C_{13}-Norisoprenoid Glucoconjugates from Lulo (*Solanum quitoense* L.) Leaves

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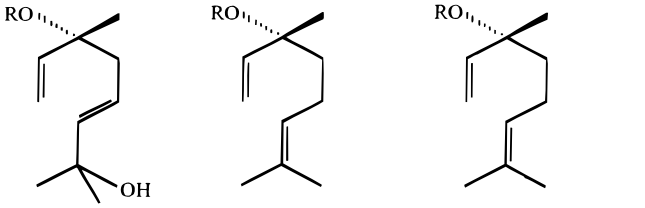
With the aid of multilayer coil countercurrent chromatography, subsequent acetylation, and liquid chromatographic purification of a glycosidic mixture obtained from lulo (*Solanum quitoense* L.) leaves, three C_{13}-norisoprenoid glucoconjugates were isolated in pure form. Their structures were elucidated by NMR, MS, and CD analyses to be the novel (6*R*,9*R*)-13-hydroxy-3-oxo-α-ionol 9-*O*-*β*-D-glucopyranoside (**4a**), the uncommon (3*S*,5*R*,8*R*)-3,5-dihydroxy-6,7-megastigmadien-9-one 5-*O*-*β*-D-glucopyranoside (citroside A) (**5a**), and the known (6*S*,9*R*)-vomifoliol 9-*O*-*β*-D-glucopyranoside (**6a**). Enzymatic treatment of compound **5a** showed the formation of 3-hydroxy-7,8-didehydro-*β*-ionone (**7**), an important lulo peeling volatile, which in its turn after chemical reduction and heated acid catalyzed rearrangement generates *β*-damascenone (**9**) and 3-hydroxy-*β*-damascone (**10**).

Keywords: *Lulo; Solanum quitoense; (6R,9R)-13-hydroxy-3-oxo-α-ionol 9-O-β-D-glucopyranoside; new C_{13}-norisoprenoid glucoconjugate; (3S,5R,8R)-3,5-dihydroxy-6,7-megastigmadien-9-one 5-O-β-D-glucopyranoside; (6S,9R)-vomifoliol 9-O-β-D-glucopyranoside; aroma precursors; 3-hydroxy-7,8-didehydro-β-ionone; β-damascenone; 3-hydroxy-β-damascone*

INTRODUCTION

Lulo plant (*Solanum quitoense* L.), native to South America, is an important fruit species in Colombia due to the pleasant and delicate aroma of its fruit. During recent years, its cultivation has been increasing significantly because the Colombian government included this species in a particular fruit export promotion program.

After our first studies on the aroma composition of the fruit pulp and peelings (Suárez and Duque, 1991; Suárez et al., 1993), further investigations reported on the glycosidically bound aroma substances (Suárez et al., 1991). Our latter research revealed the presence of the glycoconjugated aroma compounds **1**−**3** as precursors of monoterpenols in the peelings of this fruit (Wintoch et al., 1993; Duque et al., 1993).



1 **2** **3**

As a continuation to the above-mentioned studies, we performed a preliminary screening of bound volatiles in lulo leaves, which revealed a high amount of bound C_{13}-norisoprenoids (Osorio and Duque, 1995). This paper describes, for the first time, the isolation and characterization of the novel glucoconjugated C_{13}-norisoprenoid (6*R*,9*R*)-13-hydroxy-3-oxo-α-ionol 9-*O*-*β*-D-glucopyranoside (**4a**) as well as the presence of the uncommon (3*S*,5*R*,8*R*)-3,5-dihydroxy-6,7-megastigmadien-9-one 5-*O*-*β*-D-glucopyranoside (**5a**) and its role as precursor in the formation of 3-hydroxy-7,8-didehydro-*β*-ionone, *β*-damascenone, and 3-hydroxy-*β*-damascone and the known (6*S*,9*R*)-vomifoliol 9-*O*-*β*-D-glucopyranoside (**6a**).

