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Hydroxyester disaccharides from fruits of cape gooseberry (*Physalis peruviana*)

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Abstract

The 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside of ethyl 3-hydroxyoctanoate and the diastereomeric 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosides of (3*R*) and (3*S*)-butyl 3-hydroxybutanoate, respectively, were isolated by chromatographic methods from fruits of cape gooseberry (*Physalis peruviana*) harvested in Colombia. Their structures were identified by ESI–MS/MS and NMR spectroscopy. The three glycoconjugates can be considered as immediate precursors of ethyl 3-hydroxyoctanoate and butyl 3-hydroxybutanoate, which are important aroma volatiles found in the fruit.

Keywords: *Physalis peruviana*; Solanaceae; Uchuva; Cape gooseberry; Aroma precursors; Glycosides; Hydroxyester glycosides

1. Introduction

During the last decade a considerable part of flavour research has been directed not only to the free volatiles as important contributors to the overall aroma of the fruits but also to the examination of glycoconjugates. The latter are known to act as volatile generators by enzymatic or chemical pathways during fruit homogenization, e.g. in the course of consumption, plant maturation, industrial pretreatment or processing (Crouzet and Chassagne, 1999). In the course of our studies on the aroma of Colombian fruits, we have isolated several glycoconjugates and established their role as aroma progenitors in fruits such as lulo (*Solanum quitoense*) (Wintoch et al., 1993; Osorio et al., 1999), piñuela (*Bromelia plumieri*) (Parada and Duque, 1998), and melón de olor (*Sicana odorifera*) (Parada et al., 2000).

Recently we have examined the bound aroma compounds of cape gooseberry (*Physalis peruviana*) cultivated in Colombia. Most importantly, during this study a considerable number of a homologous series of 3- and 5-hydroxyesters could be identified (Mayorga et al., 2001). Our results were in line with previous reports on the aroma of cape gooseberry fruits harvested in Germany (Berger et al., 1989). Thus, to expand our knowledge on flavour formation of *Physalis peruviana* fruit, we decided to look for hydroxyester glycoconjugates which could liberate volatile hydroxyesters as a mechanism of aroma generation. The present paper describes the isolation and characterization of three new hydroxyester glycosides: 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside of ethyl 3-hydroxyoctanoate **1;** 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside of butyl (*3R*)-hydroxybutanoate **2**; and 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside of butyl (*3S*)-hydroxybutanoate **3**.

2. Results and discussion

2.1. Characterization of glycoside *1*

Compound **1** was characterized in its acetylated form **1a** by ESI–MS/MS, as well as by ^{1}H and ^{13}C NMR spectroscopy. The molecular mass of **1a** was determined to be 806 amu as the ESI mass spectrum revealed the

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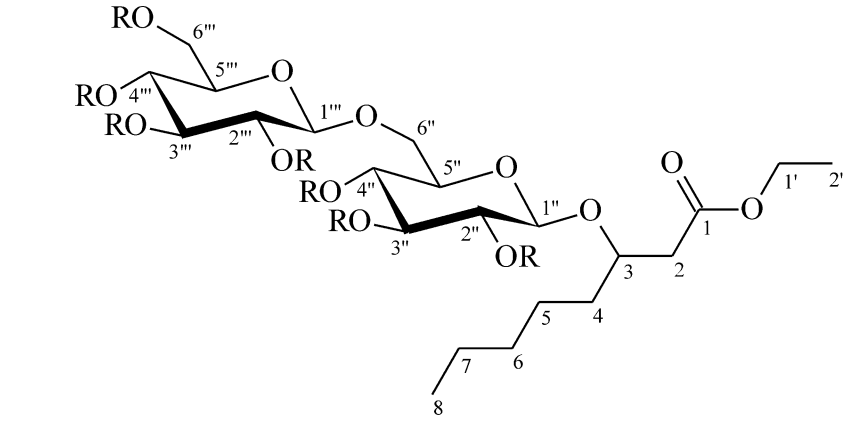

