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Free and bound flavour components of Amazonian fruits: 3-glycosidically bound components of cupuacu

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Abstract

The total quantity (11.7 mg/kg of pulp) of aglycones released by enzymatic hydrolysis of the glycosidic extract indicates a significant aroma potential for cupuacu. Among the 47 aglycones identified, 24 are not present in the free volatile fraction, among them, 4-methylguaiacol, 4-propylguaiacol, 2,6-dimethyl-octa-1,7-dien-3,6-diol, 2,6-dimethyl-oct-7-en-1,6-diol, homovanillic acid, 2-methyl-but-3-en-1-ol and acetic acid. The quantitatively most important aglycones are 3-methyl-butan-1-ol, 2-phenylethanol, linalol, (*Z*)-2,6-dimethyl-octa-2,7-dien-1,6-diol, butan-1-ol and hexan-1-ol. The methylated alditol acetates analysis of monosaccharides released by acid hydrolysis of the heterosidic extract, indicates that glucose is involved in glucosidic and glycosidic structures. Moreover, rhamnopyranose and xylopyranose units, bound in the terminal position, suggest the presence of rutinosides and small amounts of primeverosides. Six glucosides, hexyl, benzyl, 2-phenylethyl, (*R*) and (*S*)-linalyl and geranyl β-D-glucopyranosides, five rutinosides, benzyl, (*R*) and (*S*)-linalyl, α-terpineyl and 2-phenylethyl rutinosides and 3-methyl-but-2-enyl vicianoside were identified by GC–EIMS after TFA derivatization of the crude heterosidic fraction. Moreover, four linalol oxides, octyl, 3-methyl-butyl β-D-glucopyranosides and hexyl, octyl, two linalol oxides and 3-methyl-butyl rutinosides, have been tentatively identified from their TFA mass spectra. Some glucosides are probably substituted by malonyl and unidentified acyl residues.

Keywords: Cupuacu; *Theobroma grandiflorum*; Glycosides; Enzymatic hydrolysis; Aglycones; TFA derivatives; GC–MS

1. Introduction

Free volatile compounds, extensively studied over the last 30 years, are also present in plants as glycosidically bound components. These compounds were first reported by Bourquelot and Bridel (1913) who identified a geranyl-β-D-glucoside in *Pelargonium odoratissimum*. To date, glycoconjugates have been detected in almost 170 plants belonging to 50 families (Crouzet & Chassagne, 1999; Stahl-Biskup, Intert, Holthuijzen & Stengele, 1993; Winterhalter & Skouroumounis, 1997).

Aroma compounds can be released from these non-volatile precursors by enzymatic or chemical reactions during maturation, storage, industrial pretreatment or processing. As an example, vanillin, the characteristic aroma compound of vanilla, is released during the fermentation process by the action of vanilla β-glucosidase on vanillin glucoside (Arana, 1943).

There has been much recent research on glycosidically bound compounds present in fruit and, particularly, in tropical fruit such as purple and yellow passion fruit (Chassagne, Crouzet, Bayonove, & Baumes, 1998; Engel & Tressl, 1983; Winterhalter, 1990), papaya (Heidlas, Lehr, Idstein & Schreier, 1984; Schwab, Mahr & Schreier, 1989) pineapple (Wu, Kuo, Hartman, Rosen & Ho, 1991), mango (Adedeji, Hartman, Lech & Ho, 1992; Koulibaly, Sakho & Crouzet, 1992; Sakho, Chassagne & Crouzet, 1997), hog plum (Adedeji, Hartman, Rosen & Ho, 1991), granadilla (Agudelo, Suarez & Duque, 1996), lulo fruit (Wintoch, Morales, Duque & Schreier, 1993) and bacuri (Boulanger, Chassagne & Crouzet, 1999).

