Glycosidically Bound Flavor Compounds of Cape Gooseberry (*Physalis peruviana* L.)

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The bound volatile fraction of cape gooseberry (*Physalis peruviana* L.) fruit harvested in Colombia has been examined by HRGC and HRGC-MS after enzymatic hydrolysis using a nonselective pectinase (Rohapect D5L). Forty bound volatiles could be identified, with 21 of them being reported for the first time in cape gooseberry. After preparative isolation of the glycosidic precursors on XAD-2 resin, purification by multilayer coil countercurrent chromatography and HPLC of the peracetylated glycosides were carried out. Structure elucidation by NMR, ESI-MS/MS, and optical rotation enabled the identification of (1*S*,2*S*)-1-phenylpropane-1,2-diol 2-*O*-*β*-D-glucopyranoside **(1)** and *p*-menth-4(8)-ene-1,2-diol 1-*O*-α-L-arabinopyranosyl-(1−6)-*β*-D-glucopyranoside (**2**). Both glycosides have been identified for the first time in nature. They could be considered as immediate precursors of 1-phenylpropane-1,2-diol and *p*-menth-4(8)-ene-1,2-diol, typical volatiles found in the fruit of cape gooseberry.

Keywords: *Cape gooseberry; Physalis peruviana; tropical fruits; aroma precursors; bound volatiles*

INTRODUCTION

Cape gooseberry (*Physalis peruviana*) is a tropical bush native to South America. The fruit has been widely used as a source of vitamins A and C and minerals, mainly iron and potassium, and in folk medicine as a diuretic, as an antiparasitic remedy, and as a cure for throat infections (*1*). In Colombia, *P. peruviana* is cultivated in regions between 1500 and 3000 m above sea level. The round fruit with an average diameter of 20−25 mm and an approximate weight of 4−5 g is protected by an accrescent calyx and covered by a brilliant resinous yellow peel. The pulp has a soft and exotic aroma and is very much appreciated on the international markets, particularly in France and Germany where the fruit is considered a delicacy. Presently, cultivation of cape gooseberry in Colombia is steadily increasing to satisfy the growing export demands, ranking it second after banana fruit exports from Colombia.

Previous work on *P. peruviana* has focused on the isolation and characterization of several bioactive withanolides from the whole plant (*2*, *3*), leaves (*4*), and roots (*5*), as well as berries and the surrounding calyx (*6*). Although the fruit is highly esteemed by consumers, knowledge about its flavor is scarce. To our knowledge, there is only one report in 1989 on the flavor of cape gooseberry fruits (*7*). More recently, 1-*O*-*trans*-cinnamoyl-*β*-D-glucopyranosyl-(1−6)-*β*-D-glucopyranoside was identified as a natural precursor of cinnamic acid-derived volatiles in the fruits (*8*).

As part of our continuing studies on Colombian fruits (*9*−*12*), we report the identification of glycosidically bound compounds in cape gooseberries harvested in Colombia. In addition, the isolation and characterization of two novel glycoconjugates, which can be considered as immediate progenitors of *p*-menth-4(8)-ene-1,2-diol and 1-phenyl-1,2-propanediol, important aroma constituents of *P. peruviana*, are described.

EXPERIMENTAL PROCEDURES

General. ^{1}H and ^{13}C NMR spectral data were recorded on a Fourier transform Bruker AMX 300 spectrometer with $CDCl_3$ or CD_3OD as solvent and TMS as internal reference standard.

