Glycosidically Bound Aroma Compounds from the Pulp and the Peelings of Lulo Fruit (*Solanum vestissimum* D.)

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HRGC and HRGC–MS identifications of bound aroma compounds from the pulp and the peelings of lulo fruit (*Solanum vestissimum* D.) were achieved after isolation of extracts obtained by Amberlite XAD-2 adsorption and methanol elution followed by hydrolysis with commercial pectinase enzymes. Whereas the aglycons identified in the peelings mainly consisted of terpenoids, with enantiomerically pure (*R*)-(−)-linalool as the main constituent, aglycons exhibiting aromatic structures, in particular, benzoic acid and methyl benzoate, predominated in the pulp. After per-O-methylation of a fraction obtained from the glycosidic extract by rotation locular countercurrent chromatography (RLCCC), HRGC–MS revealed linalool β-D-glucoside as the bound conjugate of linalool in the peelings.

INTRODUCTION

Lulo plant (*Solanum vestissimum* D.), native of South America, is an important fruit species in Colombia, due to the pleasant and delicate aroma of its fruit. During the past decade, its domestic consumption has steadily increased until reaching significant figures, i.e., from 12 000 tons in 1988 to 27 000 tons in 1990. Its production is expected to increase even further in the near future, as the Colombian government recently included this species in a particular fruit export promotion program. In addition to its consumption as fresh fruit, lulo shows a great potential for processed food production, i.e. jellies, juices, ice creams, and yogurts.

Recently, a number of studies have been carried out on the qualitative and quantitative aroma composition of lulo pulp (Suárez und Duque, 1991a,b) and peelings (Suárez et al., 1991). As a continuation to the former studies, we undertook the present investigation for where we report, for the first time, the occurrence of glycosidically bound aroma substances from the pulp and the peelings of this fruit.

EXPERIMENTAL PROCEDURES

Solvents. All solvents used were of high purity at purchase (Aldrich) and were redistilled before use.

Fruits. Fresh lulo fruits (*S. vestissimum* D.) came by air freight from Granada, Cundinamarca, Colombia, and were analyzed within 2 days after arrival.

Isolation of Glycosidic Extracts (Gunata et al., 1985). After the pulp (4.8 kg) was separated from the peelings (2.8 kg), both were studied individually by using the same preparation steps. These comprised homogenization in phosphate buffer (pH 7.0) (5 and 3 L, respectively) containing 0.2 M glucono-δ-lactone as glycosidase inhibitor, centrifugation at 4000*g*, and concentration of the supernatants under reduced pressure (rotavapor) each to 2 L. Each of the aqueous residues was subjected to liquid chromatography on Amberlite XAD-2 adsorbent (glass column, 25 × 500 mm). After washing with 1500 mL of H_2O, elution was performed with 500 mL of methanol. The eluate was concentrated to dryness under reduced pressure (rotavapor), redissolved in 50 mL of 0.2 M citrate–phosphate buffer (pH 5.0), and extracted with diethyl ether to remove any remaining volatiles.

Enzymatic Hydrolysis. In a typical experiment, a nonselective pectinase [a, 500 μL of Rohapect D5L (Röhm, Darmstadt, FRG); b, 1 mL of Macerozym R-10 (Serva, Heidelberg, FRG)] was added to the glycosidic extract (50 mL) from the peelings (a) and the pulp (b), respectively, and the mixture incubated at 37 °C overnight. After addition of 2-undecanol (40 μg) as standard, the liberated aglycons were continuously extracted (16 h) with diethyl ether and the dried (anhydrous Na_2SO_4), filtered, and concentrated (Vigreux column, 45 °C) extract subjected to HRGC and HRGC–MS analyses. In the same manner, blank tests without addition of enzyme were carried out.

Rotation Locular Countercurrent Chromatography (RLCCC). The lyophilisate obtained from the glycosidic extract isolated from 1 kg of peelings was dissolved in 5 mL of distilled water and subjected to a prefractionation using RLCCC. The apparatus (Eyela RLCCC, Tokyo Rikakikai Co.) was operated in the descending mode employing a solvent system made up from the two phases produced by mixing $CHCl_3$–MeOH–H_2O (9:12:8) with the upper layer as stationary phase and the more dense, less polar layer as the mobile phase. The flow rate was 1 mL/min, rotation speed 80 rpm, and slope 30°. Twelve 100-mL fractions were separated that were analyzed by TLC using SiO_2 GF_{254} (Merck, Darmstadt, FRG) as stationary and $CHCl_3$–MeOH–H_2O (80:20:1.5) as mobile phase (detection, 254 nm and vanillin/H_2SO_4). Glycosides were not detectable in fractions 1–4; they were discarded. Fractions 5–7 and 8–12 were combined, resulting in fractions I and II, respectively, whose compositions of (i) aglycons and (ii) glycosides were analyzed as follows. (i) A 10th part of each of the fractions was treated by Rohapect D5L as described above, and the liberated aglycons were studied by HRGC and HRGC–MS. (ii) The rest of each of the fractions was per-O-methylated according to the method of Finne et al. (1980).