EXPERIMENTAL PROCEDURES

General. NMR spectra were taken on a Fourier transform JEOL JNM-EX500 spectrometer and on a Bruker AC 500 spectrometer with $CDCl_3$ as solvent and Me_4Si as internal standard. UV spectra were obtained with a Merck Hitachi diode array detector L-4500. CD spectra were recorded on a JASCO J-500C polarimeter. Fast atom bombardment mass spectrometry (FAB-MS) was carried out with a JEOL JMS-AX505HA spectrometer using glycerol matrix and ion source temperature at 305 °C. Positive ions over a range *m*/*z* 10−650 were scanned. For chemical ionization mass spectrometry (CI-MS), a Shimadzu 9020 DF mass spectrometer was used (reactant gas, isobutane). Positive ions over a range of *m*/*z* 70−700 were scanned. For flash chromatography Merck silica gel 60 (0.032−0.063 nm) was employed. All solvents used were of high purity at purchase (Merck) and were redistilled before use.

Plant Material. Fresh lulo (*S. quitoense* L.) leaves came from a commercial orchard located in the rural area La Vega, Cundinamarca, Colombia. Voucher specimens were coded COL 352780 at the Instituto de Ciencias Naturales de la Universidad Nacional de Colombia.

Isolation of a Glycosidic Extract. Lulo leaves (10 kg) were cut blended with 1 L of methanol (adjusted to pH 7.0). After centrifugation (10000*g*, 30 min), the supernatant was concentrated under reduced pressure (rotavapor) and then extracted with petroleum ether and diethyl eter to eliminate

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Table 1. MS Spectral Data for Acetylated Glucoside 4b

fragment (*m*/*z*)	FAB-MS (%)	CI-MS[a] (%)	EI-MS[b] (%)	interpretation
597	7	6		$[M + H]^+$
331	17	100	70	$[(\text{hexose} + 4\ \text{acetyl} - H_2O) + H]^+$
271	2	14	10	$[(\text{hexose} + 4\ \text{acetyl} - H_2O - \text{AcOH}) + H]^+$
249	14	78	20	$[(\text{aglycon} + \text{acetyl} - H_2O) + H]^+$
211	2	3	8	$[331 - 2\ \text{AcOH}]^+$
207	4	7	10	$[(\text{aglycon} - H_2O) + H]^+$
169	19	28	100	$[(211 - \text{acetyl}) + H]^+$

[a] Reactant gas isobutane. [b] Electron impact at 20 eV.

Table 2. ^{1}H NMR Spectral Data for Acetylated Glucoside 4b ($CDCl_3$, 500 MHz, Coupling Constants in Hertz, δ Relative to TMS)

δ	signal	*J*	assignment[a]
0.99/1.04	6 H, 2s		H_3 C11/H_3 C12
1.24	3 H, d	6.4	H_3 C10
2.01−2.12	15 H, 5 s		H_3 acetates (5×)
2.15	1 H, d	17.4	H_a C2
2.40	1 H, d	17.4	H_b C2
2.60	1 H, d	9.0	H C6
3.65	1 H, dxdxd	10.1/4.1/2.3	H C5′
4.08	1 H, dxd	12.0/2.3	H_a C6′
4.23	1 H, dxd	12.0/4.1	H_b C6′
4.25	1 H, quintet	6.4	H C9
4.58	1 H, d	8.3	H C1′
4.60	2 H, br s		H_2 C13
4.99	1 H, dxd	10.1/8.3	H C2′
5.10	1 H, dxd	10.1/9.6	H C4′
5.20	1 H, dxd	10.1/9.6	H C3′
5.60	1 H, dxd	15.1/9.0	H C7
5.67	1 H, dxd	15.1/6.4	H C8
6.04	1 H, br s		H C4

[a] Assignments were based on a ^{1}H−^{1}H COSY experiment.

chlorophyll pigments and remaining volatiles (Humpf and Schreier, 1991). The aqueous residue was divided in five portions, and each was passed through an Amberlite XAD-2 column (45 × 800 mm, 10 mL/min) (Gunata et al., 1985). After washing with 2 L of distilled water, elution was performed with 1.5 L of methanol. The combined methanol eluates were concentrated to dryness under vaccuum and lyophilyzed to afford 15 g of crude glycosides.

Multilayer Coil Countercurrent Chromatography (ML-CCC). Portions of ∼1.5 g of glycosidic extract were placed in an Ito multilayer countercurrent chromatograph (P.C. Inc., Potomac, MD) with 75 m × 2.6 mm i.d. PTFE tubing (total volume = 400 mL) for separation of glycosides. The MLCCC apparatus was operated at a rotation speed of 800 rpm, using $CHCl_3$/MeOH/H_2O (7:13:8) as solvent system with the less dense layer as mobile phase at a flow rate of 1 mL/min. Fifty fractions (each 10 mL) were collected.