Electrospray ionization ion trap multiple mass spectrometric (ESI-MS/MS) data were obtained using a Bruker-Esquire-LC-MS/MS system with electrospray ionization in the positive mode. Direct introduction of samples into the electrospray ion source was done by syringe pump (dilution of sample = 1−2 mg/10 mL in acetonitrile/H_2O, 9:1) at a flow rate of 4 μL/min. Electrospray parameters were as follows: nebulizing gas, 10 psi, and drying gas, 4 L/min (both nitrogen); drying temperature, 320 °C; end plate voltage, −3000 V; capillary voltage, −3500 V; skimmer 1 voltage, +50 V; skimmer 2 voltage, +10 V; capillary exit voltage, +150 V; multiplier voltage, 1500 V; detection mode, positive; acquisition, accumulation cutoff, 40 *m*/*z*; scan range, 50−1500 *m*/*z*; summation, 8 spectra; pressure, $\sim 1 \times 10^{-5}$ mbar in the ion trap.

TLC was carried out using silica gel 60 F_{254} plates (Merck). All solvents were of high purity at purchase and were redistilled before use. Commercial chemicals used were of analytical grade quality.

Plant Material. Fresh cape gooseberries (*P. peruviana*) were obtained from a local market in Bogotá, Colombia, in 1997. Intact fruits were carefully selected according to the degree of ripeness measured by fruit color (brilliant orange) and the pH value of the pulp (pH 3.6).

Isolation of Glycosidic Extract. The fruit (15 kg) was homogenized in 15 L of water (pH adjusted to 7.0) and centrifuged (5000*g* for 30 min). The supernatant was subjected to LC on Amberlite XAD-2 resin (glass column, 40 × 700 mm, 10 mL/min) in portions of 1.5 L (*14*). After the column had been rinsed with 3 L of distilled water, retained material was eluted with 1.5 L of methanol. The combined methanolic eluates were concentrated to dryness in vacuo, extracted with

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Table 1. Glycosidically Bound Volatiles Liberated by Enzymatic Hydrolysis of Cape Gooseberry (*P. peruviana* L.) Fruit

		retention indices		
no.	compound	exptl	ref[a]	amount[b]
1	2-methylpropanol	1091	1083	+++
2	butanol	1148	1139	++++
3	2-methylbutanol	1209	1203	++++
4	3-hydroxy-2-butanone[f]	1277	1268	++
5	2-heptanol[f]	1320	1318	+
6	hexanol	1355	1353	++++
7	(*E*)-2-hexenol[f]	1406	1408	+
8	acetic acid[f]	1439	1430	++++
9	methyl 3-hydroxybutanoate[f]	1468	1460	+
10	ethyl 3-hydroxybutanoate[f]	1525	1522	++
11	2-methylpropanoic acid[f]	1569	1563	+
12	butanoic acid[f]	1629	1627	+
13	2-methylbutanoic acid[f]	1665	1662	+
14	ethyl 3-hydroxyhexanoate	1670	1675	++
15	α-terpineol	1675	1679	++
16	butyl 3-hydroxybutanoate[f]	1684	1678	+++
17	unknown (55, 73, 45, 43, 72, 57, 115, 131)[c]	1750		+++
18	methyl 2-hydroxybenzoate[f]	1762	1754	+
19	unknown (71, 43, 87, 102, 59, 41, 74, 131)[c]	1783		+
20	unknown (109, 41, 119, 43, 134, 85, 91, 152)[c]	1832		++
21	hexanoic acid[f]	1839	1834	+
22	guaiacol[f]	1853	1840	++
23	benzyl alcohol	1865	1862	++++
24	ethyl 3-hydroxyoctanoate	1880	1886	+++
25	γ-octalactone and 2-phenylethanol	1888 1888	1883 1896	+ +
26	δ-octalactone	1955	1956	+
27	unknown (71, 43, 59, 42, 41, 57, 89, 101)[c]	1984		++
28	phenol (I.S.)[g]	2009	1996	++++
29	ethyl 5-hydroxyoctanoate	2036	2030	+
30	3-phenylpropanol[f]	2040	2039	++
31	octanoic acid[f]	2050	2050	+
32	unknown (117, 71, 43, 88, 89, 41, 55, 127)[c]	2079		++
33	4-vinylguaiacol[f]	2190	2181	+++
34	*p*-menth-4(8)-ene-1,2-diol[d,f]	2249		+++
35	decanoic acid	2270	2266	+
36	geranoic acid	2328	2319	+
37	4-vinylphenol[f]	2384	2372	+
38	benzoic acid	2419	2417	++++
39	3,4-dimethylbenzoic acid[e,f]	2447		+
40	1-phenyl-1,2-propanediol[d,f]	2455		+++
41	4-vinylsyringol	2526	2543	++
42	9-(*Z*)-octadecenoic acid[e]	2576		+++
43	cinnamic acid	>2700	2869	++++
44	hexadecanoic acid	>2700	2890	+++

