derivatives [9-hydroxymegastima-4,6-dien-3-one (two isomers), 9-hydroxymegastima-4,7-dien-3-one, and vomifoliol glucosides and arabinoglucosides] were tentatively identified.

Keywords: *Mango; glycosidically bound compounds; TFA derivatives; GC/MS*

INTRODUCTION

The presence of glycosidically bound volatile compounds in plants has been well established (Stahl-Biskup, 1987). These compounds are able to release free aroma compounds by enzymatic or acid hydrolysis and in fruits can be considered as aroma precursors. There has been much recent research on fruit and in particular on tropical fruit glycosidically bound compounds (Engel and Tressl, 1983; Schwab et al., 1989; Winterhalter, 1990; Wu et al., 1991; Adedeji et al., 1992; Koulibaly et al., 1992).

Glycosidically bound volatiles were simultaneously detected in African mango by Adedeji et al. (1992) and Koulibaly et al. (1992). Thirty-three volatile compounds were detected by gas chromatography/mass spectromety (GC/MS) analysis of the enzymatically released aglycons: 8 monoterpene alcohols, 5 aldehydes, 5 acids, 7 hydroxy esters, 5 C_{13} norisoprenoids, and 3 miscellaneous compounds (Adedeji et al., 1992). Bound volatile compounds were present in differing quantitaties in six mango varieties, the ratio between free and bound forms varying from 1.6 to 3.8. These values are similar to those reported for aromatic grape and apricot cultivars (Gunata et al., 1985; Salles et al., 1989). Several aglycons were identified in an acid-hydrolyzed heterosidic pooled isolate obtained from two component-rich varieties, mango obtained from ungrafted trees and mango of Governor variety (Koulibaly et al., 1992). Research into mango glycosidically bound compounds has been hampered by the lack of knowledge concerning the structure of heterosidic compounds.

Knowledge of the saccharidic moiety structure is necessary to control the enzymatic hydrolysis of glycosidically bound volatile compounds. It has been shown (Gunata et al., 1988) that hydrolysis takes place in a sequential manner: the terminal carbohydrate unit is released by the action of a specific glycosidase; then a carbohydrate unit, reported today as a glucose unit linked to the aglycon, is hydrolyzed and released by the action of a *β*-glucosidase.

This study investigates glycosidically bound volatile compounds, isolated from African mangoes obtained from ungrafted trees (Sakho et al., 1985a,b; Koulibaly et al., 1992). TLC, GC, and GC/MS of saccharides and aglycons released by acid and enzymatic hydrolysis of heterosidic compounds and GC/MS of trifluoracetylated derivatives were used.

MATERIALS AND METHODS

Reagents. The solvents (*n*-pentane, diethyl ether, 2-propanol, and chloroform) were of pure grade (purity >97.7%) from Merck (Darmstadt, Germany); acetonitrile, methanol, and ethyl acetate were of HPLC grade from Rathburn (Walkerburn, Scotland). Ammonia was from Merck.

n-Paraffins C_8-C_{30}, purity >95.5%, were from Fluka (Buchs, Switzerland).

Trimethylsilylating (TMS) reagent [*N*,*O*-bis(trimethylsilyl)-trifluoroacetamide chloromethylsilane, (99:1)] and trifluoracetylating (TFA) reagent [*N*-methylbis(trifluoroacetamide)] were Pierce (Rockford, IL).

Amberlite XAD-2 (20−60 mesh), obtained from Röhm and Hass (Philadelphia, PA) was treated according to the procedure of Gunata et al. (1985).

Chromagel 60 A GC silica gel (230−400 mesh) was from Solvants, Documentation, Synthèse (Peypin, France).

Insoluble poly(vinylpyrrolidone) Polyclar AT was a Serva (Heidelberg, Germany) product.

TLC was performed on 0.2 mm precoated silica plates (Kieselgel 60, Merck). Saccharidic compounds were revealed using *N*-(1-naphthyl)ethylenediamine dihydrochloride, Nediac reagent (Merck).

Aspergillus niger pectinase and cellulase were from Sigma (St. Louis, MO) and Rohapect D5L was from Röhm (Darmstadt, Germany).