Acetylation and Purification of Acetylated Glycosides. Combined MLCCC fractions (13−16) were concentrated under reduced pressure to dryness. The dry residue (1 g) was acetylated with 20 mL of Ac_2O/pyridine and 500 mg of 4-(dimethylamino)pyridine at room temperature overnight. The peracetylated glycosides were further separated by flash chromatography using 150 mL of hexane/diethyl ether (1:5) as solvent. Combined fractions (10 mL each) 71−77, 89−103, and 119−130 were concentrated in vacuo to 1 mL for subsequent HPLC purification.

Preparative HPLC. The semipurified acetylated glycosides **4b**−**6b** obtained above were finally purified by preparative HPLC using an Ultrasphere Si column (5 μm, 250 mm × 4.6 i.d., Beckman), with diethyl ether as mobile phase at a flow rate of 1 mL/min, thus yielding pure compounds as white crystals: **4b** (30 mg), **5b** (2 mg), and **6b** (26 mg).

Glycoside **4b** showed the following spectral data: UV (Et_2O) λ_{max} 228 nm; MS, cf. Table 1; ^{1}H NMR, cf. Table 2; ^{13}C NMR, cf. Table 3; CD spectrum showed a positive maximum at 240 nm ($\Delta\epsilon$ = +17.7, MeOH).

Table 3. ^{13}C NMR Spectral Data for Acetylated Glucoside 4b ($CDCl_3$, 125 MHz, δ Relative to TMS)

1b δ	DEPT	assignment[a]
20.56−20.69	CH_3	CH_3CO (5×)
20.86	CH_3	C10
26.89[b]	CH_3	C11
27.67[c]	CH_3	C12
36.25	C	C1
48.01	CH_2	C2
51.16	CH	C6
61.87	CH_2	C6′
64.06	CH_2	C13
68.26	CH	C4′
71.56	CH	C2′
71.86	CH	C5′
72.73	CH	C3′
76.52	CH	C9
99.61	CH	C1′
123.63	CH	C4
127.21	CH	C7
136.28	CH	C8
157.44	C	C5
169.14	C	CH_3**C**O
169.31	C	CH_3**C**O
170.12	C	CH_3**C**O
170.28	C	CH_3**C**O
170.55	C	CH_3**C**O
198.43	C	C3

[a] Assignments were based on a ^{1}H−^{13}C COSY experiment. [b,c] Interchangeable values.

For glycoside **5b** the following spectral data were obtained: UV (Et_2O) λ_{max} 227 nm; FAB-MS, *m*/*z* (%) 597 (8, $[M + H]^+$), 331 (6, $[\text{hexose} + 4\ \text{acetyl} - H_2O + H]^+$), 249 (5, $[\text{aglycon} + \text{acetyl} - H_2O + H]^+$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (3H, s, CH_3-11), 1.31 (3H, s, CH_3-12), 1.42 (1H, dd, Ha-2)*, 1.42 (3H, s, CH_3-13), 1.47 (1H, t, J = 13.3 Hz, Ha-4), 2.00−2.10 (15H, 5s, 5 acetates), ∼2.00 (1H, Hb-2)*, 2.18 (3H, s, CH_3-10), 2.47 (1H, dd, J = 13.3, 3.2 Hz, Hb-4), 3.66 (1H, ddd, J = 9.8, 5.9, 2.9 Hz, H-5′), 4.08 (1H, dd, J = 12.0, 5.9 Hz, Hb-6′), 4.17 (1H, dd, J = 12.0, 2.9 Hz, Ha-6′), 4.78 (1H, d, J = 7.8 Hz, H-1′), 5.01 (1H, dd, J = 9.8, 7.8 Hz, H-2′), 5.02 (1H, dd, J = 9.8, 9.7 Hz, H-4′), 5.16 (1H, m, H-3), 5.21 (1H, t, J = 9.8 Hz, H-3′), 5.91 (1H, s, H-8) (asterisks indicate that the signals are obscured due to partial overlapping); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.62−20.75 (5 acetates), 25.81 (CH_3-13), 26.62 (CH_3-10), 28.79 (CH_3-12), 31.81 (CH_3-11), 35.76 (C-1), 43.22 (C-4), 44.54 (C-2), 62.22 (C-6′), 66.98 (C-3), 68.51 (C-4′), 71.13 (C-2′), 71.90 (C-5′), 72.90 (C-3′), 77.81 (C-5), 95.29 (C-1′), 100.88 (C-8), 116.87 (C-6), 169.46−170.49 (5 acetates), 197.68 (C-7), 210.74 (C-9). Signals were assigned on the basis of H−H and H−C COSY spectra.