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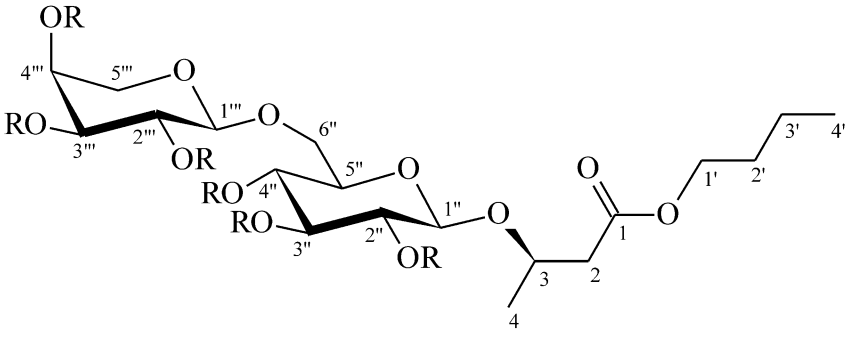
1a: R= Ac



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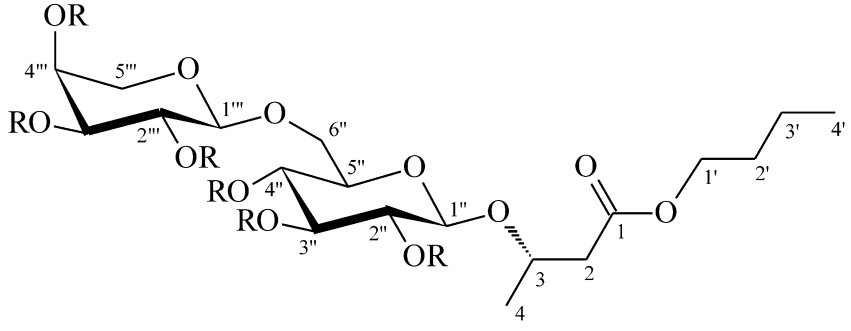
2a: R= Ac



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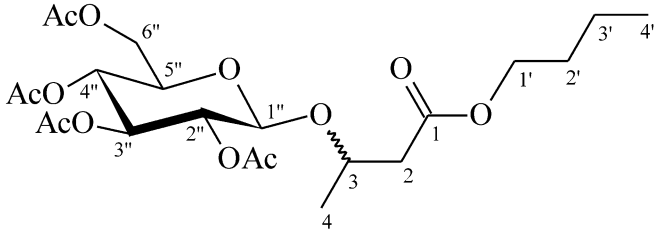

4: (3*R*)

5: (3*S*)

Fig. 1. Hydroxyester glycosides isolated from cape gooseberry (*Physalis peruviana*).

adduct ions m/z 845 $[M+K]^+$ and 829 $[M+Na]^+$. Furthermore, the ion spectrum of m/z 829 showed the fragment m/z 769 $[M+Na–AcOH]^+$ confirming the above mentioned molecular weight. The presence of the fragment ion m/z 528 in the ESI–MS spectrum of **1a** evidenced a peracetylated disaccharide (dihexose) as a sugar moiety. The molecular mass of the aglycon moiety was calculated to be 188 amu (difference between the molecular weight of the glycoside and the weight of the peracetylated disaccharide residue).

From ^{1}H (Table 1) as well as ^{13}C NMR spectral data (Table 2) the presence of a disaccharide moiety in compound **1a** was confirmed. The ^{1}H NMR spectrum exhibited two doublets at δ_H 4.63 ($J=8.0$ Hz) (δ_C 100.3) and at δ_H 4.64 ($J=8.0$ Hz) (δ_C 100.8) for two anomeric protons indicating the presence of two β-glycosidic linkages. The ^{1}H–^{1}H COSY evidenced two sequences of hydrogen connectivities corresponding to the axial protons of two β-glucopyranoside units linked to each other. Furthermore, the ^{13}C NMR spectral data were in good agreement with data published for the per-*O*-acetylated β-D-gentiobioside residue (Straubinger et al., 1998). In addition, the NMR spectroscopy data showed a methyl group (δ_H 1.27, δ_C 14.0) coupling in the ^{1}H–^{1}H COSY with an oxymethylene group (δ_H 4.13, δ_C 60.5) indicating a CH_3–CH_2–O– unit, which in turn is connected to a carbonyl group (δ_C 171.1) according to the HMBC data (correlation between the signal at δ_H 4.13 and δ_C 171.1). On the other hand, the ^{1}H–^{1}H COSY, DEPT and HMBC experiments allowed us to assign the partial structure CH_3–CH_2–CH_2–CH_2–CH_2–CH–O– whose oxymethine proton (δ_H 4.08–4.20, δ_C 76.8) is connected to the carbonyl function at δ_C 171.1 via a methylene group (δ_H 2.41 and 2.50, δ_C 40.0), thus indicating the structure of ethyl 3-hydroxyoctanoate for the aglycon moiety. Finally, the HMBC correlation between the anomeric proton (δ_H 4.63, δ_C 100.3) and the signal at δ_C 76.8 lead us to link the sugar unit to C-3 of the aglycon moiety. Thus, compound **1a** has been established to be the heptaacetate of 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside of ethyl 3-hydroxyoctanoate (Fig. 1). The stereochemistry at C-3 remains unsolved.