Capillary Gas Chromatography (HRGC). (*a*) *Analysis of Aglycons.* A Carlo Erba Fractovap 4160 gas chromatograph with FID equipped with a J&W fused silica DB-Wax capillary column [30 m × 0.259 mm (i.d.); film thickness, 0.25 μm] was used. Split injection (1:50) was employed. The temperature program was 3 min isothermal at 50 °C, and then the temperature was raised to 240 °C at 4 °C/min. The flow rates for the carrier gas were 1.6 mL/min He, for the makeup gas 30 mL/min N_2, and for the detector gases 30 mL/min H_2 and 300 mL/min air. Injector and detector temperatures were kept at 250 °C. Volumes of 1 μL were injected.

(*b*) *Analysis of Per-O-methylated Glycosides.* A Carlo Erba Fractovap 4100 gas chromatograph with FID equipped with a J&W fused silica DB-5-30W capillary column [30 m × 0.25 mm (i.d.), film thickness, 0.25 μm] was used. Split injection (1:50) was employed. The temperature program was 60–300 °C at 5 °C/min. The flow rates for the carrier gas were 1.8 mL/min He, for the makeup gas 30 mL/min N_2, and for the detector gases 30 mL/min H_2 and 300 mL/min air. The injector temperature

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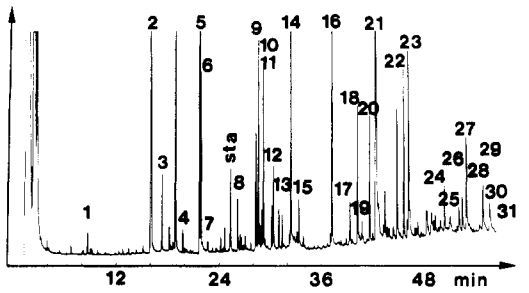

Figure 1. HRGC–MS separation of aglycons from lulo fruit pulp on a J&W DB-Wax WCOT capillary column (30 m × 0.25 mm i.d.; d_f = 0.25 μm). The numbers correspond to the numbers outlined in Table I. (sta, standard.)

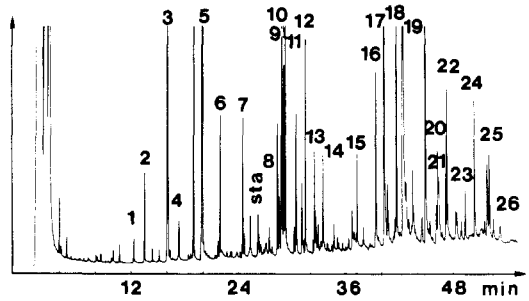

Figure 2. HRGC–MS separation of aglycons from the peelings of lulo fruit on a J&W DB-Wax WCOT capillary column (30 m × 0.25 mm i.d.; d_f = 0.25 μm). The numbers correspond to the numbers outlined in Table II. (sta, standard.)

was kept at 250 °C and the detector temperature at 300 °C. Volumes of 1 μL were injected.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 1440 gas chromatograph equipped with a Carlo Erba water-cooled on-column injection system was combined by direct coupling to a Finnigan MAT 44 mass spectrometer. The same types of columns as mentioned above for HRGC analyses were used. The conditions were as follows: temperature program (DB-Wax), 3 min isothermal at 50 °C and then from 50 to 240 °C at 4 °C/min; temperature program (DB-5), 60–300 °C at 5 °C/min; carrier gas flow rates, each 1.6 mL/min He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA. Volumes of 1 μL were used.

Results of qualitative analyses were verified by comparison of HRGC retention (R_t) and mass spectral data with those of authentic reference substances.

Chiral Analysis of Linalool. Chirality evaluation of linalool was carried out by on-line coupled multidimensional gas chromatography (DB-5/Lipodex C)–mass spectrometry (MDGC–MS) as recently described (Bernreuther and Schreier, 1991).

Reference Substances. 3-Hydroxy-5,6-epoxy-β-ionol was kindly provided by Dr. Winterhalter, Würzburg. 2-*exo*-Hydroxy-1,8-cineole was synthesized according to the method of Bitteur et al. (1990).