The spectral data of glycoside **6b** were as follows: UV (Et_2O) λ_{max} 230 nm; CI-MS, *m*/*z* (%) 555 (5, $[M + H]^+$), 537 (2, $[M - H_2O + H]^+$), 331 (100), 271 (16), 207 (66), 206 (12), 169 (32), 150 (24); ^{1}H and ^{13}C NMR data were in good agreement with those showed by an authentic sample of peracetylated (6*S*,9*R*)-vomifoliol 9-*O*-β-D-glucopyranoside. CD data of the glucoside **6a** correspond to those published by Otsuka et al. (1995), showing 6*S* as the absolute configuration at C-6.

Deacetylation and Enzymatic Hydrolysis. To 1 mg of **4b** in 5 mL of MeOH was added 5 mL of 0.02 M NaOMe solution. After 12 h, the mixture was neutralized by adding 50 mg of Amberlite IR-120 (15−50 mesh, H^+ form). After removal of the ion-exchange resin by filtration, the solvent was evaporated in vacuo, and the deacetylated glycoside was dissolved in 5 mL of H_2O. To this solution was added 10 mL of citric acid−phosphate buffer (pH 5.5) as well as 500 μg of a commercially available β-glucosidase (almond, emulsine), and the mixture was incubated over 24 h at 37 °C. The liberated aglycon (woody, balsamic, and pleasant odor) was extracted with diethyl ether and subjected to HRGC/and HRGC/MS analyses: Ri (DB-5) 1785, Ri (HP1) 1704; EI-MS (70 eV), *m*/*z* (%) 224 (M, 1), 209 (M − CH_3, 1), 206 (M − H_2O, 1), 191 (1), 181 (1), 168 (1), 167 (3), 163 (3), 150 (3), 125 (11), 109 (5), 107

(9), 95 (19), 83 (25), 81 (11), 79 (12), 69 (5), 67 (7), 55 (19), 53 (8), 45 (19), 43 (100), 41 (30), 39 (19). In the same way, after deacetylation and enzymatic hydrolysis, compound **5b** yielded an aglycon (green-woody odor), which was also analyzed by HRGC and HRGC/MS: Ri (HP1) 1609, Ri (DB-5) 1689, Ri (DB-Wax) 2682; EI-MS (70 eV), *m*/*z* (%) 206 (M^+, 29), 191 (26), 173 (58), 147 (16), 131 (14), 130 (10), 129 (9), 119 (27), 115 (11), 105 (14), 91 (31), 79 (13), 78 (8), 77 (21), 65 (10), 63 (9), 55 (5), 53 (11), 51 (12), 45 (2), 43 (100), 41 (22), 39 (22). MS and ^{1}H NMR data were in good agreement with those published by Sannai et al. (1984). In the same way as described above, compound **6b** was also deacetylated and subjected to enzymatic hydrolysis, and the liberated aglycon (sweet, woody, and fruity odor) was analyzed by HRGC and HRGC/MS: Ri (DB-5) 1806; Ri (HP1) 1749; EI-MS (70 eV) data as reported by Achenbach et al., (1981).