This study investigates glycosidically-bound volatile compounds, isolated from an amazonian fruit, cupuacu, recovered by solid phase extraction. GC and GC–MS of saccharides and aglycones released by acid and enzymatic

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hydrolysis of heterosidic compounds and GC–MS of trifluoracetylated derivatives were used.

2. Materials and methods

2.1. Reagents

The solvents *n*-pentane, dichloromethane and methanol were pure grade (purity more than 97.7 %) from Carlo Erba (Rodano, Italy) and were distilled before use. *n*-Paraffins C8 to C32, purity more than 95.5%, were obtained from Sigma (St Quentin Fallavier, France).

Trifluoroacetylating (TFA) reagent [*N*-methyl-bis (trifluoroacetamide)] was obtained from Pierce (Rockford, IL, USA).

Amberlite XAD-2 (20–60 mesh), obtained from Röhm and Hass (Philadelphia, PA, USA), was treated according to the procedure of Günata, Bitteur, Brillouet, Bayonove and Cordonnier (1985).

Hemicellulase REG-2 (Gist-Brocades, Seclin, France) and sweet almond glucosidase-emulsin (Sigma) were used.

Reference volatile compounds were obtained from a commercial firm or received as a gift; glucosides and rutinosides, were synthesized in our laboratory (Salles, Jallageas & Crouzet, 1990); 3-methyl-but-2-enyl vicianoside was isolated from purple passion fruit (Chassagne, Crouzet, Bayonove, Brillouet & Baumes, 1996).

2.2. Plant material

Mature cupuacu fruits were purchased at Belem market and transported to France by plane. After seed separation, the pulp was frozen and stored at −18°C until used. The thawed pulp was homogenized in a Waring blender in the presence of distilled water (1:1, w/v) and centrifuged (30 min, 10 000 *g*) at 4°C. The precipitate was diluted in distilled water (1:1, w/v), and the solution centrifuged under the same conditions. The two clear supernatants were pooled and used in the next separation step.

2.3. Volatile component and glycoside separation

Clear juice (100 ml) was poured onto a 9×1 cm i.d. column filled with solvent-washed XAD-2 at 1.5 ml/min and the separation of volatile compounds and glycosides was achieved as indicated by Boulanger et al. (1999).

2.4. Enzymatic hydrolysis

The glycosidic extracts obtained from 95 ml of clear juice were concentrated in vacuum to dryness, redissolved in 0.3 ml of 0.2 M citrate-phosphate buffer (pH 5) and extracted five times using pentane-dichloromethane (2:1) and the residue hydrolyzed by hemicellulase REG-2 almond glucosidase at pH 5 (Boulanger et al., 1999).

2.5. Estimation of glycosidically bound compounds

The glycosidically bound compounds were estimated by the determination of the total area of identified and unidentified aglycones released by enzymatic treatment, as indicated above, of the crude glycosidically bound fraction (Günata et al., 1985).

2.6. Methyl alditol acetates analysis

The method used for methyl alditol acetates preparation was reported in detail in Boulanger et al. (1999).

2.7. Trifluoroacetylation

An aliquot of the methanolic solution obtained after elution of the XAD-2 column, corresponding to 1 ml of clear juice, was concentrated to dryness in a screw-capped vial at 60°C under a stream of nitrogen. Anhydrous pyridine (20 μl) and 20 μl of trifluoroacetylating (TFA) reagent were added and the vial tightly closed, stirred, heated at 60°C for 20 min, and then allowed to cool to room temperature (Sweeley, Bentley, Makita & Wells, 1963).