[a] Retention indices for authentic reference substances measured on a DB-Wax column. [b] Amount in micrograms per kilogram of fruit: + = <300; ++ = 300−700; +++ = 700−1300; ++++ = >1300. [c] Prominent MS peaks (in order of decreasing intensity). [d] Identified by using the MS and NMR data of the glycosidic precursor. [e] Tentatively identified by MS. [f] Newly identified bound volatiles. [g] I.S., internal standard.

diethyl ether to eliminate the remaining volatiles, and lyophilized to afford 25.2 g of a crude isolate.

Enzymatic Hydrolysis. Aliquots of the isolate (200 mg) were dissolved in 25 mL of 0.2 M citric acid−phosphate buffer (pH 5.0), and a nonselective glycosidase (300 μL of Rohapect D5L, Röhm, Darmstadt, Germany) was added, together with phenyl β-D-glucopyranoside (internal standard). The mixture was incubated at 37 °C for 36 h, and the liberated aglycons (bound volatiles) were extracted with diethyl ether. After drying over anhydrous sodium sulfate, the organic layer was concentrated (Vigreux column, 38 °C) to 0.2 mL and subjected to capillary GC (HRGC) and HRGC-MS analyses. For enzymatic hydrolysis either fractions obtained by countercurrent

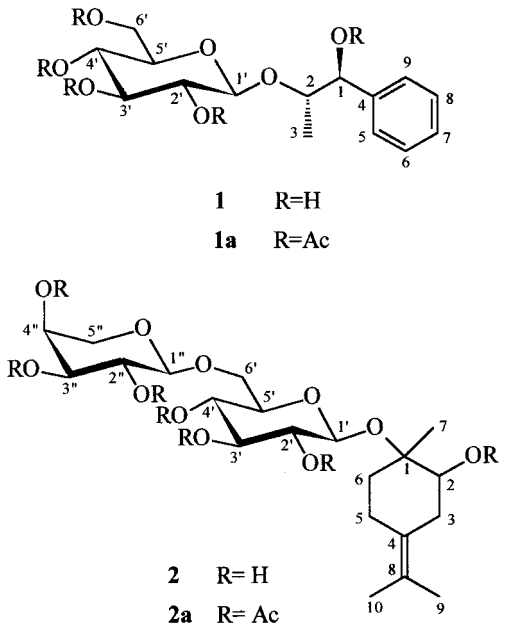

Figure 1. Structures of newly identified glycosides of cape gooseberry: (1*S*,2*S*)-1-phenylpropane-1,2-diol 2-*O*-β-D-glucopyranoside, **1**; *p*-menth-4(8)-ene-1,2-diol 1-*O*-α-L-arabinopyranosyl-(1−6)-β-D-glucopyranoside, **2**.

chromatographic separations or pure glycoconjugates, 1 mg of substrate, and 40 μL of Rohapect D5L were used under the same conditions as described above.