Isolation of Natural Compound 5a. Five grams of crude glycosidic extract was subjected to MLCCC using $CHCl_3$/MeOH/H_2O (7:13:8) as mentioned above. Combined fractions (13−16) was refractionated by MLCCC using EtOAc/*n*-BuOH/H_2O (3:2:5) as solvent system with the more dense layer acting as mobile phase at a flow rate of 1 mL/min. Fifty-five fractions (each 5 mL) were collected. Subfractions (21−28) were finally purified by subsequent preparative HPLC on RP-18 and Lichrosorb Diol columns with methanol (flow rate = 1 mL/min) and hexane/*n*-butanol/methanol/water (65:25:9:1) (flow rate = 1 mL/min) as solvent systems, respectively, to yield 1 mg of pure **5a**. Glucoside **5a** showed the following spectral data: UV (MeOH) λ_{max} 227 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 1.12 (3H, s, CH_3-11), ∼1.31 (1H, Ha-2)*, 1.34 (3H, s, CH_3-12), ∼1.42 (1H, Ha-4)*, 1.43 (3H, s, CH_3-13), 1.88 (1H, dd, J = 11.5, 3.9 Hz, Hb-2), 2.16 (3H, s, CH_3-10), 2.45 (1H, dd, J = 11.3, 3.9 Hz, Hb-4), 3.10 (1H, dd, J = 8.5, 7.8 Hz, H-2′), 3.15−3.19 (2H, m, H-4′ and H-5′), 3.21 (1H, dd, J = 8.5, 8.5 Hz, H-3′), 3.57 (1H, dd, J = 11.6, 5.2 Hz, Hb-6′), 3.77 (1H, dd, J = 11.7, 1.8 Hz, Ha-6′), 4.29 (1H, tt, J = 11.3, 3.9 Hz, H-3), 4.48 (1H, d, J = 7.8 Hz, H-1′), 5.86 (1H, s, H-8) (asterisks indicate that the signals are obscured due to partial overlaping). In the same way as described above, natural glucoside **5a** was enzymatically hydrolyzed with emulsin (β-glucosidase), producing compound **7** as hydrolysis product. This time compound **7** was characterized by HRGC and HRGC/MS. The data obtained were in good agreement with the data mentioned above for this compound.

Model Reactions. *(a) Reduction of 3-Hydroxy-7,8-didehydro-β-ionone.* To 2 mg of 3-hydroxy-7,8-didehydro-β-ionone (**7**) dissolved in 5 mL of ethanol was added 2 mg of $NaBH_4$, and the reaction was allowed to continue for 24 h. After extraction with ethyl acetate, the organic phase was dried and concentrated, yielding 1 mg of 3-hydroxy-7,8-didehydro-β-ionol (**8**), which was characterized by HRGC and HRGC/MS: Ri (DB-Wax) 2763; EI-MS (70 eV), *m*/*z* (%) 208 (M^+, 9), 193 (13), 175 (9), 149 (7), 133 (8), 131 (11), 121 (6), 119 (8), 105 (18), 93 (7), 91 (20), 79 (12), 77 (15), 69 (16), 67 (5), 65 (9), 55 (14), 53 (10), 45 (10), 43 (100), 41 (27), 39 (19). The chromatographic and mass spectral data are in agreement with those showed by an authentic sample.

(b) Acid Treatment of 3-Hydroxy-7,8-didehydro-β-ionol. A solution of 0.5 mg of compound **8** dissolved in 10% aqueous ethanol was adjusted to pH 3.0 and placed in a sealed ampule for 1 day at 100 °C (Sefton et al., 1989). The reaction products were extracted with ethyl ether and characterized by HRGC and HRGC/MS as β-damascenone (**9**) (Ri DB-Wax 1817) and 3-hydroxy-β-damascone (**10**) (Ri DB-Wax 2554) in a 13:87 ratio. The mass spectral data for compounds **9** and **10** are in good agreement with those of authentic samples.

Capillary Gas Chromatography (HRGC). A Hewlett-Packard 5890 gas chromatograph with FID equipped with a J&W DB-5 fused silica capillary column (30 m × 0.31 mm i.d.; film thickness = 0.52 μm) was used. A Hewlett-Packard HP1 fused silica capillary column (12 m × 0.2 mm i.d.; film thickness = 0.33 μm) was also used. The DB-5 column was operated with a temperature program starting at 60 °C, raised to 300 °C at 5 °C/min, and kept at 300 °C for 10 min. The conditions for the HP1 column were as follows: temperature program starting at 50 °C, raised to 300 °C at 5 °C/min, and kept at 300 °C for 10 min. Injector and detector temperatures were kept at 300 °C. The flow rate for the carrier gas was 1.1 mL/min He, the flow rate for the make up gas was 30 mL/min, and those for the detector gases were 30 mL/min H_2 and 30 mL/min air. Split injection 1:10 and 2 μL injection volumes were used. The linear retention index (Ri) is based on a series of *n*-hydrocarbons.