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S0021-8561(96)00277-4 CCC: $14.00 © 1997 American Chemical Society

Plant Material. Mango fruit used in this study were obtained from ungrafted trees growing in an orchard located near Dakar, Sénégal. Fruits, gathered when fully ripe, were washed, crushed, and refined using a 1 mm diameter screen on a pilot pulper−refiner−pitter (Institut de Technologie Alimentaire, Dakar, Sénégal).

The purée obtained was homogenized, poured into 1 kg bags, and frozen at −20 °C for transport and storage.

Clear Juice Preparation. To obtain clear juice, the mango purée was processed as follows: (a) After dilution with distilled water (1:1, v/v) the mixture was centrifuged at 10000*g* for 15 min and the supernatant filtered. (b) The mango pulp homogenate was treated for 90 min at 25 °C by Rohapect D5L (3.5 g/L) and cellulase (0.2 g/L) in the presence of Polyclar AT (0.2 g/L); the clear juice was obtained by two successive centrifugations at 2500*g* for 30 min and at 10000*g* for 15 min.

The pellet obtained in (a) was treated in the same way.

Isolation of the Glycosidically Bound Fraction. Clear juice (150 mL) was poured in a 30 × 2 cm i.d. column filled with solvent-washed XAD-2 at 40 mL/min. The column was rinsed with 200 mL of distilled water, and the free volatile compounds were eluted with 300 mL of pentane. The glycosidically bound fraction was then eluted using 300 mL of methanol, and the eluate was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under vaccuum at 40 °C. The residue, dissolved in 2 mL of methanol, constitutes the crude glycosidically bound fraction.

Silica Gel Chromotography. The crude glycosidically bound fraction was separated on Chromagel 60 Å GC silica gel in a 450 × 15 mm i.d. column. Elution was carried out successively with 150 mL of chloroform, 440 mL of chloroform/acetonitrile/32% ammonia (15:85:10, v/v), and 660 mL of the same mixture (12.5:87.5:12.5, v/v) (Salles et al., 1990). Eight milliliter fractions were collected and checked by thin layer chromatography (TLC). Fractions with the same TLC patterns were pooled to give four fractions (F1−F4), these fractions were evaporated under vacuum, and the obtained residues were dissolved in 2 mL of methanol.

TLC. TLC was performed with ethyl acetate/2-propanol/water (65:30:15, v/v) as eluent; saccharidic compounds were revealed using Nediac reagent (Salles et al., 1990).

GC. A Varian 3300 (Walnut Creek, CA) apparatus fitted with a flame ionization detector, a 30 m × 25 mm i.d. silica capillary column, DB-5 (J&W Scientific, Folsom, CA), and a Shimadzu (Kyoto, Japan) CR 3 A printer−plotter was used. The operating conditions were as follows: For the aglycon analysis, injector and detector temperatures were 250 and 300 °C, respectively, and hydrogen carrier flow rate was 1.7 mL/min. The column temperature was held at 60 °C for 3 min and then programmed at 2 °C/min to 220 °C. For TFA glycoside derivatives, injector and detector temperatures were 280 and 300 °C, respectively, and helium carrier flow rate was 1.8 mL/min. The column temperature was raised from 125 to 280 °C at 3 °C/min and held for 15 min.

Authentic samples used for identification were obtained from a commercial supply house (phenyl glucoside) or synthesized in our laboratory [benzyl, 2-phenylethyl, and α-terpineyl glucosides and rutinosides) (Salles et al., 1990); eugenyl, vanillyl, and furaneyl glucosides (Chassagne, 1996)]. α-Terpineyl arabinoglucoside was received as gift.

Linear retention indices were calculated using *n*-paraffin standards (Van der Dool and Kratz, 1963).

GC/MS. A Varian 3400 gas chromatograph coupled to an Automass 020 (Unicam, Argenteuil, France) mass spectrometer and a Hewlett-Packard (Palo Alto, CA) 5890 chromatograph coupled to a HP 5889A mass spectrometer were used for electron impact (EI) spectra recording. The column and the temperature program were the same as described above. Helium was used as carrier gas at 2 mL/min. The temperature of the ion source was 160 °C and the ionization voltage 70 eV. The filament current was 0.376 mA, and the mass range was 35−250 scanned at 1.0 s/decade for aglycons and 60−600 scanned at 1.0 s/decade for trifluoroacetylated derivatives.