Capillary Gas Chromatography (HRGC). HRGC analyses were performed with a Hewlett-Packard 5890 series II gas chromatograph equipped with an FID detector. A DB-Wax (J&W Scientific Inc., Folsom, CA) fused silica column (30 m × 0.25 mm i.d., 0.25 μm) was used with the following temperature program: 4 min isothermal at 50 °C, then raised to 130 °C at 4 °C/min, from 130 to 190 °C at 1 °C/min, then again from 190 °C to 220 °C at 4 °C/min, and finally kept at this temperature for 20 min. Injector and detector temperatures were maintained at 220 °C. The carrier gas flow was 1.0 mL/min He. Volumes of 1 μL were injected with a split ratio of 1:10. Retention indices were calculated using a mixture of *n*-hydrocarbons as standards. Quantitative data for enzymatically released compounds were obtained by the internal standard method using phenol (released by enzymatic hydrolysis of phenyl β-D-glucopyranoside) without consideration of calibration factors, that is, $F = 1.00$ for all compounds.

Capillary Gas Chromatography/Mass Spectrometry (HRGC-MS). Experiments were carried out on a Hewlett-Packard 5970 mass selective detector directly coupled to an HP 5890 gas chromatograph with the same type of column and temperature conditions as used for HRGC. Mass spectra were scanned at 70 eV in the range of 30−300 amu. Results of qualitative analysis (mass spectral data studies) were verified by comparing the retention indices and mass spectral data with those of authentic reference substances.

Multilayer Coil Countercurrent Chromatography (ML-CCC). Portions of ∼1.8 g of the XAD-2 isolate were separated by preparative MLCCC (P.C. Inc., Potomac, MD) using a 75 m × 2.6 mm i.d. PTFE tubing (total volume = 400 mL). The MLCCC apparatus was operated at a rotational speed of 800 rpm, using $CHCl_3$/MeOH/H_2O (7:13:8) as solvent system with the less dense layer acting as mobile phase at a flow rate of 1 mL/min. Sixty-six fractions (each 5 mL) were collected. Each fraction was concentrated to dryness and lyophilized. An aliquot of each fraction was enzymatically hydrolyzed, and the released volatiles were analyzed by HRGC-MS.

Acetylation and Purification of Acetylated Glycosides. Combined MLCCC fractions 35−46 (580 mg) were acetylated overnight with 5 mL of Ac_2O/pyridine. The peracetylated glycosides were subjected to flash chromatography (*13*) using a silica gel column (63−200 μm) and the following

Table 2. MS Spectral Data for Compound 1a

fragment (*m*/*z*)	ESI-MS/MS (%)	interpretation
563.0	3	$[M + K]^+$
547.3	100	$[M + Na]^+$
542.0	6	$[M + H_2O]^+$
485.1	12	$[M + Na - AcOH - 2H]^+$
331.0	6	$[M -$ aglycon(Ac) $+ H]^+$
271.1	1	$[331.0 - AcOH]^+$
169.1	1	$[271.1 - AcOH - CH_2{=}C{=}O]^+$
109.2	3	$[169.1 - AcOH]^+$
daughter ion spectrum of *m*/*z* 547.2		
547.2	100	$[M + Na]^+$
487.1	97	$[M + Na - AcOH]^+$
427.1	15	$[M + Na - 2AcOH]^+$
385.1	2	$[427 - CH_2{=}C{=}O]^+$
daughter ion spectrum of *m*/*z* 487.1		
427.1	100	$[487.1 - AcOH]^+$
385.1	6	$[427.1 - CH_2{=}C{=}O]^+$
295.0	4	$[487.1 -$ aglycon(Ac) $+ 2H]^+$

discontinuous pentane/diethyl ether gradient (8:2, 6:4, 1:1, 4:6, and 2:8 in volumes of 200 mL). One hundred fractions (each ~10 mL) were collected. Combined fractions 38−51 and 72−85 were concentrated in vacuo to dryness for subsequent purification. Combined fractions 38−51 (58 mg) were purified by crystallization using *tert*-butyl methyl ether (TBME) as solvent to afford 9 mg of pure **1a** (white needles). After deacetylation of **1a** and liberation of the aglycon by enzymatic hydrolysis, evaluation of the absolute stereochemistry of the aglycon was made using HPLC with chirospecific on-line detection (Chiralyzer, IBZ Messtechnik, Hannover, Germany) (Eurospher Si 100-5 column, 5 μm, 250 mm × 4 mm, Knauer, Germany; eluent, TBME/hexane 7:3, 1 mL/min). The sugar moiety was similarly identified by HPLC on-line coupled chirospecific detector using a Lichrospher NH_2 column (5 μm, 250 × 4 mm, Knauer; eluent, CH_3CN/H_2O 7:3, 1 mL/min).