Capillary Gas Chromatography/Mass Spectrometry (HRGC/MS). A Hewlett-Packard 5890 gas chromatograph with split injector (1:10), equipped with a selective mass 5970 Hewlett-Packard detector, was used. The same type of columns and temperature conditions as mentioned above for HRGC were used, temperature of ion source and all connecting parts, 300 °C; electron energy, 70 eV; mass range, 30−350.

RESULTS AND DISCUSSION

Isolation of Glycosides. A glycosidic extract from lulo leaves was obtained by Amberlite XAD-2 adsorption (Gunata et al., 1985) and methanol elution. It was subsequently subjected to a prefractionation using MLCCC (Roscher and Winterhalter, 1993). Monitoring of separated MLCCC fractions by TLC revealed major products in MLCCC fractions 13−16, which after acetylation and flash chromatography gave three semipurified fractions 71−77, 89−103, and 119−130. Each of the latter mentioned fractions was finally purified by HPLC on SiO_2 to afford pure peracetylated glucosides **4b**, **5b**, and **6b**, respectively.

Characterization of Glucoside 4a. The characterization was made in its acetate **4b** form by UV and MS as well as by ^{1}H and ^{13}C NMR mono- and bidimensional spectroscopy. Compound **4b** showed a UV absorption maximum at 228 nm, indicating an enone structure (Hesse et al., 1987). FAB-MS as well as CI-MS data (cf. Table 1) yielded a molecular mass of 597 and diagnostic ions *m*/*z* 331 and 271, thus indicating a monosacharide (hexose) as sugar moiety. From both ^{1}H (cf. Table 2) and ^{13}C NMR data (cf. Table 3) the presence of a glucose moiety was confirmed. The ^{1}H NMR exhibited a doublet at δ 4.58 (J = 8.3 Hz) for the anomeric proton, indicating a β-glycosidic linkage. Furthermore, from these NMR data obtained for the sugar moiety was evident a coincidence with data published for per-O-acetylated β-glucoside (Guldner and Winterhalter, 1991).

The ^{1}H NMR data (Table 2) analysis led us to conclude that the aglycon part of **4b** resembles the 3-oxo-α-ionol structure except for the absence of the CH_3-13 signal and the presence of an oxymethylene signal at δ 4.60 (br s), which correlates with the olefinic H-4 at δ 6.04 in the H−H COSY experiment. The latter correlates in its turn with the signal at δ 64.06 in the H−C COSY experiment. This analysis indicates that compound **4b** holds an acetylated hydroxyl group at C-13. The ^{13}C NMR data (Table 3) are also in good agreement with the structure proposed for **4b**, that is, the peracetylated 13-hydroxy-3-oxo-α-ionol β-D-glucopyranoside. The chemical shift of H-9 resonating upfield (∼1.1 ppm) in glucoside **4b** from the corresponding signal for acetylated hydroxyl group (Neugebauer et al., 1995) clearly indicates that the sugar is attached to the hydroxyl group in the C-9 position. Deacetylation of **4b** and subsequent treatment with β-glucosidase (emulsine) led to the release of the corresponding aglycon, the structure of which was confirmed as 13-hydroxy-3-oxo-α-ionol by HRGC/MS analysis. Among the two stereogenic centers found in **4b**, absolute stereochemistry at C-6 was determined on the basis of CD. Compound **4b**

exhibited a positive maximum at 240 nm ($\Delta\epsilon = +17.7$, MeOH), thus establishing its 6*R* absolute stereochemistry, which correlates well with the data published for the (6*R*)-3-oxo-α-ionol isomer (Pabst et al., 1992). The chemical shift of C-9 (δ 76.52, Table 3) in glucoside **4b** clearly indicates *R* configuration for this stereogenic center. Pabst et al. (1992) reported the ^{13}C NMR chemical shifts of the β-D-glucopyranoside of (9*R*)- and (9*S*)-3-oxo-α-ionol to be δ 77.0 and 74.7, respectively.

On the basis of the overall results for compound **4b**, we assign the structure (6*R*,9*R*)-13-hydroxy-3-oxo-α-ionol 9-*O*-β-D-glucopyranoside for compound **4a**. As far as we know, this is the first time that compound **4a** has been found in nature. Experiments to investigate the role of this glucoside as precursor of the C_{13}-norisoprenoids present in lulo aroma are under way.

Characterization of 5a and 6a. This characterization was also performed in their acetate **5b** and **6b** forms.