2.8. Aglycone analysis

A Varian 3300 (Walnut Creek, CA, USA) chromatograph equipped with split injector (1/10) and flame ionization detector was used in this case and for all GC analysis. Two fused silica capillary columns (J&W Scientific, Folsom, CA, USA) were employed: (a) DB-WAX and (b) DB-5MS (30 m×0.25 mm i.d., film thickness, 0.25 μm). The temperature programs were (a) 3 min isothermal at 60°C and then increased at 2°C/min to 220°C, then from 220 to 250°C at 5°C/min and maintained for 15 min, (b) 5 min isothermal at 40°C, increased from 40 to 200°C at 2°C/min, then from 200 to 250°C at 5°C/min and maintained for 15 min. In both cases, the carrier gas was H_2 at 1.8 ml/min; N_2 at 30 ml/min was used for the make-up. The injector temperature was maintained at 250°C and the detector temperature was (a) 250°C and (b) 300°C. Nonan-4-ol was the internal standard, linear retention indices were calculated using *n*-paraffin standards (Van den Dool & Kratz, 1963).

2.9. TFA glycoside analysis

A DB-5MS fused silica capillary column (30 m×0.25 mm i.d.; 0.25 μm) was used. The column temperature was raised from 125 to 220°C at 3°C/min then increased to 280°C at 2°C/min; injector temperature was 280°C, and detector temperature 300°C. The flow rate for the carrier gas and the make-up were the same as those mentioned above. Identifications were based on RRt (retention time relative to phenyl glucoside, used as

internal standard) on DB-5MS, using authentic reference compounds and confirmed by GC–MS.

2.10. Methylated alditol acetate analysis

DB-5MS and DB-225 capillary column were used. For the two columns, the operating conditions were as follows: split injection (1/10), carrier gas H_2 at 1.8 ml/min, injector and detector temperature 250°C. The column temperature was for DB-5MS: an isotherm at 145°C for 10 min then increased to 210°C at 2°C/min, and for DB-225: an isotherm at 170°C for 15 min then increased to 210°C at 5°C/min. Identifications were based on RRt (retention time relative to inositol, used as internal standard) on DB-5MS, using authentic reference compounds and confirmed by GC–MS (Bjorndal, Hellerqvist, Lindberg & Svenson, 1970).

2.11. GC–MS analysis

EI-mass spectra were recorded by coupling a Varian 3400 gas chromatograph equipped with a DB-5MS fused silica capillary column (30 m×0.25 mm i.d.; 0.25 μm bonded phase, J&W Scientific) to an Automass 020 (Unicam, Argenteuil, France) mass spectrometer under the experimental conditions previously described (Boulanger et al., 1999).

2.12. Bound malonic acid determination

A methanolic solution (20 ml) of crude heterosidic extract was concentrated under vacuum to dryness, redissolved in 10 ml of distilled water acidified to pH 1.5 and refluxed for 2 h. After diethyl ether extraction, methylation was performed using diazomethane and the organic phase was dried, concentrated and analysed by GC and GC–MS (Schwab & Schreier, 1988). A sample of malonic acid was derivatized under the same conditions for identification.

2.13. Repeatability

Three analyses, extraction and measurement, were carried out on each extract to determine the variation coefficient for each component identified.

3. Results and discussion

3.1. Estimation of glycosidically-bound compounds

The determination of total quantity of aglycones released by enzymatic hydrolysis of the glycosidic extract indicated a significant aroma potential for cupuacu (11.7 mg/kg of pulp). This potential was greater than those determined for bacuri (7.9 mg/kg of pulp), and acerola (6.3 mg/kg of pulp). However, this potential was lower than that found for other tropical fruit such as mango (19.6 mg/kg of pulp), purple (30 mg/kg of pulp) and yellow (37.6 mg/kg of pulp) passion fruits.

3.2. Acid hydrolysis of the glycosidic extract

GC of alditol acetates showed that glucose (66%) and rhamnose (25%) were the most abundant carbohydrates released by acid hydrolysis of cupuacu crude heterosidic extract. Small amounts of xylose (4.5%), galactose (2.5 %), mannose (1.5%) and arabinose (0.5%) were also found.