Deacetylation of **1a** and subsequent treatment with Rohapect D5L (nonselective glycosidase) led to the liberation of ethyl 3-hydroxyoctanoate **6**. Ester **6** is an important free aroma volatile in the fresh fruit exhibiting fruity, green and sweet odor notes. The identity of **6** was confirmed by HRGC and HRGC–MS analyses.

Table 1
^{1}H NMR (300 MHz, $CDCl_3$) spectral data for compounds **1a**–**3a**

H[a]	**1a**	**2a**	**3a**
2a	2.41, 1H, *dd* (16.0/5.0)	2.42, 1H, *dd* (16.0/5.5)	2.36, 1H, *dd* (16.0/4.5)
2b	2.50, 1H, *dd* (16.0/8.0)	2.66, 1H, *dd* (16.0/7.0)	2.54, 1H, *dd* (16.0/8.5)
3	4.08–4.20, 1H[b]	4.25–4.37, 1H, *m*	4.14–4.26, 1H, *m*
4	1.50–1.63, 2H, *m*	1.20, 3H, *d* (6.5)	1.30, 3H, *d* (6.5)
5/6/7	1.24–1.36, 6H, *m*		
8	0.91, 3H, *t* (7.0)		
1′	4.13, 2H, *q* (7.0)	4.08, 2H, *t* (6.5)	3.99–4.14, 2H, *m*
2′	1.27, 3H, *t* (7.0)	1.62, 2H, *q* (7.0)	1.61, 2H, *q* (7.0)
3′		1.39, 2H, *s* (7.5)	1.38, 2H, *s* (7.5)
4′		0.94, 3H, *t* (7.5)	0.94, 3H, *t* (7.5)
1″	4.63, 1H, *d* (8.0)	4.56, 1H, *d* (8.0)	4.62, 1H, *d* (8.0)
2″	4.88, 1H, *dd* (9.5/8.0)	4.88, 1H, *dd* (9.5/8.0)	4.88, 1H, *dd* (9.5/8.0)
3″	5.15, 1H, *dd* (9.5/9.5)	5.18, 1H, *dd* (9.5/9.5)	5.17, 1H, *dd* (9.5/9.5)
4″	4.89, 1H, *dd* (9.5/9.5)	4.92, 1H, *dd* (9.5/9.5)	4.92, 1H, *dd* (9.5/9.5)
5″	3.62–3.71,1H, *m*[c]	3.63, 1H, *br dd* (9.5/7.0)	3.68, 1H, *ddd* (9.5/7.0/2.0)
6a″	3.62–3.71,1H, *m*[c]	3.70, 1H, *dd* (11.0/7.0)	3.62, 1H, *dd* (11.0/7.0)
6b″	3.82, 1H, *br d* (9.5)	3.78, 1H, *br d* (11.0)	3.84, 1H, *dd* (11.0/2.0)
1‴	4.64, 1H, *d* (8.0)	4.79, 1H, *d* (6.5)	4.52, 1H, *d* (6.5)
2‴	4.98, 1H, *dd* (9.5/8.0)	5.14, 1H, *dd* (9.0/6.5)	5.14, 1H, *dd* (9.0/6.5)
3‴	5.18, 1H, *dd* (9.5/9.5)	5.21, 1H, *dd* (9.0/3.5)	5.03, 1H, *dd* (9.0/3.5)
4‴	5.06, 1H, *dd* (9.5/9.5)	5.24, 1H, *ddd* (3.5/3.5/2.0)	5.24, 1H, *ddd* (3.5/3.5/2.0)
5‴	3.62–3.71, 1H, *m*[c]		
5a‴		3.80, 1H, *br d* (13.0)	3.60, 1H, *dd* (13.0/2.0)
5b‴		4.00, 1H, *dd* (13.0/3.5)	4.02, 1H, *dd* (13.0/3.5)
6a‴	4.12, 1H, *dd* (12.5/2.5)		
6b‴	4.27, 1H, *dd* (12.5/5.0)		
CH_3CO	1.98, 1.99, 2.08, 2.10, 12 H, 4×*s*, 2.02, 9H, *s* (7X)	1.98, 2.01, 2.08, 2.13, 12 H, 4×*s*, 2.02, 6H, *s* (6X)	1.97, 2.04, 2.07, 2.12, 12 H, 4×*s*, 2.02, 6H, *s* (6X)