Enzymatic Hydrolysis. *A. niger* pectinase (0.2 mL) containing β-D-glucosidase, α-L-arabinase, and α-L-rhamnosidase activities (Reyné et al., 1992), in 0.5 mL of 0.1 M phosphate−citrate buffer (pH 5.0), were added to the residue obtained by elimination of the solvent, under a stream of nitrogen, from 0.5 mL of the crude glycosidically bound fraction. The reaction was performed in an hermetically sealed flask for 16 h at 45 °C. The liberated aglycons were extracted with pentane, and the extract was dried over anhydrous sodium sulfate and concentrated to about 0.5 mL by microdistillation.

Acid Hydrolysis. Acid hydrolysis of the crude glycosidically bound fraction (0.1 mL) was performed using 2 M trifluoroacetic acid (0.04 mL) at 120 °C for 1 h (Alberstein et al., 1967). After cooling, the released aglycons were extracted with pentane and the aqueous solution was analyzed by TLC and GLC after trimethylsilylation.

Trimethylsilylation. The method described by Sweely et al. (1963) was used: 100 μL of the aqueous solution obtained after acid hydrolysis of the crude glycosidically bound fraction or after enzymatic hydrolysis of the F4 fraction was concentrated to dryness in a screw-capped vial at 60 °C under a nitogen stream; 20 μL of anhydrous pyridine and 20 μL of TMS reagent were added, the vial was tightly closed, and the contents were stirred, heated at 60 °C for 20 min, and then allowed to cool at room temperature.

Trifluoroacetylation. The crude glycosidically bound extract and the fractions recovered after silica gel separation were evaporated to dryness and treated as above using 20 μL of anhydrous pyridine and 20 μL of TFA reagent instead of the TMS reagent.

RESULTS AND DISCUSSION

Silica Gel Fractionation. The main objective of the prefractionating of the crude mango glycosidically bound extract using silica gel chromatography was to facilitate the separation and the identification of aglycons released by enzymatic hydrolysis and of derivatized glycosidic compounds. However, the TLC analysis of the four fractions (increasing polarity F1−F4) revealed, after Nediac revelation, several spots with R_f values of >0.5. This result indicates the presence of glucosides as major derivatives in the crude extract as well as in F1−F3 fractions, as previously demonstrated using synthetic glycosidically bound compounds and grape and apricot glycosidic fractions (Salles et al., 1990). The presence of glucosides is confirmed by the violet-red color that most of the spots developed after Nediac coloration. Fraction F3 would appear to contain mostly polar derivatives, diglycosides, or polyhydroxylated derivatives. C_{13} norisoprenoid glycosides have been previously reported in bound form in mango (Adedeji et al., 1992).

Acid Hydrolysis. TLC and GC after trimethylsilylation show that glucose (80%) is the most important carbohydrate released by acid hydrolysis of the whole crude heterosidic fraction and of the four isolated fractions. This result concurs with results obtained in the above section and with previously reported data. In particular, the significant aglycon release obtained by the action of β-glucosidase on mango heterosidic compounds (Adedeji et al., 1992) indicates the presence of glucosides. The significant amounts of arabinose (10%) and the trace amounts of rhamnose (1%) detected show that arabinoglucosides and rutinosides are also present.

Enzymatic Hydrolysis. Analysis of aglycons released by enzymatic hydrolysis of the different heterosidic fractions should furnish interesting information concerning the possible nature of glycosidically bound compounds present in these fractions. However, according to the sequential enzymatic mechanism of hydrolysis and the enzyme specificity (Gunata et al., 1988) the nature of the released aglycons can be dependent of the enzyme preparation used. As indi-

Table 1. Volatile Compounds Released by Enzymatic Hydrolysis of Glycosidically Bound Fractions Isolated from African Mango