Among the methylated alditol acetates derivatized from the cupuacu heterosidic fraction, the identification of 2,3,4,6-tetra-*O*-methylglucose and 2,3,4-tri-*O*-methylglucose (Table 1) indicated that glucose was involved in glucosidic and glycosidic structures. Moreover, the presence of 2,3,4-tri-*O*-methylrhamnose and 2,3,4-tri-*O*-methylxylose, characteristic of rhamnopyranose and xylopyranose units bound in the terminal position, suggested the presence of rutinosides and small amounts of primeverosides; these results showed that the occurrence of gentiobiosides was also possible.

3.3. Enzymatic hydrolysis of the glycosidic extract

The analysis of aglycones released by enzymatic hydrolysis of the glycosidic extract should give relevant information about the possible nature of cupuacu glycosidically-bound compounds. According to the sequential enzymatic mechanism described by Günata, Bitteur, Brillouet, Bayonove and Cordonnier (1988), several enzymatic activities were required to perform the total hydrolysis of glycosides. Previously reported data (Cordonnier, Günata, Baumes & Bayonove, 1989) indicated that hemicellulase REG-2 possessed both high β-glucosidase and arabinofuranosidase activities, fair rhamnopyranosidase activity but low arabinopyrano-sidase activity; and sweet almond glucosidase had a high

Table 1
Identification and relative molar percentages of partially methylated alditol acetates isolated from cupuacu fruit glycosides

Alditol acetates	Relative retention time[a]	Relative molar percentage
2,3,4-tri-*O*-Methyl rhamnose	0.263	23.2
2,3,4-tri-*O*-Methyl xylose	0.435	2.8
2,3,4,6-tetra-*O*-Methyl glucose	0.472	27.1
2,3,4,6-tetra-*O*-Methyl galactose	0.511	tr
3,4,6-tri-*O*-Methyl mannose	0.629	tr
2,3,4-tri-*O*-Methyl glucose	0.680	46.9

[a] Relative to inositol.

arabinopyranosidase secondary activity. According to the results previously reported by Chassagne, Bayonove, Crouzet and Baumes (1995) and Chassagne, Boulanger and Crouzet (1999) on passion fruit glycosides, a mixture of these two preparations was able to hydrolyse cupuacu glycosides.

The volatile compounds released by the action of this mixture, and identified by GC–MS are listed in Table 2. Among the 47 compounds newly identified, 24, as indicated in this table, were not present in the free volatile fraction. However, among the 24 compounds, except for the phenolic compounds (4-methylguaiacol, 4-propylguaiacol and homovanillic acid), 2-methyl-but-3-en-1-ol, oct-1-en-3-ol, 2,6-dimethyl-octa-1,7-dien-3,6-diol, 2,6-dimethyl-oct-7-en-1,6-diol and acetic acid, all the aglycones were present in low amounts. It has previously been suggested (Williams, Sefton & Wilson 1989; Williams, Sefton & Francis 1992), that, because of the low concentration of some free aroma compounds, flavour precursor analysis can be used, in a strategic approach, to investigate flavour compounds. It can be assumed that the cytotoxicity of phenolic compounds is lowered through the glycosylation process (Stahl-Biskup et al., 1993).

The concentration of bound 3-methyl-butan-1-ol (1.7 mg/kg) was considerably greater than the concentration found in the free fraction (0.07 mg/kg). The odour of this compound reported by Fischer, Hammerschmidt and Brunke (1995) as chocolate-like, was not perceived by us on sniffing the aglycone fraction or the free cupuacu volatile extract.

Oxygenated terpene compounds represented an important part of the compounds released by enzymatic hydrolysis, linalol and linalol oxides constituting 56% of this fraction. A considerable amount of (*Z*)-2,6-dimethyl-octa-2,7-dien-1,6-diol and low amount of its (*E*) isomer, compounds previously identified in bacuri (Boulanger et al., 1999) and in free form in cupuacu (Boulanger & Crouzet, submitted), were found. As small amount of 2,6-dimethyl-oct-7-en-1,6-diol, recently reported as a component of *Rosa damascena* petal, constituted a potential aroma precursor (Knapp, Straubinger, Fornari, Oka, Watanabe & Winterhalter, 1998). Moreover, 2,6-dimethyl-octa-1,7-dien-3,6-diol, also present in bacuri and rose petals, can also acted as aroma precursor (Knapp et al., 1998).