[a] Assignments were based on ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY.
[b] Obscured.
[c] Signal overlapped.

Table 2
^{13}C NMR (75 MHz, $CDCl_3$) spectral data for compounds **1a**–**3a**

C[a]	**1a**		**2a**	**3a**	
	^{13}C	DEPT	^{13}C	^{13}C	DEPT
1	171.1	C	171.0	170.8	C
2	40.0	CH_2	42.0	42.1	CH_2
3	76.8	CH	71.9	74.2	CH
4	35.4	CH_2	19.6	21.9	CH_3
5	24.6	CH_2			
6	31.8	CH_2			
7	22.5	CH_2			
8	14.2	CH_3			
1′	60.5	CH_2	64.4	64.4	CH_2
2′	14.0	CH_3	30.6	30.7	CH_2
3′			19.1	19.2	CH_2
4′			13.7	13.6	CH_3
1″	100.3	CH	98.8	101.1	CH
2″	71.5	CH	71.6	71.5	CH
3″	73.0	CH	73.0	73.0	CH
4″	69.3	CH	69.0	69.2	CH
5″	73.5	CH	74.2	73.3	CH
6″	68.2	CH_2	67.1	67.5	CH_2
1‴	100.8	CH	100.4	100.4	CH
2‴	71.2	CH	69.6	69.1	CH
3‴	73.0	CH	70.0	69.9	CH
4‴	68.5	CH	68.0	67.4	CH
5‴	72.1	CH	63.0	62.7	CH_2
6‴	62.0	CH_2			
$\underline{C}H_3CO$	20.5, 1C; 20.6, 5C; 20.7, 1C (7X)	CH_3	20.6, 3C; 20.7, 1C; 20.8, 1C; 20.9, 1C (6X)	20.5, 1C; 20.6, 3C; 20.7, 1C; 20.9, 1C (6X)	CH_3
$CH_3\underline{C}O$	169.2, 169.3, 169.4, 169.5, 170.1, 170.2, 170.6 (7X)	C	169.2, 169.3, 169.5, 170.1, 170.2, 170.3 (6X)	169.2, 169.3, 169.5, 170.0, 170.1, 170.2 (6X)	C

[a] Assignments were based on DEPT, HMQC and HMBC experiments.

Compound **1** is, to our best knowledge, reported here for the first time. In contrast, the aglycon is a known bound aroma compound of cape gooseberry (Berger et al., 1989; Mayorga et al., 2001), and piñuela (*Bromelia plumieri*) (Parada and Duque, 1998). The aglycon has also been reported as free volatile in fruits such as mountain papaya (*Carica pubescens*) (Idstein et al., 1985), pineapple (*Ananas comosus*) (Umano et al., 1992), caja fruit (*Spondias lutea*) (Allegrone and Barbeni, 1992), bachang (*Mangifera foetida*), kuini (*Mangifera odorata*) (Wong and Ong, 1993), apple (*Malus silvestris*) (Beuerle et al., 1996), pear (*Pyrus communis*) (Beuerle and Schwab, 1997), and yellow passion fruit (*Passiflora edulis flavicarpa*) (Werkhoff et al., 1998).

