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ALIPHATIC β-D-GLUCOSIDES FROM FRUITS OF *CARICA PUBESCENS*

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Key Word Index—*Carica pubescens*; Caricaceae; mountain papaya fruit; glucosides; ethyl and butyl 3-hydroxybutanoate; 1-hydroxyoctan-3-one; bound octane-1,3-diol; enantiodifferentiation.

Abstract—Ethyl 3-*O*-β-D-glucopyranosylbutanoate, butyl 3-*O*-β-D-glucopyranosylbutanoate and 3-oxo-octyl 1-*O*-β-D-glucopyranoside were isolated from *Carica pubescens* fruit pulp by liquid chromatography on XAD. Identifications were performed after peracetylation by comparison of HRGC and HRGC-mass spectral data with those of synthesized reference compounds. Chiral evaluation of glycosidically-bound 3-hydroxybutanoates and octane-1,3-diol was achieved by multidimensional gas chromatography, combining a polar achiral column (DB-Wax) with a chiral main column (heptakis-2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin/OV 1701 and 2,3-di-*O*-acetyl-6-*O*-tert-butyl-dimethylsilyl-β-cyclodextrin/OV 1701, respectively). Comparison of retention times of synthesized, optically-enriched reference compounds with enzymically-released aglycones revealed enantiomeric excesses of the (*S*)-3-hydroxybutanoates, i.e. 96% and 24% for the ethyl and the butyl ester, respectively. Octane-1,3-diol exhibited an enantiomeric excess of (*R*)-90%.

INTRODUCTION

Previous studies on the volatile compounds of mountain papaya (*Carica pubescens*) fruit have characterized a considerable number of them as derivatives of fatty acid metabolism [1, 2]. Among them, were found a homologous series of 3-hydroxyesters, constituents which are also common in other tropical fruits, such as pineapple [3], mango [4–6], cape gooseberry [7], tamarillo [8] and *Spondias* spp. [9, 10]. In the course of our studies on glycosidically-bound aroma precursors [11, 12], we describe herein for the first time, the identification of glucosides of ethyl and butyl 3-hydroxybutanoates, as well as the newly characterized natural compound, 1-hydroxyoctan-3-one. Furthermore, the chirality of the glycosidically-bound 3-hydroxybutanoates and octane-1,3-diol is evaluated.

RESULTS AND DISCUSSION

After enzymic hydrolysis of a glycosidic fraction from *C. pubescens* fruit pulp, obtained by liquid chromatography on XAD, HRGC and HRGC-mass spectrometric analyses revealed the occurrence of benzyl alcohol, aliphatic alcohols, such as butan-1-ol and hexan-1-ol, 3-hydroxyesters, such as ethyl 3-hydroxybutanoate (**1b**) and butyl 3-hydroxybutanoate (**2b**), as well as a new natural compound, 1-hydroxyoctan-3-one (**3b**), as main constituents. The structure of **3b** was elucidated by means of EI and CI mass spectrometry, and HRGC-FTIR data, and confirmed by synthesis. Ketone **3b** seems to be biogenetically related to the minor aglycone, octane-1,3-diol (**4**), which has been described only in apple fruits to date [13–15].

Preparative separation by multilayer coil countercurrent chromatography (MLCCC) of the glycosidic isolate into six pooled fractions led to two fractions, in which **1** (Fr. 4) and **2**, together with **3** (Fr. 5), were detected by on-line coupled HPLC-atmospheric pressure CI tandem mass spectrometry (HPLC-APCIMS/MS). The presence of peaks at *m/z* 312 $[M+NH_4]^+$, 295 $[M+H]^+$ and 133 [aglycone+H]$^+$ for **1**, 340 $[M+NH_4]^+$, 323 $[M+H]^+$ and 161 [aglycone+H]$^+$ for **2**, 324 $[M+NH_4]^+$, 307 $[M+H]^+$ and 145 [aglycone+H]$^+$ for **3**, respectively, demonstrated that the aliphatic aglycones **1b**, **2b** and **3b** were conjugated to a hexose. Identifications of **1a**, **2a** and **3a** were performed after peracetylation of the two fractions by comparison of HRGC and HRGC-EI mass spectral data with those of synthesized ref-

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Table 1. ^{1}H NMR spectral data of compounds **1a**, **2b**, **3a**, **3b** ($CDCl_3$, 400 MHz) and **2a** ($CDCl_3$, 600 MHz)