3.4. Identification of TFA glycoside derivatives

Previous data (Voirin, Baumes, Günata, Bitteur, Bayonove & Tapiero, 1992; Voirin, Baumes, Sapis & Bayonove, 1992) indicate that glycoside structures can be tentatively established by direct GC/EI-MS of their TFA derivatives. According to these reports, no interference between the fragment ions resulting from the saccharidic moiety and those resulting from the aglycone moiety

Table 2
Aglycones released by enzymatic hydrolysis from cupuacu crude heterosidic extract

Compound	RI[a]	RI[b]	Concentration µg/kg of pulp
Aromatic compounds			
Benzaldehyde	1497	960	41±2
Benzyl alcohol	1851	1033	333±54
2-Phenylethanol	1883	1103	672±73
4-Methyl guaiacol[d]	1890	1181	188±31
Anisaldehyde[d]	1980	1239	26±5
4-Propyl guaiacol[c,d]	2041	1356	42±7
4-Ethyl phenol[d]	2118	1153	tr[e]
Eugenol[d]	2122	1349	tr
Ethyl 4-hydroxy-3-methoxybenzoate[d]	2479	–	tr
Vanillin[d]	2520	1393	tr
Vanillin methyl ether[c]	2539	–	tr
Coniferylic alcohol[d]	3105	1729	tr
4-Hydroxy-3-methoxyphenyl acetate[c,d] (homovanillic acid)	–	1633	209±39
Aliphatic alcohols			
2-Methyl-but-3-en-1-ol[d]	–	778	133±23
Butan-1-ol	1119	–	534±81
3-Methyl-butan-1-ol	1215	729	1699±237
3-Methyl-but-3-en-1-ol[d]	1225	720	18±3
Pentan-1-ol	1230	770	47±6
3-Hydroxy-butan-2-one	1256	–	tr
2-Ethyl-butan-1-ol[c,d]	1291	–	tr
3-Methyl-but-2-en-1-ol	1315	768	255±38
Hexan-1-ol	1336	871	497±50
(*Z*)-Hex-3-en-1-ol	1372	856	15±3
Oct-1-en-3-ol[d]	1419	984	45±8
Octan-1-ol[d]	1540	1074	21±4
Oxygenated terpenics compounds			
(*Z*)-Linalol furanoxide	1434	1068	11±0
(*E*)-Linalol furanoxide	1457	1082	14±2
Linalol	1543	1096	904±91
α-terpineol	1693	1188	58±6
(*Z*)-Linalol pyranoxide[c]	1720	1167	9±1
(*E*)-Linalol pyranoxide[c]	1747	1173	66±4
Citronellol[d]	1755	1228	13±1
Geraniol[d]	1831	1254	14±1
2,6-Dimethyl-octa-1,7-dien-3,6-diol[d]	2134	1277	50±8
2,6-Dimethyl-oct-7-en-1,6-diol[c,d]	2184	–	74±19
(*E*)-2,6-Dimethyl-octa-2,7-dien-1,6-diol	2281	1338	21±3
(*Z*)-2,6-dimethyl-octa-2,7-dien-1,6-diol	2325	1362	550±143
Esters			
Methyl 3-methyl 3-hydroxybutanoate[c,d]	1363	–	15±2
Methyl 3-methyl 2-hydroxybutanoate[c,d]	1383	889	14±2
Ethyl 3-hydroxybutanoate[c]	1483	911	tr
Ethyl 3-hydroxyhexanoate[c]	1664	1126	tr
Acids			
Acetic acid[d]	1413	–	40±4
Butanoic acid	1594	–	tr
Nonanoic acid[d]	2130	1280	tr
Hexadecanoic acid	–	1970	tr
Miscellaneous compounds			
δ-Decalactone[d]	2141	1493	tr
Dehydrovomifoliol[d]	3000	1765	tr