2.2. Structure elucidation of glycosides *2* and *3*

Glycosides **2** and **3** were similarly characterized in their peracetylated forms **2a** and **3a**. A molecular formula of $C_{31}H_{46}O_{18}$ for **2a** could be deduced from HR–FABMS. The ESI–MS spectrum of compound **2a** showed strong adduct ions at *m*/*z* 766 $[M+AcOH]^+$, 745 $[M+K]^+$ and 729 $[M+Na]^+$ from which a molecular mass of 706 amu could be calculated in agreement with HR–FABMS measurements. In ESI–MS^n experiments, product ion spectra of *m*/*z* 729, 669 and 609 (see Experimental), respectively, confirmed the molecular weight. These data also indicated the presence of a terminal triacetylated pentosyl unit as part of the structure of compound **2a** due to the presence of a characteristic fragment at *m*/*z* 239 $[pentose(Ac)_3+Na\text{-}AcOH]^+$ in the product ion spectrum of *m*/*z* 609. Further, the latter spectrum reveals ions at *m*/*z* 507 $[M+Na-3AcOH-CH_2=C=O]^+$, 407 $[pentose(Ac)_3$ $hexose(Ac)_3+Na-2AcOH-CH_2=C=O-H_2O]^+$ and 347 $[407-AcOH]^+$; which is in accordance with the presence of a disaccharide unit as sugar moiety. Additionally, the fragment ion at *m*/*z* 142 $[aglycon-H_2O]^+$ suggested a weight of 160 amu for the aglycone moiety and the fragment ion at *m*/*z* 551 $[M+Na-2AcOH-C_4H_9-H]^+$ provided a hint for the presence of a C_4H_9 alkyl chain in the aglycone structure.

The presence of two doublets at δ_H 4.56 ($J=8.0$ Hz) (δ_C 98.8) and at δ_H 4.79 ($J=6.5$ Hz) (δ_C 100.4) in the NMR spectra indicated a disaccharide as sugar moiety containing one β- and one α-glycosidic linkage. In both the ^1H and ^{13}C NMR spectra the characteristic signals for a terminal α-L-arabinopyranosyl moiety linked to a

β-D-glucopyranosyl unit was observed (Straubinger et al., 1999). A correlation between the protons at C-6 of the hexose and the C-1 in the pentose moiety revealed a 1→6 linkage. In addition, the NMR spectral data showed one carbonyl (δ_C 171.0), one oxymethine (δ_H 4.25-4.37, δ_C 71.9) and one low-field shifted methylene group (δ_H 2.42 and 2.66, δ_C 42.0). In the ^1H–^1H COSY spectrum, the methylene group displayed a coupling to the oxymethine proton indicating a $-CHO–CH_2–CO-$ unit, which in turn is connected to a methyl group (δ_H 1.20, δ_C 19.6). A methylene group at δ_H 4.08 (δ_C 64.4) correlating in the HMBC spectrum to the above mentioned carbonyl group indicates an ester structure in the aglycon. Since there are three additional carbon signals left, butanol can be assumed as the alcohol moiety of the ester. This assumption is in accordance with the observed loss of a C_4H_9-chain in the mass spectrum and with the observed molecular mass of 706 amu. Thus, the structure of compound **2a** could be assigned as the peracetate of 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside of butyl 3-hydroxybutanoate (Fig. 1). The stereochemistry of carbon **3** could be deduced as *R* by comparison of the NMR spectroscopy data with those published by Krajewski et al. (1997) for a similar glycoside containing glucose instead of arabinose–glucose as the sugar moiety. The two diastereoisomers differ in the coupling constants of the diastereotopic protons of C-2 and in the ^1H and ^{13}C NMR chemical shifts of positions 2, 3 and 4 as well as of the anomeric signal of the glucose moiety (cf. Table 3).

HR–FABMS measurements of **3a** indicated a molecular formula of $C_{31}H_{46}O_{18}$. The ESI–MS/MS data of **3a** was identical to that of compound **2a** suggesting an isomeric structure for both compounds. ^1H and ^{13}C NMR chemical shifts of glycoside **3a** were also very similar to those of **2a** (cf. Tables 2 and 3) except for the signals of C-2, C-3 and C-4, as well as for the anomeric signals of the disaccharide. The values clearly indicated *S* configuration at C-3 when compared with the synthetic glucosides described by Krajewski et al. (1997). On the basis of the overall results for compound **3a** the structure of 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside of butyl (3*S*)-3-hydroxybutanoate (Fig. 1) could be assigned to compound **3**.

Enzymatic hydrolysis (Rohapect D5L) of the glycosides **2** and **3** liberated butyl 3-hydroxybutanoate **7** which was unambiguously identified by HRGC and HRGC–MS. By using HRGC–O, the aroma of **7** was determined as fruity, estery, sour and gooseberry-like.