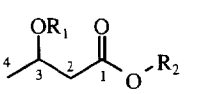
H	1a	2a*†	2b	3a*	3b
1a/b				3.82 *ddd* (9.7/8.3/5.5) 4.01 *dt* (9.9/5.5)	3.82 *m*
2a/b	2.35 *dd* (16.04/4.5) 2.53 *dd* (16.2/8.4) 2.40 *dd* (15.2/7.3) 2.73 *dd* (15.8/5.9)	(*S*) 2.35 *dd* (16.0/4.5) (*S*) 2.54 *dd* (16.0/8/5) (*R*) 2.40 *dd* (15.7/7.3) (*R*) 2.76 *dd* (15.7/5.8)	2.38 *dd* (16.6/8.5) 2.45 *dd* (16.2/3.7)	2.55 *dt* (16.9/5.2) 2.74 *ddd* (17.1/8.2/5.6)	2.64 *t* (5.0)†
3	4.13 *m*	4.22 *m*	4.16 *m*		
4	120 *m*	(*S*) 1.28 *d* (6.3) (*R*) 1.20 *d* (6.3)	1.19 *d* (6.3)	2.37 *dd* (8.3/6.9)	2.41 *t* (7.5)
5				1.52 *quin* (7.4)	1.56 *quin* (7.4)
6/7				1.25 *m*	1.25 *m*
8				0.86 *t* (7.0)	0.86 *t* (7.0)
1′	4.15 *m*	4.08 *m*	4.08 *t* (6.7)		
2′	1.24 *m*	(*S*) 1.60 *quin* (6.8) (*R*) 1.59 *quin* (6.7)	1.58 *m*		
3′		(*S*) 1.35 *sex* (7.4) (*R*) 1.36 *sex* (7.4)	1.35 *sex* (7.4)		
4′		(*S*) 0.93 *t* (7.2) (*R*) 0.92 *t* (7.2)	0.92 *t* (7.0)		
			(OH) 2.96 *br s*		(OH) 2.64‡
G-1	4.62 *d* (8.1) 4.59 *d* (8.1)	(*S*) 4.62 *d* (8.1) (*R*) 4.58 *d* (8.1)		4.49 *d* (8.1)	
G-2	4.92 *dd* (9.6/8.1)	4.92 *dd* (9.6/8.1)		4.92 *dd* (9.6/8.1)	
G-3	5.06 *t* (9.6)	5.06 *t* (9.6)		5.17 *t* (9.6)	
G-4	5.18 *t* (9.6)	5.18 *t* (9.6)		5.04 *t* (9.6)	
G-5	3.66 *m*	3.66 *ddd* (10.0/4.8/2.5)		3.67 *ddd* (9.6/4.7/2.2)	
G-6a	4.22 *m*	4.13 *dd* (12.2/2.3)		4.10 *dd* (12.3/2.4)	
G-6b		4.25 *dd* (12.2/4.9)		4.24 *dd* (12.3/4.8)	
MeCO	1.98–2.08 *s*	1.98–2.08 *s*		1.98–2.08 *s*	

* Assignments based on ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-COSY.
† Absolute configuration relates to C-3 of aglycone.
‡ Signals overlapped.

erence compounds. β-D-Glucosides of 3-hydroxyesters, such as ethyl and butyl 3-hydroxybutanoates but also of 1-hydroxyoctan-3-one have not been found in nature to date.

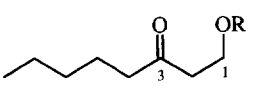
The synthesized reference compounds **1a**, **2a** and **3a** formed, on APCI mass spectrometry, the ammonium adducts $[M+NH_4]^+$ *m/z* 480, 508 and 492 as molecular ions. Collision-induced dissociation (CID) of the adduct ions resulted in product-ion spectra, which were dominated entirely by fragments of a peracetylated hexose (*m/z* 331 $[M-\text{aglycone}+H]^+$, 271 $[331-HAc]^+$, 228 $[271-CH_2{=}C{=}O]^+$, 211 $[271-HAc]^+$, 169 $[211-CH_2{=}C{=}O]^+$, and 109 $[169-Hac]^+$) [11, 12]. ^{1}H and ^{13}C NMR spectra of **1a** and **2a** gave rise to the signals for two diastereomeric glycosides. For **2a**, the assignments related to the absolute configuration at chiral C-3 of the aglycone were possible, since the glucoside synthesis was carried out using butyl 3-hydroxyhexanoate (**2b**) with a defined ratio of (*S*)- and (*R*)-enantiomers of 65:35 (Tables 1 and 2). For reference compound **3a**, we observed complex signal patterns of the prochiral C-1 and C-2 protons next to the anomeric carbon.