RESULTS AND DISCUSSION

Aglycons. Typical HRGC–MS separations of the bound aroma compounds from the pulp and the peelings of lulo fruit (*S. vestissimum* D.) are outlined in Figures 1 and 2, respectively. Tables I and II show the individual aglycons including the range of their amounts determined. In total, in the pulp 31 and in the peelings 26 aglycons were identified. Quantitative HRGC revealed in the pulp 6.9 mg/kg and in the peelings 16.2 mg/kg aglycons. The extract obtained from the peelings showed a pleasant fruity–flowery odor, whereas that of the pulp was less pleasant and more phenolic-like.

Distinct differences were found in the composition of aglycons between the pulp and the peelings of lulo fruit.

Table I. Compounds Identified in Lulo Fruit Pulp by HRGC and HRGC–MS after Enzymatic Hydrolysis (Macerozym R-10) of a Methanolic Eluate Obtained from an XAD-Separated Fraction

peak no.	R_t[b] sample	R_t[b] ref	compound	amount[c]
1	1198	1210	3-methyl-1-butanol	+
2	1418	1415	acetic acid	++++
3	1460	1460	methyl 3-hydroxybutanoate	+
4	1540	1544	linalool	+
5	1595	1598	methyl benzoate	++++
6	1599	1596	butanoic acid	++
7	1632	1637	methyl 3-hydroxyhexanoate	+
sta	1712	1717	2-undecanol (standard)	++
8	1740	1744	(*E*)-2-butenoic acid	+
9	1829	1840	guaiacol	++
10	1834	1836	2-*exo*-hydroxy-1,8-cineole	+
11	1846	1853	benzyl alcohol	+
12	1888	1899	2-phenylethanol	+
13	1926	1928	2,6-dimethyl-3,7-octadiene-2,6-diol	+
14	1965	1973	phenol	++
15	2000	1997	2,5-dimethyl-4-hydroxy-2*H*-furan-3-one	++
16	2166	2181	4-vinylguaiacol	+++
17	2248	2254	2,6-dimethyl-2(*Z*),7-octadiene-1,6-diol	+
18	2290	2294	2,6-dimethyl-2(*E*),7-octadiene-1,6-diol	++
19	2310	2322	*cis*-isoeugenol	+
20	2340	2352	4-vinylphenol	++
21	2391	2408	benzoic acid	++++
22	2535	2543	4-vinylsyringol	++
23	2567	2578	methyl vanillate	++
24	2767	2776	3-hydroxy-5,6-epoxy-β-ionol	+
25	2846	2850	3-oxo-*retro*-α-ionol	+
26	2865	2871	hexadecanoic acid	+
27	2887	2895	methyl 4-hydroxybenzoic acid	++
28	2976	2996	1,3-benzenediol	+
29		*d*	methyl ferulate (tent.)	+
30		*d*	vomifoliol (tent.)	+
31		*d*	4-hydroxy-3-methoxybenzoic acid (tent.)	+

[a] The peak numbers correspond to the numbers in Figure 1. [b] R_t, linear retention index based on a series of *n*-hydrocarbons. [c] + = <50; ++ = 50–300; +++ = 300–1000; ++++ = >1000 μg/kg. [d] Tentatively assigned by mass spectral data only.

Table II. Compounds Identified in Lulo Fruit Peelings by HRGC and HRGC–MS after Enzymatic Hydrolysis (Rohapect D5L) of a Methanolic Eluate Obtained from an XAD-Separated Fraction

peak no.[a]	R_t[b]		compound	amount[c]
1	1310	1320	3-methyl-2-buten-1-ol	+
2	1348	1353	1-hexanol	++
3	1419	1415	acetic acid	+++
4	1459	1460	methyl 3-hydroxybutanoate	++
5	1539	1544	linalool	++++
6	1600	1598	butanoic acid	++
7	1684	1679	α-terpineol	++
sta	1712	1717	2-undecanol (standard)	++
8	1781	1790	nerol	++
9	1830	1832	geraniol	++++
10	1834	1836	2-*exo*-hydroxy-1,8-cineole	++
11	1846	1853	benzyl alcohol	++
12	1927	1928	2,6-dimethyl-3,7-octadiene-2,6-diol	++
13	1965	1973	phenol	++
14	2001	1997	2,5-dimethyl-4-hydroxy-2*H*-furan-3-one	++
15	2160	2181	4-vinylguaiacol	++
16	2247	2254	2,6-dimethyl-2(*Z*),7-octadiene-1,6-diol	++
17	2287	2294	2,6-dimethyl-2(*E*),7-octadiene-1,6-diol	++++
18	2350	2352	4-vinylphenol	+++
19	2392	2408	benzoic acid	++++
20	2585	2600	3,7-dimethyl-2(*Z*),6(*E*)-octadiene-1,8-diol	+++
21	2627	2640	3,7-dimethyl-2(*E*),6(*E*)-octadiene-1,8-diol	++
22	2626	2637	3-oxo-α-ionol	++
23	2715	2720	3-oxo-*retro*-α-ionol, isomer I	++
24	2768	2776	3-hydroxy-5,6-epoxy-β-ionol	++
25	2846	2854	3-oxo-*retro*-α-ionol, isomer II	++
26		*d*	vomifoliol (tent.)	+