To the best of our knowledge this is the first time that these diastereoisomeric glycosides have been found in nature. The aglycones, however, are known constituents of several fruits e.g. hog plum (*Spondias mombins* L.) (Adedeji et al., 1991), mango (*Mangifera indiga* L.) (Adedeji et al., 1992), mountain papaya (Krajewski et al., 1997), caja fruit (Allegrone and Barbeni, 1992), kuini (Wong and Ong, 1993), and banbangan (*Mangifera panjang* Kostermans) (Wong and Siew, 1994).

Finally, it has to be stressed that glycoconjugates **1**–**3** could be considered as immediate precursors of ethyl 3-hydroxyoctanoate and butyl 3-hydroxybutanoates which are important aroma contributors in cape gooseberry fruits.

3. Experimental

3.1. General

MLCCC was performed in the head to tail mode at a rotational speed of 800 rpm, using a 75 m × 2.6 mm i.d. PTFE tubing (total volume = 400 ml) and $CHCl_3$–MeOH–H_2O (7:13:8) as solvent system. ^1H–^{13}C NMR spectra were recorded in $CDCl_3$ at 300/75 or 500/125 MHz, respectively. HR–FABMS was taken in a glycerol matrix. ESI–MS/MS was employed in the positive mode by direct introduction of samples with acetonitrile–H_2O (9:1) at a flow rate of 4 μl/min. Electrospray parameters: nebulizing gas, 10 psi; drying gas, 4 l/min (both nitrogen); drying temperature 320 °C. Voltages: end plate, −3000 V; capillary, −3500 V; skimmer 1, + 50 V; skimmer 2, + 10 V; capillary exit, + 150 V; multiplier, 1500 V; detection mode: positive. Acquisition: accumulation cut-off, 40 *m*/*z*; scan range, 50–1500 *m*/*z*; summation, 8 spectra. Pressure: about 1×10^{-5} mbar in the ion trap. For flash chromatography, Merck silica gel 60 (0.063–0.200 mm) was used. HRGC of the aglycones was carried out with

Table 3
Selected NMR spectral data of compounds **2a**, **3a** (300 MHz, $CDCl_3$) and **4**[a] and **5**[a]

Position	**2a** (3*R*)	**3a** (3*S*)	**4** (3*R*)	**5** (3*S*)
H-2a	2.42 (16.0/5.5)	2.36 (16.0/4.5)	2.40 (15.7/7.3)	2.35 (16.0/4.5)
H-2b	2.66 (16.0/7.0)	2.54 (16.0/8.5)	2.76 (15.7/5.8)	2.54 (16.0/8.5)
H-4	1.20 (6.5)	1.30 (6.5)	1.20 (6.3)	1.28 (6.3)
H-1″	4.56 (8.0)	4.62 (8.0)	4.58 (8.1)	4.62 (8.1)
C-2	42.0	42.1	42.4	42.1
C-3	71.9	74.2	73.4	74.4
C-4	19.6	21.9	20.2	21.6
C-1″	98.8	101.1	99.9	101.1

[a] Compounds **4** and **5:** synthetic 3-*O*-(tetra-*O*-acetyl-β-D-glucopyranoside) of butyl 3-hydroxybutanoates (400 MHz, $CDCl_3$) (Krajewski et al., 1997).

a fused-silica WCOT column (30 m×0.25 mm i.d., 0.25 μm film thickness) coated with DB-Wax. The column temperature was programmed 4 min isothermally at 50 °C, then raised to 130 °C at 4 °C/min, from 130 to 190 °C at 1 °C/min and then raised again from 190 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 normal paraffins as standard. HRGC–MS of the aglycones was employed with the same type of column and temperature conditions as mentioned above for HRGC. Mass spectra were scanned at 70 eV in the range of 30–300 amu. Results of qualitative analyses (mass spectral data studies) were verified by comparing the retention indices and mass spectral data with those of authentic reference substances. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter.

3.2. Plant material

Fresh cape gooseberry fruits (*Physalis peruviana* L.) were obtained from a local market in Bogotá, Colombia in 1997. Fruits fully healthy were carefully selected according to the degree of ripeness measured by fruit color (brilliant orange) and pH = 3.6 of its pulp.