In the case of **5b**, FAB-MS indicates a molecular weight of 596 and the presence of a peracetylated hexose as sugar unit. ^{1}H NMR suggested the presence of a tetraacetyl-β-D-glucopyranosyl moiety, with data in good agreement with those reported for other β-glucopyranosides (Guldner and Winterhalter, 1991). Furthermore, the ^{1}H NMR data showed the signals assignable to three methyl singlets (δH 1.15, 1.31, 1.42), an acetyl (δH 2.18 and δC 26.62 and 210.74), and two oxymethines, one being tertiary at δC 77.81 and the other secondary, presumably in the acetate form, at δH 5.16 and δC 66.98. These facts, the signal at δ 5.91 correlating with the signal at δ 100.88 in the H−C COSY experiment and the further signals in the ^{13}C NMR spectrum at δ 116.87 and 197.68, clearly indicated the presence of an allenic group in the aglycon part of **5b**. The position of the glucose moiety was settled as follows. The 3-*O*- and 5-*O*-glucosides of **5a** are known compounds, and their ^{13}C NMR data were reported (Miyase et al., 1987; Umehara et al., 1988). The former showed C-3 and C-5 signals at δ 72.0 and 71.3, respectively, while the latter exhibited them at δ 62.5 and 78.1, respectively. Taking into consideration an acylation shift (downfield shift would be several parts per million), the data of compound **5b** (δ 66.98 and 77.81) are much more consistent with those of an acetylated 5-*O*-glucoside. Thus, compound **5b** has been established to be the pentaacetate of 5-*O*-β-D-glucopyranoside of 3,5-dihydroxy-6,7-megastigmadien-9-one. The stereochemistry of the three chiral centers of this compound was determined as follows. The ^{1}H NMR spectrum of **5b** clearly showed axial configuration (δ 5.16, multiplet, $W_{1/2} = 21$ Hz) for H-3. Irradiation of protons of CH_3-12 (δ 1.31) produced an NOE on the signal at δ 5.16, indicating a CH_3-12 axial configuration. Further NOE experiments irradiating CH_3-13 (δ 1.42) did not have any effect on the signal of H-3 at δ 5.16, thus demonstrating an equatorial position for this methyl group. Assuming that the aglycon of compound **5a** is being formed by biodegradation of allenic carotenoids such us fucoxanthine or neoxanthine (Isoe et al., 1971), the stereochemistry at C-3 must be *S*. This fact and the results of the abovementioned NOE experiments clearly showed (3*S*,5*R*) stereochemistry for compound **5b**. In relation to the other chiral center, we assigned *R* configuration at C-8 on the basis of the NMR data in deuteropyridine of the natural **5a**, which were in good agreement with those published by Umehara et al. (1988) for the (3*S*,5*R*,8*R*)-

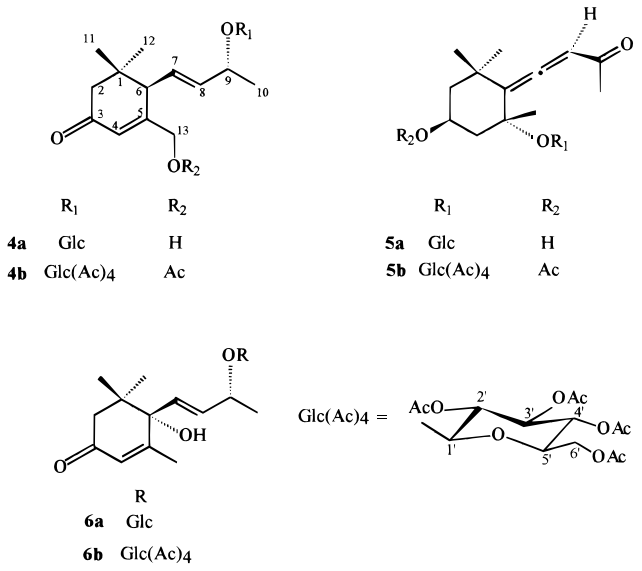

Figure 1. Structures of the major glucoconjugated C_{13}-norisoprenoids identified in lulo (*S. quitoense* L.) leaves.