[a] RI, Linear retention index on DB-WAX.
[b] RI, Linear retention index on DB-5MS.
[c] Compound tentatively identified.
[d] Compound not previoulsy identified in the volatile fraction (Boulanger & Crouzet, in press).
[e] tr, traces.

occurs. Fragment ions characteristic of the saccharidic moiety were generally less intense that those corresponding to the aglycones. Fragment ions at *m/z* 319, 265, 205, 177 and 193 indicated the presence of a glucose unit, whereas fragment ions at *m/z* 435, 292, 207 and 179 and fragment ions at *m/z* 421, 193 and 165 were characteristic of rhamnose and arabinose or apiose, respectively. However, low intensity fragment ions at *m/z* 278 and 298 can be used to distinguish arabinofuranosyl and apiofuranosyl derivatives. For example, as indicated in Fig. 1, fragment ions at *m/z* 435, 319, 265, 207, 205, 193 and 179, showed the occurrence of glucose and rhamnose units. The results obtained after acid hydrolysis, showing that rhamnose was in a terminal position, were suggestive of a rutinose unit. Otherwise, fragment ions at *m/z* 136, 121, 93, 81, and 69 can be attributed to α-terpineol. α-Terpineyl rutinoside was formally identified by comparison of the relative retention time of its TFA derivative to that of an authentic sample. Hexyl, benzyl, 2-phenylethyl, (*R*) and (*S*)-linalyl and geranyl β-D-glucopyranosides and benzyl, (*R*) and (*S*)-linalyl and 2-phenylethyl rutinosides were identified using the same method (Table 3). Moreover, four linalol oxides, octyl, 3-methyl-butyl β-D-glucopyranosides and hexyl, octyl, two linalol oxides and 3-methyl-butyl rutinosides were tentatively identified from their TFA mass spectra. A good correlation between the aglycone moieties, constitutive of the identified glycosidic compounds and the volatile compounds present in the fraction released by enzymatic hydrolysis, was found. More particularly, 3-methyl-butan-1-ol was the most abundant compound of this fraction. Moreover, 3-methyl-but-2-enyl vicianoside, previously isolated from passion fruit (Chassagne et al., 1996), was found to be a constituent of the cupuacu glycosidic extract. The presence of (*R*) and (*S*) isomers of linalyl glucoside and rutinoside with an enantiomeric excess of 20% in favour of the (*S*) occurs but only the (*S*) derivative was detected in passion fruit (Chassagne et al., 1998) and in bacuri (Boulanger et al., 1999); conversely, in contradiction with the results obtained for the structural composition of the saccharidic moiety, no gentiobiosides were detected.

The chromatogram of cupuacu TFA glycoconjugates (not given) showed that glycosides with important retention times (greater than 17 min) were few compared to glucosides with low retention times (lower than 17 min). The presence of 6′-*O*-malonyl glucoconjugates in cupuacu can be explained the discrepancy between the considerable amount found for 2,3,4-tri-*O*-methyl-glucose (49.6%) and the failure to identify gentiobiosides