compound	RI (DB-5)	ID	fraction[a]
(*Z*)-hex-3-en-1-ol	854	MS, RT	C
hexanol	867	MS, RT	C
hexanoic acid	959	MS, RT	C
benzyl alcohol	1033	MS, RT	C, F2
furaneol	1050	MS, RT	C
(*Z*)-linalool furanoxide	1068	MS, RT	C, F2
(*E*)-linalool furanoxide	1082	MS, RT	C, F2
2-phenylethyl alcohol	1111	MS, RT	C, F2
linalool pyranoxide	1163	MS, RT	C
α-terpineol	1179	MS, RT	C, F1, F2, F3
benzoic acid	1191	MS, RT	C, F1, F2, F3
p-cymen-7-ol	1278	MS, RT	F1
1,8-*p*-menthadien-7-ol	1295	MS, RT	C
carvacrol	1297	MS, RT	C
cis-6-*p*-menthen-2,8-diol	1324	MS, RT	F2, F3
eugenol	1353	MS, RT	C
vanillin	1365	MS, RT	F3
9-hydroxymegastigma-4,7-dien-3-one	1372	MS	F3, C
trans-6-*p*-menthen-2,8-diol	1386	MS, RT	C
1-*p*-menthen-7,8-diol	1469	MS	F3
9-hydroxymegastigma-4-en-3-one	1668	MS	C
9-hydroxymegastigma-4,6-dien-3-one (isomer 1)	1700	MS, RT	C, F3
9-hydroxymegastigma-4,6-dien-3-one (isomer 2)	1757	MS, RT	C, F3
myristic acid	1763	MS, RT	F1
vomifoliol	1778	MS, RT	C, F2, F3
palmitic acid	1951	MS, RT	F1
stearic acid	2118	MS, RT	F1

[a] C, crude glycosidically bound fraction; F1, F2, F3, fractions isolated after silica gel separation.

cated under Materials and Methods, a pectinase preparation containing β-D-glucosidase, α-L-arabinase, and α-L-rhamnosidase activities (Reyné et al., 1992) was used and the reaction was performed for a long reaction time, 16 h at 45 °C, to obtain strong hydrolysis conditions.

The volatile compounds identified by GC/MS analysis after enzymatic hydrolysis of the different mango glycosidically bound fractions are listed in Table 1. The presence of linalool oxide isomers, α-terpineol, and phenolic compounds (carvacrol, vanillin, and eugenol) is consistent with previously published results (Adedeji et al., 1926; Koulibaly et al., 1992).

Linalool and geraniol identified after acid hydrolysis are not present among the compounds reported in Table 1; linalool and geraniol are problably artifacts or rearrangement products of free or bound polyols. Several polyols are reported in Table 1, and indeed several unidentified polyols have been detected by GC/MS after enzymatic hydrolysis and trimethylsilylation of the polar F4 fraction.

Menthol previously identified as free (Indstein and Schreier, 1989; Koulibaly et al., 1992) and bound compound (Koulibaly et al., 1992) was not detected after enzymatic hydrolysis of the heterosidic pool. However, several compounds having a *p*-menthane skeleton were detected, such as *p*-cymen-7-ol and carvacrol. Some of these [*cis*- and *trans*-6-*p*-menthen-2,8-diol (sobrerol)] are reported for the first time as mango aroma compounds. 1-*p*-Menthen-7,8-diol (8-hydroxyperillyl alcohol) (Figure 1) was also tentatively indentified.

Detected in hydrolysates obtained from fractions F1 and F3 were 9-hydroxymegastigma-4,7-dien-3-one, 9-hydroxymegastigma-4,6-dien-3-one (two isomers), and vomifoliol, previously identified among mango (Adedeji et al., 1992) and purple passion fruit (Winterhalter, 1990) bound volatiles, as well as 9-hydroxymegastigma-4-en-3-one, reported as a purple passion fruit bound C_{13} norisoprenoid (Winterhalter, 1990).

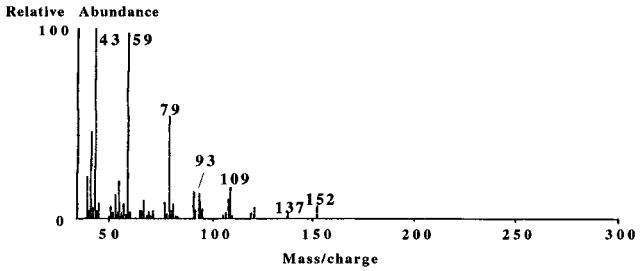

Figure 1. EI spectum of 1-*p*-menthenen-7,8-diol.

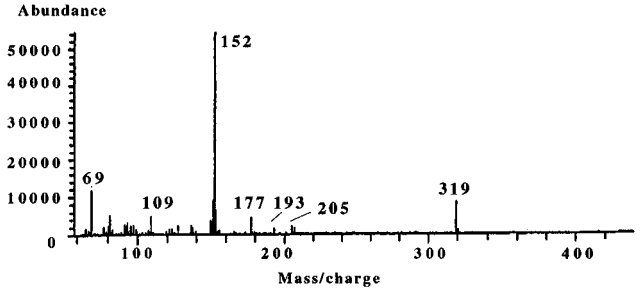

Figure 2. EI spectrum of trifluoroacetylated vanillyl glucoside.