Component **2a** was isolated of fractions 72−85 (227 mg) by preparative HPLC using a Eurospher Si 100-5 column (5 μm, 250 mm × 16 mm, Knauer) with TBME as mobile phase at a flow rate of 3 mL/min, followed by a Eurospher 100-C18 column (5 μm, 250 mm × 16 mm, Knauer) and MeOH/H_2O (7:3) as solvent at a flow rate of 5 mL/min. Final purification of **2a** (1 mg) was achieved on an analytical Lichrocart RP18 column (5 μm, 250 mm × 4 mm, Merck) using an MeOH/H_2O gradient from 75:25 to 85:15 in 20 min, at a flow rate of 0.5 mL/min.

RESULTS AND DISCUSSION

Analysis of Bound Volatiles. The volatile compounds (aglycons) released by enzymatic hydrolysis of the glycosidic fraction obtained from cape gooseberry fruit are listed in Table 1, together with their concentration in the fruit and their retention indices on a DB-Wax column. Approximately 40 aglycons were identified in this fruit, with 21 of them being reported for the first time as bound aroma constituents. The isolated aglycons exhibited compounds with an aromatic structure (34.5%), followed by acids (31.5%), aliphatic alcohols (19%), hydroxyesters (6.5%), and terpenoids (3.2%). The pattern of products obtained for cape gooseberry harvested in Colombia significantly differs from the composition of a German variety (*7*).

Isolation of Glycoconjugates. A glycosidic extract of cape gooseberries, obtained by Amberlite XAD-2 adsorption and subsequent methanol elution (*14*), was fractionated by the liquid−liquid chromatographic technique of MLCCC (*11*). Monitoring of separated MLCCC fractions by enzymatic hydrolysis revealed major products in MLCCC fractions 38−51 and 72−85. After acetylation, flash chromatography, and HPLC, the peracetylated glycosides **1a** and **2a** (Figure 1) were obtained in pure form.

Characterization of (1*S*,2*S*)-1-Phenylpropane-1,2-diol 2-*O*-*β*-D-Glucopyranoside (1). Glucoconjugate **1** was characterized in its acetylated form **1a** and in the free form **1** by ESI-MS/MS and ^1H and ^{13}C NMR spectroscopy. The molecular mass of **1a** was determined to be 524 amu (pseudomolecular ions at *m*/*z* 563 $[M + K]^+$, 547 $[M + Na]^+$, and 542 $[M + H_2O]^+$, cf. Table 2). The fragments at *m*/*z* 331, 271, and 169 revealed the presence of a tetraacetylated hexose unit (*11*) in **1a**. The molecular mass of the aglycon moiety was calculated to be 194 amu (difference between the molecular weight of the glycoside and the weight of the tetraacetylated hexose).

Table 3. ^1H and ^{13}C NMR Spectral Data for Compounds 1 (CD_3OD, 300 MHz) and 1a ($CDCl_3$, 300 MHz)