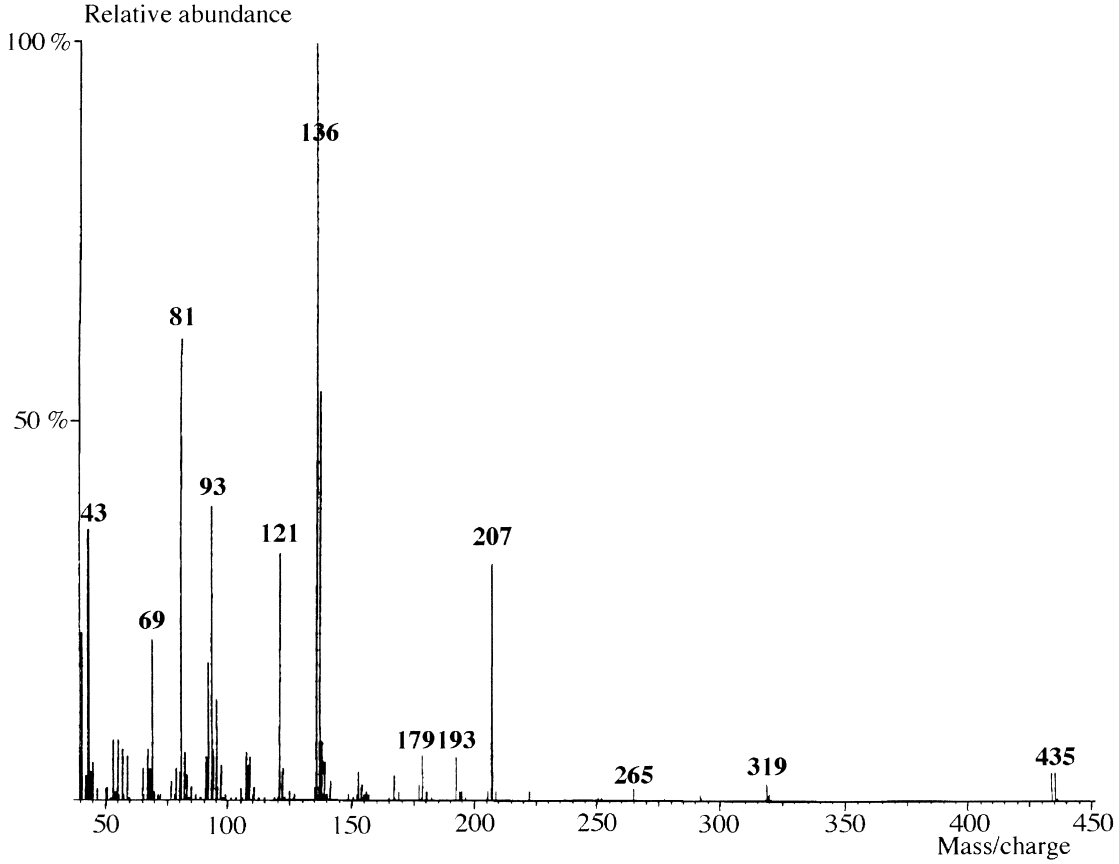

Fig. 1. EI mass spectrum of TFA α-terpineyl rutinoside isolated from cupuacu pulp.

Table 3
Electronic impact mass spectrometric data of cupuacu trifluoroacetylated glycosides identified or tentatively identified