3.3. Extraction and isolation of compounds *1–3*

The fruits (15 kg) were homogenized in 15 l of water (adjusted to pH 7.0) and centrifuged (5000 *g* for 30 min). The supernatant divided in ten portions was subjected to LC on Amberlite-XAD-2 (glass column, 40×700 mm, 10 ml/min) (Gunata et al., 1985). After rinsing the column with 3 l of distilled water, the glycosides were eluted with 1.5 l of methanol. The combined methanol eluates were concentrated to dryness under reduced pressure, extracted with diethyl ether to eliminate remaining volatiles and lyophilized to afford 25.2 g of crude glycosides. Portions of ~1.8 g of the glycosidic extract were subjected to MLCCC for separation of glycosides. Sixty-six fractions (each 5 ml) were collected. Each fraction was concentrated to dryness, lyophilized, and an aliquot was enzymatically hydrolyzed (Rohapect D5L). The released volatiles (aglycons) were analyzed by HRGC–MS. Combined MLCCC fractions (35–46, 580 mg) (containing hydroxyester precursors, according to the results of enzymatic hydrolysis) were acetylated overnight with 5 ml of Ac_2O-pyridine. The peracetylated glycosides thus obtained were subjected to flash chromatography (Still et al., 1978), using a silica gel column and the following 200 ml pentane–Et_2O discontinuous gradients (8:2, 6:4, 1:1, 4:6, 2:8). One hundred fractions (each ~10 ml) were collected. Combined fractions 72–85 (227 mg) were concentrated in vacuum to dryness and subjected for subsequent purification to preparative HPLC using first an Eurospher Si 100-5 column (5 μm, 250×16 mm) with TBME as mobile phase at a flow rate of 3 ml/min, followed by chromatography on a Eurospher 100 C18 column (5 μm, 250×16 mm) with MeOH–H_2O (7:3) at a flow rate of 5 ml/min. The fraction obtained from the latter column in a range of 50–55 min was finally purified on a LichroCart RP-18 column (5 μm, 250 mm×4 mm) by a gradient solvent system MeOH–H_2O (80:20 to 90:10) during 20 min at a flow rate of 0.5 ml/min to yield pure compound **1a** (2.5 mg).

The fraction eluted from the above mentioned Eurospher 100 C18 preparative column in a range of 20–25 min was finally purified by HPLC using this time an analytical column Eurospher Si 100-5 (5 μm, 250×4 mm) with hexane–TBME (1:1) at a flow rate of 2 ml/min as well as a chiral column LichroCart (R,R)-Whelk-01 (5 μm, 250×4 mm) with hexane–*iso*-PrOH (7:3) as mobile phase at 1 ml/min. Crystallization in hexane–*iso*-PrOH (7:3) of the two peaks which eluted from the chiral column yielded pure **2a** (6 mg) and **3a** (9 mg).

3.4. Enzymatic hydrolysis

Three mg of each MLCCC fractions were dissolved in 25 ml of 0.2 M citrate–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 as internal standard (105 μg). Then, the mixture was incubated at 37 °C over 36 h and the liberated aglycones were extracted with diethyl ether. The organic phase was dried over anhydrous sodium sulfate, concentrated (Vigreux column, 38 °C) to 0.2 ml, and subjected to HRGC and HRGC–MS analyses. For enzymatic hydrolyses of pure compounds, 1 mg of each compound was used.

3.5. Data for compounds *1a–3a, 6 and 7*

Heptaacetate **1a**. Colorless gum, $[\alpha]_D^{22}$ −4.6° (MeOH, *c* 0.0867). For ^{1}H NMR and ^{13}C NMR spectra (see Tables 1 and 2). HR–FAB–MS: *m/z* 829.3077 $[M+Na]^+$ ($C_{36}H_{56}O_{20}Na$ requires 829.3105). ESI–MS/MS *m/z* (rel. int.): 845 $[M+K]^+$ (19), 829 $[M+Na]^+$ (100), 528 [hexose(Ac)$_4$ hexose(Ac)$_3$ + Na—AcO–AcOCH$_2$ + H]$^+$ (5). Daughter ion spectrum of *m/z* 829: 769 $[M+Na–AcOH]^+$ (100), 709 $[769\text{-}AcOH]^+$ (38), 667 $[709\text{-}CH_2CO]^+$ (11), 607 $[667\text{-}AcOH]^+$ (9), 547 $[607\text{-}AcOH]^+$ (4). Daughter ion spectrum of *m/z* 769: 769 $[M+Na–AcOH]^+$ (100), 709 $[769\text{-}AcOH]^+$ (98), 649 $[709\text{-}AcOH]^+$ (9), 607 $[709\text{-}AcOH–CH_2=C=O]^+$, 547 $[607\text{-}AcOH]^+$ (7).