Several acids (hexanoic, myristic, palmitic, stearic, and benzoic) were also detected in bound form in mango; palmitic acid has been previously reported (Adedeji et al. 1992). Fatty acids are presumed to play a significant metabolic role in the mango ripening; more particularly the ratio of palmitic acid to palmitoleic acid was correlated with mango aroma and flavor (Bandyopadhayay and Gholap, 1973).

Moreover, it is worth drawing attention to the identification of 2,5-dimethyl-4-hydroxy-3-(2*H*)furanone (Furaneol) in bound form, since this result indicates clearly that this compound, previously identified as free component in canned mangoes (Hunter et al., 1974), is a natural product. Results of flavor threshold determination and aroma comparison studies suggest, however, that this compound does not contribute significantly to mango aroma and flavor, with the exception of mango cultivars possessing pineapple-like aroma (Wilson et al., 1990).

The chromatographic profiles obtained for the aglycons released by enzymatic hydrolysis of the glycosidically bound fractions isolated from the enzymatically liquified pellet or from the whole pulp are the same as the profile obtained from the serum resulting from pulp centrifugation.

GC/MS Study of TFA Derivatives. Several glycosically bound compounds have been identified by ^{1}H and ^{13}C NMR spectroscopy (Williams et al., 1982; Winterhalter et al., 1991; Chassagne et al., 1996) or by MS/MS (Salles et al., 1991) after isolation of these compounds. Grape monoterpene glycoside structures have been established by a combination of enzymatic hydrolysis followed by aglycon GC/MS analysis, and direct GC/EI-MS of TMS and TFA derivatives, trifluoroacetylation gives the best results (Voirin et al., 1992a,b). According to these authors, no interference between the fragment ions resulting from the saccharidic moiety and those resulting from the aglycon moiety is observed. The EI-MS characteristic fragment ions determined by Voirin et al. (1992a,b) for TFA derivatives of β-D-glucopy-

Table 2. TFA Derivatives of β-D-Glucopyranosides Identified or Tentatively Identified in African Mango

compound	RI (DB-5) unknown	RI (DB-5) ref	EI-MS of sugar moiety	EI-MS of aglycon moiety
furaneyl	1668	1672	319 (18), 177 (9), 193 (1), 205 (0.8)	128 (100), 69 (44), 85 (24), 72 (12), 148 (7), 147 (6)
benzyl	1770	1770	319 (5), 193 (4), 205 (1), 265 (0.8)	91 (100), 92 (20), 107 (2.5), 108 (1.7)
linalyl oxide	1784		319 (26), 193 (19), 205 (17), 265 (0.9)	111 (100), 93 (76), 69 (48), 71 (28), 153 (26), 94 (9)
linalyl oxide	1794		319 (32), 193 (18), 177 (3.8), 265 (1.4)	111 (100), 93 (78), 69 (48), 71 (29), 153 (23), 94 (9)
linalyl oxide	1809		319 (20), 193 (12), 177 (9), 265 (1.4)	111 (100), 93 (73), 69 (49), 71 (25), 153 (15), 94 (4.5)
linalyl oxide	1814		319 (26), 177 (10), 193 (2), 265 (0.8)	111 (100), 93 (96), 69 (47), 71 (2), 153 (17), 94 (9)
2-phenylethyl	1868	1856	319 (8.5), 193 (2.6), 177 (0.5)	105 (100), 104 (53), 106 (31), 91 (40)
α-terpineyl	1946	1936	319 (38), 177 (10), 193 (9)	136 (100), 81 (100), 69 (70), 93 (48), 121 (28)
eugenyl	2046	2039	193 (10), 177 (5), 205 (4), 179 (3)	164 (100), 71 (55), 81 (25), 149 (15), 103 (13)
vanillyl	2101	2092	319 (16), 177 (9), 205 (5), 193 (3)	152 (100), 151 (17), 153 (15), 109 (10), 81 (6)
9-hydroxymegastima-4,6-dien-3-one (isomer 1)	2201		319 (70), 193 (6), 177 (3), 265 (2.5)	164 (100), 149 (67), 45 (27), 91 (26), 121(25)
9-yydroxymegastima-4,6-dien-3-one (isomer 2)	2371		319 (66), 193(4), 177(3), 265 (0.5)	164 (100), 149 (52), 45 (16), 105 (8), 121 (6)
vomifoliol	2319		319 (33), 193 (6), 177 (5), 205 (4), 265 (2)	124 (100), 150 (62), 69 (37), 95 (13), 79 (10), 206 (6)