	1a		**1**	
position[a]	δ_H[b]	δ_C[b]	δ_H[b]	δ_C[b]
1	5.66, 1H, d (7.5)	79.3	4.50, 1H, d (7.5)	83.4
2	4.06, 1H, dq (7.5/6.5)	77.5	3.93, 1H, dq (7.5/6.5)	79.9
3	1.03, 3H, d (6.5)	18.2	1.04, 3H, d (6.5)	18.8
4		137.1		142.2
5	7.28−7.37, 1H, m	127.3	7.24−7.40, 1H, m	128.2−129.5, m
6	7.28−7.37, 1H, m	128.5	7.24−7.40, 1H, m	128.2−129.5, m
7	7.28−7.37, 1H, m	128.5	7.24−7.40, 1H, m	128.2−129.5, m
8	7.28−7.37, 1H, m	128.5	7.24−7.40, 1H, m	128.2−129.5, m
9	7.28−7.37, 1H, m	127.3	7.24−7.40, 1H, m	128.2−129.5, m
1′	4.78, 1H, d (8.0)	101.0	4.51, 1H, d (8.0)	105.7
2′	5.01, 1H, dd (9.5/8.0)	71.3	3.21−3.40,1H, m	75.7
3′	5.18, 1H, dd (9.5/9.5)	73.0	3.21−3.40,1H, m	78.1
4′	5.09, 1H, dd (9.5/9.5)	68.5	3.21−3.40,1H, m	71.6
5′	3.72, 1H, ddd (9.5/5.0/2.5)	71.6	3.21−3.40,1H, m	78.0
6a′	4.16, 1H, dd (12.0/2.5)	61.9	3.85, 1H, dd (12.0/2.0)	62.8
6b′	4.26, 1H, dd (12.0/5.0)		3.67, 1H, dd (12.0/4.5)	
5 × $\underline{CH_3}CO$	2.00−2.10, 15H	20.5		
		20.6 (2C)		
		20.7		
		21.3		
5 × $CH_3\underline{C}O$		169.4		
		169.8		
		170.4		
		170.7		

[a] Assignments are based on $^1H-^1H$ COSY, HMBC, and HMQC experiments. [b] Coupling constants in hertz; δ_H and δ_C relative to TMS.

Table 4. MS Spectral Data for Compound 2a

fragment (*m*/*z*)	ESI-MS/MS (%)	interpretation
829.5	9	$[M + K + MeOH]^+$
797.3	16	$[M + K]^+$
781.5	100	$[M + Na]^+$
739.4	11	$[M + Na - CH_2{=}C{=}O]^+$
697.4	4	$[M + Na - 2(CH_2{=}C{=}O)]^+$
259.1	2	$[M - \{^-O\text{-hexose (Ac)}_3\text{aglycon (Ac)}\}]^+$ $[\text{pentose(Ac)}_3]^+$
daughter ion spectrum of *m*/*z* 781.4		
781. 3	98	$[M + Na]^+$
721.3	65	$[M + Na - AcOH]^+$
661.3	5	$[M + Na - 2AcOH]^+$
587.2	100	$[M + Na - \text{aglycon (Ac)} + H]^+$ $[\text{pentose(Ac)}_3\text{hexose(Ac)}_3OH + Na]^+$
527.3	44	$[587 - AcOH]^+$
467.1	16	$[587 - 2AcOH]^+$
425.0	5	$[467.1 - CH_2{=}C{=}O]^+$
daughter ion spectrum of *m*/*z* 527.2		
527.1	100	$[587.1 - AcOH]^+$
467.0	24	$[527.1 - AcOH]^+$
407.1	9	$[467.0 - AcOH]^+$
97.1	4	$[407 - \{^-\text{O-hexose(Ac)}_3OH - H_2O\} - Na]^+$ $[\text{pentose (Ac)}_3OH - 3AcOH + H]^+$ $[C_5H_5O_2]^+$