[a] The peak numbers correspond to the numbers in Figure 2. [b] R_t, linear retention index based on a series of *n*-hydrocarbons. [c] + = <50; ++ = 50–300; +++ = 300–1000; ++++ = >1000 μg/kg. [d] Tentatively assigned by mass spectral data only.

Whereas the aglycons identified in the peelings mainly consisted of terpenoids, with enantiomerically pure (*R*)-(−)-linalool (ee 100%) as the main constituent, aglycons exhibiting aromatic structures, in particular, benzoic acid

Table III. Major Compounds Identified by HRGC–MS after Enzymatic Hydrolysis (Rohapect D5L) of RLCCC-Separated Fraction I and Fraction II

compounds identified in	
fraction I	fraction II
benzoic acid	acetic acid
3-oxo-α-ionol	linalool
3-oxo-*retro*-α-ionol, isomer I	α-terpineol
3-oxo-*retro*-α-ionol, isomer II	geraniol
hexadecanoic acid	2-*exo*-hydroxy-1,8-cineole
1,3-benzenediol	benzoic acid

and methyl benzoate, predominated in lulo fruit pulp. Apart from a number of monoterpene diols identified (cf. Tables I and II), HRGC–MS revealed the occurrence of six additional terpenoid diols among the aglycons of the peelings, but their exact identities could not be evaluated due to lack of authentic reference material.

The composition of aglycons from both the pulp and the peelings of lulo fruit consisted of compounds arising from the fatty acid, shikimate, and terpenoid metabolism. Insofar, it did not differ from the findings described in previous studies performed with various plant sources (Strauss et al., 1987; Williams et al., 1989; Pabst et al., 1991; Krammer et al., 1991). In the peelings of lulo fruit a number of monoterpene diols was identified. These compounds have been found previously as monoterpene metabolites in plant tissues (Williams et al., 1980; Winterhalter et al., 1986; Strauss et al., 1988) and microorganisms (Brunerie et al., 1988).

Glycosides. After enzymatic treatment with Rohapect D5L of RLCCC-separated and combined fractions I and II from lulo fruit peelings (cf. Experimental Procedures) followed by subsequent solvent extraction of the liberated aglycons, HRGC and HRGC–MS revealed a distinct number of compounds, the major ones of which are listed in Table III. Whereas in fraction I norisoprenoids were detected, in fraction II several monoterpene alcohols, with linalool as the main constituent, were identified.

After per-O-methylation of fractions I and II, linalool β-D-glucoside was identified in fraction II by HRGC and HRGC–MS using an authentic reference sample (Schwab, 1989). The per-O-methylated monoterpene glucoside showed the following chromatographic and spectroscopic data: R_t (DB-5) 2072; MS (*m/z*, %), 45 (100), 41 (52), 69 (50), 101 (48), 115 (43), 80 (26), 81 (26), 93 (25). Linalool β-D-glucoside, previously found in *Solanum tuberosum* herb (Moede, 1985) and, more recently, in *Arachniodes maximowiczii* (Tanaka et al., 1986) as well as in the aerial parts of *Pluchea indica* (Uchiyama et al., 1989), is the real precursor of linalool in lulo fruit. Whereas both lulo fruit and peelings homogenized at pH 7 did not exhibit linalool, it was the major constituent of the pulp (Suárez and Duque, 1991a) and the peelings held, e.g., for juice production, at natural pH (3.5). Structural elucidation of the glycosides present in fraction I is still under investigation.

ACKNOWLEDGMENT

M.S. thanks the BID-ICFES-U. NAL Program for a doctoral fellowship to pursue research work in Germany.

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Received for review March 22, 1991. Accepted June 3, 1991.

Registry No. (*R*)-(−)-Linalool β-D-glucoside, 99096-59-0.