Table 3. TFA Derivatives of Rutinosides Identified or Tentatively Identified in African Mango

compound	RI (DB-5) unknown	RI (DB-5) ref	EI-MS of sugar moiety	EI-MS of aglycon moiety
benzyl	2118	2118	319 (18), 205 (2), 265 (1.6) 165 (1)	91 (100), 92 (69), 121 (78), 107 (11)
2-phenylethyl	2201	2191	319 (10), 177 (3.7), 207 (1.4), 179 (0.5), 193 (0.5)	105 (100), 104 (47), 106 (24), 91 (46)
α-terpineyl	2251	2241	193 (45), 319 (23), 207 (12), 205 (12), 179 (9), 265 (1.7)	81 (100), 136 (61), 137 (35), 93 (29), 121 (28)
vomifoliol	2350		207 (19), 319 (7), 177 (5), 179 (4), 193 (3), 265 (1)	124 (100), 150 (72), 69 (28), 206 (10), 95 (9), 135 (8), 79 (7)

Table 4. TFA Derivatives of 6-*O*-(α-L-Arabinofuranosyl)-β-D-glucopyranosides Tentatively Identified in African Mango

compound	RI (DB-5) unknown	RI (DB-5) ref	EI-MS of sugar moiety	EI-MS of aglycon moiety
9-hydroxymegastima-4,7-dien-3-one	2285		319 (34), 193 (6), 165 (5), 421 (2), 265 (2)	108 (100), 123 (20), 43 (14), 135 (10), 53 (7), 208 (5), 152(5)
α-terpineyl	2334	2345	193 (43), 421 (8), 278 (5), 265 (2), 319 (1), 177 (1), 165 (1)	136 (75), 81 (57), 93 (41), 121 (34)
9-hydroxymegastima-4,6-dien-3-one (isomer 1)	2636		193 (50), 165 (25), 265 (18), 319 (11), 177 (9), 205 (8), 421 (3)	164 (100), 149 (37), 121 (33), 91 (8), 45 (7)
9-hydroxymegastima-4,6-dien-3-one (isomer 2)	2684		193 (51), 165 (18), 319 (4), 421 (3.5), 177 (1.5), 265 (1)	164 (100), 45 (32), 149 (16), 91 (5), 45 (5)

ranosides, 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosides (rutinosides), and 6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranosides were used for the determination of mango glycosidically bound component saccharidic moiety. The characteristic fragments of the aglycon moiety, more numerous and more abundent than those obtained from the saccharidic moiety, generally enable glycosidically bound compounds to be tentatively identified.

The results concerning mango β-D-glucopyranosides are given Table 2. For example, fragment ions at *m*/*z* 319, 177, 205, and 193 (Figure 2) are characteristic of a glucose unit and fragment ions characteristic of another saccharide are not detected. Fragment ions at *m*/*z* 152, 151, 153, 109, and 81 can be attributed to vanillin. The presence of vanillyl β-D-glucopyranoside was confirmed using an authentic sample. Benzyl, 2-phenylethyl, α-terpineyl, eugenyl, and furaneyl β-D-glucopyranosides were identified using the same method. Furaneol bound as glucoside has been previously found in strawberries (Mayert et al., 1989).

Linalyl oxide β-D-glucopyranosides (four isomers), 9-hydroxymegastigma-4,6-dien-3-one (two isomers), and vomifoliol β-D-glucopyranosides were tentatively identified from their MS data. The linalyl oxide TFA derivative retention values agree with those previously published by Voirin et al. (1992b). Four linalyl oxide have been previously tentatively identified in apricot using ND_3 as reagent gas in negative ion chemical ionization (Salles et al., 1991).