^{1}H and ^{13}C NMR spectral data for compound **1a** (cf. Table 3) confirmed the presence of a tetraacetylated β-glucopyranose as sugar unit (*11*). The ^{1}H NMR spectrum exhibited a doublet at δ 4.78 (J = 8.0 Hz) for the anomeric proton, thus indicating a β-glycosidic linkage. NMR data for the aglycon moiety revealed the presence of a monosubstituted aromatic ring, another acetyl group, one methyl group (δ_H 1.03, J = 6.5 Hz) next to an oxymethine group (δ_H 4.06, J = 7.5 and 6.5 Hz; and δ_C 77.5), which in turn appeared to be directly connected to the β-glycosidic linkage. A doublet (J = 7.5 Hz) integrating for one proton at δ_H 5.66 revealed the presence of an additional oxymethine proton coupled to the first one. From the chemical shift value its connection to the aromatic ring and to the acetyl group was apparent. From these data the isolated compound was identified as the peracetylated 1-phenylpropane-1,2-diol 2-*O*-β-D-glucopyranoside **1a**. ^{1}H−^{1}H COSY, HMBC, and HMQC experiments confirmed the structure of **1a** and led us to the unambiguous assignment of the NMR data presented in Table 3. Deacetylation of **1a** afforded the glucoconjugate **1**, the NMR data of which (cf. Table 3) were in good agreement with the above proposed structure. The relative stereochemistry of compounds **1** and **1a** was established as *threo* through comparison of the coupling constants between H-1 and H-2 (J = 7.5 Hz) of the aglycon moiety with those reported in the literature (*15*, *16*) for the four possible stereoisomers of 1-phenylpropane-1,2-diol of synthetic origin [J = 7.3 Hz for (1*S*,2*S*) or (1*R*,2*R*) and J = 3.9 Hz for (1*S*,2*R*) or (1*R*,2*S*) stereoisomers]. Enzymatic hydrolysis of **1** with emulsin afforded the aglycon (characterized by HRGC and HRGC-MS), the absolute stereochemistry of which was elucidated by normal phase HPLC using on-line coupled chirospecific detection (Chiralyzer, IBZ Messtechnik). According to the positive rotation value measured, the absolute configuration of the aglycon was established as 1*S*,2*S* (*16*). In a similar manner using a Lichrospher-NH_2 column, the sugar moiety was confirmed to be D-glucose. On the basis of the above-mentioned results, the structure of **1** was definitely established as (1*S*,2*S*)-1-phenylpropane-1,2-diol 2-*O*-β-D-glucopyranoside. To our knowledge, this is the first time that this glycoside has been found in nature. Also, the aglycon has not yet been reported as a natural constituent of fruits. Synthetic phenylpropane-1,2-diol, however, is widely used as a chiral synthon in asymmetric syntheses of natural products (*15*−*18*).

Characterization of *p*-Menth-4(8)-ene-1,2-diol 1-*O*-α-L-Arabinopyranosyl-(1−6)-β-D-glucopyranoside (2)**.** The characterization of **2** was made in its acetate form **2a**. ESI-MS data of **2a** (cf. Table 4) showed the charac-

Table 5. ^{1}H and ^{13}C NMR Spectral Data for Compound 2a (300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR, Coupling Constants in Hertz, δ Relative to TMS)

position[a]	δ_H	δ_C	DEPT
1		77.9	C
2	4.81, 1H, br t (4.5)	75.9	CH
3	2.44, 2H, br d (4.5)	33.8	CH_2
4		125.1	C
5	2.05−2.35, 2H, m	24.5	CH_2
6	1.58−1.67, 2H[b]	29.7	CH_2
7	1.23, 3H, s	21.4	CH_3
8		124.5	C
9	1.66, 3H, br s	20.2	CH_3
10	1.59, 3H, br s	20.0	CH_3
1′	4.75, 1H, d (8.0)	100.2	CH
2′	4.95, 1H, dd (9.5/8.0)	71.7	CH
3′	5.19, 1H, dd (9.5/9.5)	73.1	CH
4′	4.94, 1H, dd (9.5/9.5)	69.3	CH
5′	3.52−3.70, 1H, m	73.2	CH
6a′	3.52−3.70, 1H, m	67.4	CH_2
6b′	3.83, 1H, br d (9.0)		
1”	4.51, 1H, d (6.0)	95.0	CH
2”	5.12, 1H, dd (9.0/6.0)	69.2	CH
3”	5.05, 1H, dd (9.0/3.5)	69.9	CH
4”	5.24, 1H, ddd (3.5/3.5/2.0)	67.5	CH
5a”	3.52−3.70, 1H, m	62.7	CH_2
5b”	4.01, 1H, dd (13.0/3.5)		
7 × $\underline{CH_3}CO$	1.98, 1.99, 2.01, 2.02, 20.3 2.04, 2.12, 21H, 7 s	20.5, 1C; 20.6, 3C; 20.8, 1C; 20.9, 1C; 21.2, 1C	CH_3
7 × $CH_3\underline{C}O$		169.1, 1C; 169.3, 1C; 169.5, 1C; 170.1, 1C; 170.2, 1C; 170.3, 2C	C