Glycoside	RRt[a]		RA[d]	EI mass spectrometric data	
	Na[b]	Ref[c]		Aglycone moiety	Saccharidic moiety
3-Methyl-butyl[e]	0.66		+ +	71(100), 69(39), 70(4), 72(4)	319(2), 193(6), 177(5), 265(1)
Hexyl	0.85	0.85	+ + +	85(100), 57(54), 61(15), 55(18)	319(2), 193(1), 177(1), 265 (0.5), 205(0.5)
Benzyl alcohol	1.19	1.19	+ + +	91(100), 92(18), 69(8),	319(2), 193(1), 205(0.8)
Linalyl furanoxide[e]	1.22		+	93(100), 69(88), 111(81), 81(45), 55(35), 153(3), 155(2), 139(6)	319(8), 193(6), 177(11)
Linalyl furanoxide[e]	1.24		+	69(100), 93(88), 111(77), 81(48), 55(43), 109(30), 153(3)	319(12), 193(5)
Octyl[e]	1.27		+	57(100), 85(70), 69(38), 109(5)	319(1), 193(1), 177(6)
(*R*)-Linalyl	1.30	1.30	+	69(100), 93(75), 81(40), 91(39), 121(15), 153(14), 109(12)	193(15), 319(8), 177(6)
(*S*)-Linalyl	1.33	1.34	+ +	69(100), 93(48), 121(4), 81(14), 97(13), 91(14), 153(3)	193(21), 319(2), 177(1),
2-Phenylethyl	1.45	1.45	+ + +	105(100), 91(33), 79(9)	319(8), 193(6), 177(6), 205(3)
Linalyl pyranoxide[e]	1.49		+	68(100), 81(78), 93(70), 95(55), 79(35), 119(18)	319(14), 193(1)
Linalyl pyranoxide[e]	1.51		+	69(100), 81(77), 93(55), 95(24), 79(45), 91(25), 119(6), 98(22), 109(20)	319(14), 193(6)
Geranyl	1.61	1.62	+	81(100), 69(68), 93(52), 79(49), 95(46), 91(23), 109(18), 55(23),123(3)	319(3), 193(7), 177(3)
Rutinoside (α-L-rhamnopyranosyl-β-D-glucopyranoside)					
3-Methyl-butyl[e]	1.48		+ + +	71(100), 69(41), 70(4), 72(4)	207(53), 179(10), 193(8), 292(1), 319(1), 435(0.8), 434(0.5)
Hexyl[e]	1.81		+	85(90), 57(48), 69(40)	207(100), 193(13), 179(7), 435(1), 434(1)
Linalyl furanoxide[e]	2.16		+ +	69(100), 111(50), 155(8)	207(60), 435(50), 434(48)
Linalyl furanoxide[e]	2.18		+ +	69(100), 111(22), 85(18), 141(8), 155(8)	207(80), 435(33), 434(32)
Benzyl alcohol	2.24	2.24	+ +	91(100), 105(14), 69(13), 108(5)	207(8), 193(17), 179(1)
(*R*)-Linalyl	2.25	2.26	+	69(100), 69(70), 91(45), 81(45), 109(24), 97(20), 121(14), 136(4), 153(2)	207(56), 193(32), 179(6)
(*S*)-Linalyl	2.28	2.28	+ + +	69(100), 93(53), 91(45), 81(48), 95(20), 97(14), 109(8), 121(4), 153(3), 136(1)	207(64), 193(84), 179(8)
Octyl[e]	2.43		+	85(82), 57(78), 109(3), 69(2)	207(100), 179(7), 193(8), 319(0.8)
2-Phenylethyl	2.46	2.46	+ +	105(100), 104(55), 91(23), 69(14), 78(12)	207(25), 179(6), 193(6)
α-Terpineyl	2.61	2.60	+	136(100), 81(60), 137(54), 93(39), 43(36), 121 (32), 69(21)	207(32), 193(6), 179(6), 435(3.5), 434(3.5), 319(2)
Vicianoside (α-L-arabinopyranosyl-β-D-glucopyranoside)					
3-Methyl but-2-enyl	2.02	2.01	+	69(100), 85(15), 97(10)	193(75), 420(2), 421(1)

[a] RRt: relative retention time compared with the internal standard (phenyl-glucoside).
[b] Na: natural compound.
[c] Ref: reference.
[d] RA: relative abundance, +: ⩽1; + +: 1–1.5; + + +: ⩾1.5 ppm.
[e] Compound tentatively identified.

from the TFA glycosides. Indeed 6′-*O*-malonyl glucoconjugates have been recently reported as common constituents of several plants, including tropical fruits such as guava, mountain papaya (Withopf, Richling, Roscher, Schwab & Schreier, 1997), and bacuri (Boulanger et al., 1999).

The determination of malonic acid, released after acid hydrolysis of the crude heterosidic extract (Withopf et al., 1997), corresponded to only 6% of 2,3,4-tri-*O*-methylglucose. In consequence, we can assumed that acyl derivatives, other than malonyl (Crouzet & Chassagne, 1999), were present in cupuacu. Methyl phenyl acetate was effectively identified by mass spectrometry at the extract.

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