As indicated in Figure 3 fragment ions at *m*/*z* 319, 265, 193, and 177 are characteristic of a glucose unit, fragment ions at *m*/*z* 207 and 179 indicate the presence of a rhamnose unit, and fragment ions at *m*/*z* 124, 150, 69, 206, 95, 135, and 79 can be attributed to a vomifoliol moiety (Winterhalter, 1990). According to these data, and the identification of vomifoliol among the compounds release after enzymatic hydrolysis of the hetrosidic extract, the presence of vomifoliol rutinoside as mango glycosidically bound compound can be postulated. Benzyl, 2-phenylethyl, and α-terpineyl rutino-

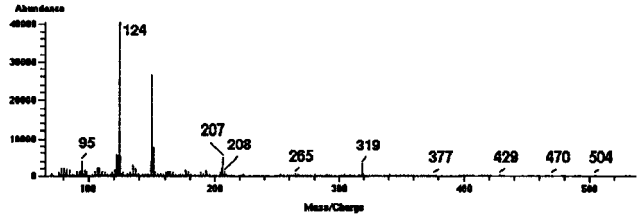

Figure 3. EI spectrum of trifluoroacetylated vomifoliol rutinoside.

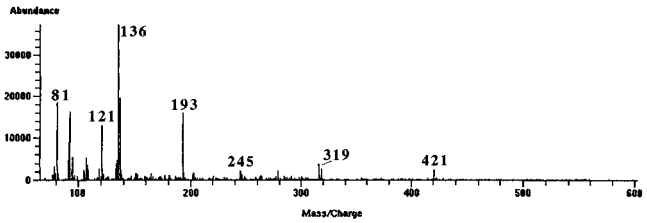

Figure 4. EI spectrum of trifluoroacteylated α-terpineyl 6-*O*-(α-L-arabinofuranosyl)-*β*-D-glucopyranoside (tentatively identified).

sides were identified on the basis of their mass spectra and their retention indices relative to those of reference compounds (Table 3).

According to the presence of fragment ions at *m*/*z* 319, 193, 177, and 265 characteristic of a glucose unit and fragment ions at *m*/*z* 421, 278, and 165 characteristic of an arabinose unit, a 6-*O*-(α-L-arabinofuranosyl)-*β*-D-glucopyranoside structure can be postulated from the spectral data given Figure 4. Fragment ions at *m*/*z* 136, 81, 93, 69, 121, and 121 can be assigned to α-terpineol. The presence of α-terpineyl 6-*O*-(α-L-arabinofuranosyl)-*β*-D-glucopyranoside was confirmed using an authentic sample. 9-Hydroxymegastima-4,7-dien-3-one, 9-hydroxymegastima-4,6-dien-3-one (two isomers), and 6-*O*-(α-L-arabinofuranosyl)-*β*-D-glucopyranosides were tentatively identified from their MS data (Table 4).

Conclusion. Ten glucosides, arabinoglucosides, and rutinosides have been identified for the first time in mango fruit using coupled GC/EI-MS; however, several compounds are still tentatively identified or unidentified according to the lack of available reference compounds. This fact underscores the limitations of the on-line analysis of glycosidically bound compounds used in the present work. The use of efficient preparative separation techniques, preparative HPLC, countercurrent chromatography, and immunoseparation is required for the isolation of labile natural compounds present in minute amounts and their identification by MS, MS/MS, and NMR. Research in this area is in progress in our laboratory.

ACKNOWLEDGMENT

We thank C. Bayonove, R. Baumes, and J. P. Lepoutre, Laboratoire des Arômes et des Substances Naturelles, INRA Montpellier, for providing reference compounds and mass spectrometer facilities.

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Registry No. Supplied by the Author: *n*-Pentane, 109-66-0; diethyl ether, 60-29-7; isopropanol, 67-63-0; chloroform, 67-66-3; acetonitrile, 75-03-8; methanol, 67-56-1; ethyl acetate, 141-78-6; ammonia, 1336-21-6; *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, 25561-30-2; chloromethylsilane, 75-77-4; *N*-methylbis(trifluoroacetamide), 815-06-5; Amberlite XAD 2, 9060-05-3; Polyclar AT, 25249-54-1; *N*-(1-naphthyl)-ethylenediamone dihydrochloride, 1465-25-4; pectinase, 9032-75-1; cellulase, 9012-54-8; phenyl glucoside, 1464-44-4.

Received for review April 16, 1996. Revised manuscript received October 22, 1996. Accepted November 21, 1996.[⊗]

JF960277B

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1997.