[a] Assignments are based on ^{1}H−^{1}H COSY and DEPT experiments. [b] Overlapped.

teristic pattern of adduct ions at *m/z* 829 $[M + K + MeOH]^+$, 797 $[M + K]^+$, and 781 $[M + Na]^+$, which indicated a molecular mass of 758 amu for **2a**. Importantly, the ESI-MS spectrum also showed the fragment of *m/z* 259 corresponding to a triacetylated pentose moiety. The ESI-MS/MS analysis for the pseudomolecular ion at *m/z* 781 revealed a hexaacetylated hexose−pentose unit (cf. Table 4). The molecular mass of the aglycon part of **2a** was calculated to be 212 amu (difference between the molecular weight of the glycoside and the weight of the disaccharide residue).

The ^{1}H NMR spectrum of **2a** (cf. Table 5) showed six acetyl groups and two doublet signals for anomeric protons, one at δ 4.75 (J = 8.0 Hz) and the other at δ 4.51 (J = 6.0 Hz), confirming the hexaacetylated disaccharide moiety. ^{1}H and ^{13}C NMR data were in good agreement with data reported for α-L-arabinopyranosyl-β-D-glucopyranoside (*19*). The absence of the typical signals for H-6′$_a$ and H-6′$_b$ of a terminal glucose unit at δ_H 4.16 and 4.26, respectively, as well as the downfield shift of C-6 from δ_C 61.9 to 67.4, confirmed the 1−6 linkage between the arabinose and glucose moiety. The ^{1}H NMR spectrum showed an additional acetyl group, three methyl groups (two of them linked to a double bond), one oxymethine, and three methylene groups being part of the aglycon structure. In the ^{13}C NMR spectrum three quaternary carbons at δ_C 77.9, 124.5, and 125.1 were observed, with the latter two signals belonging to a double bond. ^{1}H−^{1}H COSY and DEPT measurements allowed us to propose the structure of a monoacetylated *p*-menth-4(8)-ene-1,2-diol for the aglycon part of **2a**. The chemical shift of C-2 (δ_C 75.9) and a correlation between H-2 and the hydrogens of C-3 were the basis for assigning C-2 as the position for the acetate group, leaving C-1 as the sole possibility for the glycosidic linkage. After deacetylation, **2a** was enzymatically hydrolyzed with Rohapect D5L and the liberated aglycon was characterized by HRGC and HRGC-MS as *p*-menth-4(8)-ene-1,2-diol, thus confirming our NMR analyses.

On the basis of these results, the structure of compound **2a** was assigned as *p*-menth-4(8)-ene-1,2-diol 1-*O*-α-L-arabinopyranosyl-(1−6)-β-D-glucopyranoside. The absolute stereochemistry of **2a** remains to be elucidated. This is the first time that this glycoconjugate has been found in nature. In contrast, the aglycon has been reported as product of chemical synthesis (*20*) and of microbial degradation (*21*). Finally, it should be stressed that glycoconjugates **1** and **2** can be considered as immediate precursors of 1-phenylpropane-1,2-diol and *p*-menth-4(8)-ene-1,2-diol (creamy-fruity odor note), typical volatiles found in the fruit of cape gooseberry.

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