Hexaacetate **2a**. White needles, mp 104–106 °C. $[\alpha]_D^{22}$ −2.1° (MeOH, *c* 0.2867). For ^{1}H NMR and ^{13}C NMR spectra (see Tables 1 and 2). HR–FABMS: *m/z* 729.2572 $[M+Na]^+$ ($C_{31}H_{46}O_{18}Na$ requires 729.2581). ESI–MS/MS *m/z* (rel. int.): 766 $[M+AcOH]^+$ (15), 745

$[M+K]^+$ (17), 729 $[M+Na]^+$ (100). Daughter ion spectrum of *m/z* 729: 669 $[M+Na–AcOH]^+$ (100), 609 [669-AcOH]$^+$ (27), 507 [609-AcOH–CH_2CO]$^+$ (4). Daughter ion spectrum of *m/z* 669: 669 $[M+Na–AcOH]^+$ (1), 609 [669-AcOH]$^+$ (100), 507 $[M+Na\text{-}3AcOH–CH_2CO]^+$ (7). Daughter ion spectrum of *m/z* 609: 609 $[M+Na–2AcOH]^+$ (100), 551 [609-C_4H_9–H]$^+$ (49), 507 [609-AcOH–CH_2CO]$^+$ (61), 407 [pentose$(Ac)_3$hexose$(Ac)_3$ + Na-2AcOH–CH_2CO–H_2O]$^+$ (27), 347 [407-AcOH]$^+$ (20), 293 [hexose$(Ac)_3$ + Na–$2H_2O$]$^+$ (10), 239 [pentose$(Ac)_3$ + Na–AcOH]$^+$ (21), 142 [aglycon–H_2O]$^+$ (2).

Hexaacetate **3a**. White crystals, mp 101–103 °C. $[\alpha]_D^{20}$ +1.8° (MeOH, *c* 0.2267). For ^{1}H NMR and ^{13}C NMR spectra (see Tables 1 and 2). HR–FABMS: *m/z* 729.2604 $[M+Na]^+$ ($C_{31}H_{46}O_{18}Na$ requires 729.2581). ESI–MS/MS *m/z* (rel. int.): 766 $[M+AcOH]^+$ (15), 745 $[M+K]^+$ (17), 729 $[M+Na]^+$ (100). Daughter ion spectrum of *m/z* 729: 669 $[M+Na–AcOH]^+$ (100), 609 [669-AcOH]$^+$ (27), 507 [609-AcOH–CH_2CO]$^+$ (4). Daughter ion spectrum of *m/z* 669: 669 $[M+Na–AcOH]^+$ (1), 609 [669-AcOH]$^+$ (100), 507 $[M+Na\text{-}3AcOH–CH_2CO]^+$ (7). Daughter ion spectrum of *m/z* 609: 609 $[M+Na–2AcOH]^+$ (100), 551 [609-C_4H_9–H]$^+$ (49), 507 [609-AcOH–CH_2CO]$^+$ (61), 407 [pentose$(Ac)_3$hexose$(Ac)_3$ + Na-2AcOH–CH_2CO–H_2O]$^+$ (27), 347 [407-AcOH]$^+$ (20), 293 [hexose$(Ac)_3$ + Na–$2H_2O$]$^+$ (10), 239 [pentose $(Ac)_3$ + Na–AcOH]$^+$ (21), 142 [aglycon–H_2O]$^+$ (2).

Ethyl 3-hydroxyoctanoate **6**. $C_{10}H_{20}O_3$, M_r 188. R_I (DB-Wax) 1880. EIMS *m/z* (rel. int.): 43 (73), 55 (31), 60 (17), 71 (44), 83 (13), 88 (31), 89 (28), 117 (100), 125 (11), 170 (1), 187 (<1).

Butyl 3-hydroxybutanoate **7**. $C_8H_{16}O_3$, M_r 160. R_I (DB-Wax) 1684. EIMS *m/z* (rel. int.): 41 (59), 43 (100), 45 (73), 56 (49), 57 (33), 60 (28), 71 (10), 87 (88), 89 (38), 105 (6), 145 (10), 159 (<1).

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