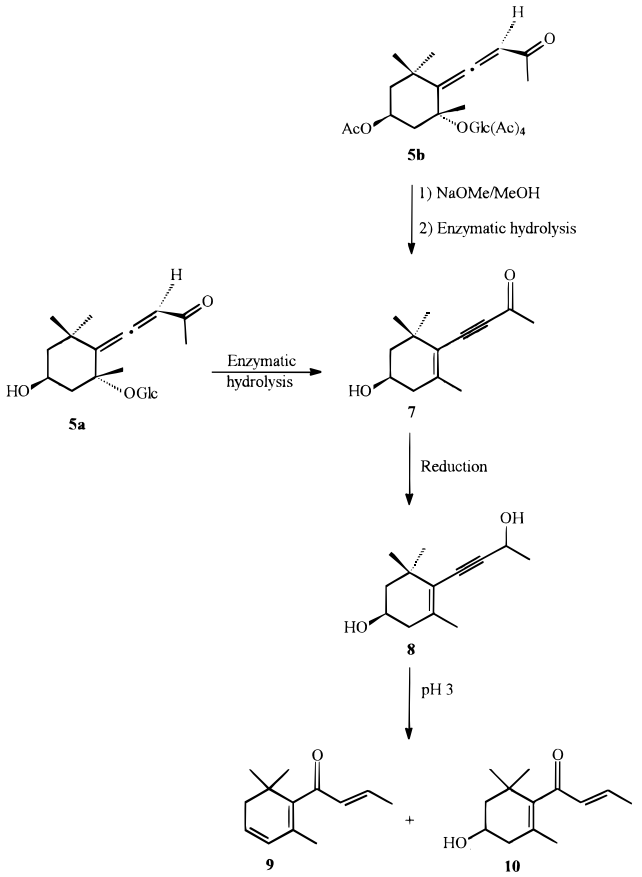

Figure 2. Generation of 3-hydroxy-7,8-didehydro-β-ionone (**7**), β-damascenone (**9**), and 3-hydroxy-β-damascone (**10**) from (3*S*,5*R*,8*R*)-3,5-dihydroxy-6,7-megastigmadien-9-one 5-*O*-β-D-glucopyranoside (**5a**).

isomer. On the basis of the above results, we have assigned the structure of (3*S*,5*R*,8*R*)-3,5-dihydroxy-6,7-megastigmadien-9-one 5-*O*-β-D-glucopyranoside for compound **5a**.

Deacetylation of **5b** and subsequent treatment with emulsin led to the liberation of the 3-hydroxy-7,8-didehydro-β-ionone (**7**) (Figure 2), the structure of which was confirmed by HRGC and HRGC/MS and ^{1}H NMR analyses. As per our observations compound **5b** is quite

labile. Thus, one could think that the acetylenic compound could come directly as a result of base treatment of **5b**. For this reason, we isolated pure glycoside **5a** from lulo leaves and submitted it to enzymatic hydrolysis with emulsin, finding 3-hydroxy-7,8-didehydro-β-ionone (**7**) as reaction product. Additional model reactions depicted in Figure 2 ($NaBH_4$ reduction of compound **7** and its further heated acid catalyzed rearrangement) showed compound **5a** as a new precursor of β-damascenone (**9**) and 3-hydroxy-β-damascone (**10**), important industrial flavor compounds. It is also important to point out that 3,5-dihydroxy-6,7-megastigmadien-9-one 5-*O*-β-D-glucopyranoside (**5a**) is the true precursor of 3-hydroxy-7,8-didehydro-β-ionone (**7**), one of the major volatiles found in lulo peelings (Suárez et al., 1993). Finally, it is important to mention that glucoside **5a** (citroside A) has been reported only in *Citrus unshius* (Umehara et al., 1988) leaves. In contrast, the peracetylated **5b** derivative has never been reported.

Analyses of the spectral data (UV, CI-MS, ^{1}H NMR and ^{13}C NMR mono- and bidimensional experiments) of **6b** and comparison with data for authentic sample allowed us to establish for this glycoside the structure of peracetylated (6*S*,9*R*)-vomifoliol 9-*O*-β-D-glucopyranoside. Glucoside **6a** is a ubiquitous natural compound that has been detected in various fruit species (Winterhalter and Schreier, 1994). Its role in the formation of theaspirones (Winterhalter and Schreier, 1994), key components of black tea aroma, has been established. The role of **6a** as an intermediate in the generation of vitispiranes has also been studied (Winterhalter and Schreier, 1988).

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