To evaluate the chiral distribution of glucosidically-bound ethyl (**1b**) and butyl 3-hydroxybutanoates (**2b**), as well as octane-1,3-diol (**4**), MDGC with modified cyclodextrin (CD) phases was used (see Experimental). For the 3-hydroxybutanoates, the (3*S*)-enantiomers predominated. Ethyl 3-hydroxybutanoate (**1b**) and butyl 3-hydroxybutanoate (**2b**) were detected with enantiomeric excesses (ees) of 96 and 24%, respectively. Analysis by multidimensional gas chromatography (MDGC) of enzymically released octane-1,3-diol (**4**) revealed the occurrence of almost enantiomerically pure (*R*)-4 (ee = 90%), as



	R_1	R_2
1	Glc	C_2H_5
1a	$Glc(Ac)_4$	C_2H_5
1b	H	C_2H_5
2	Glc	C_4H_9
2a	$Glc(Ac)_4$	C_4H_9
2b	H	C_4H_9

Glc: β-D-glucose



3 R = Glc
3a R = $Glc(Ac)_4$
3b R = H

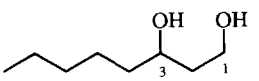


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Table 2. ^{13}C NMR spectral data of compounds **1a**, **2b**, **3a**, **3b** ($CDCl_3$, 100 MHz) and **2a** ($CDCl_3$, 150 MHz)

C	1a	2a*	2b	3a†	3b
1	170.2‡	170.1‡	172.8	65.1	57.8
2	42.1/42.3	(*S*) 42.1/(*R*) 42.4	42.8	42.3	44.4
3	73.3/74.2	(*R*) 73.4/(*S*) 74.4	64.2‡	208.5	211.7
4	20.2/21.6	(*R*)20.2/(*S*)21.6	22.4	43.5	43.3
5				23.2	23.3
6				31.3	31.3
7				22.3	22.3
8				13.7	13.8
1′	60.4	64.4	64.5‡		
2′	14.2	30.6	30.6		
3′		19.1	19.0		
4′		13.7	13.6		
G-1	99.9/101.1	(*R*)99.9/(*S*)101.1		101.1	
G-2	71.5/71.6	(*S*)71.3/(*R*)71.4		71.3	
G-3	73.0	72.8		72.8	
G-4	68.7/68.8	(*R*)68.4/(*S*)68.6		68.6	
G-5	71.8	71.6		71.9	
G-6	62.1/62.3	(*R*)61.9/(*S*)62.1		62.0	
$\underline{C}H_3CO$	20.5/20.6	20.2/20.6		20.5/20.6	
Me$\underline{C}$O	169.1–170.9‡	169.1–170.5‡		169.2–170.5	

Absolute configuration relates to C-3 of aglycone.
* Assignments based on ^{1}H-^{13}C-COSY (600 MHz ^{1}H NMR).
† Assignments based on ^{13}C–^{1}H-COSY (6.25 MHz ^{13}C NMR).
‡ Assignments may need to be reversed.

already described in apple fruits (ee% > (*R*)-99) [14, 15]. In previous studies of the composition of 3-hydroxyesters in tropical fruits, the prevalence of one of the two isomers has been observed [3, 8, 9, 20], possibly resulting from different expression of anabolic and catabolic fatty acid metabolism.

EXPERIMENTAL

General. MLCCC was performed in the tail-head mode ($CHCl_3$–MeOH–H_2O, 7:13:8). EIMS and CIMS was determined at 70 eV by HRCG-MS, scanning from *m/z* 41 to 350 with total ion current monitoring. HRGC, HRGC-MS and HRGC-FTIR were carried out using a fused-silica WCOT column (30 m × 0.25 mm i.d., df = 0.25 μm) coated with DB-Wax. The column was programmed at 50° for 3 min, then to 240° at 4° min^{-1}. Peracetylated derivatives were sepd on a fused-silica WCOT column (30 m × 0.25 mm i.d., df = 0.25 μm) coated with DB-5. The column was prog. from 60° to 300° at 5° min^{-1}. FID temp. 300°; carrier gas He 3 ml min^{-1}. Split injection (1:20) was used (1 μl). Linear R_i, MS and FTIR data were compared with those of synthesized ref. compounds. MDGC analyses were carried out with a double-oven gas chromatograph fitted with a split injector (1:20) at 250° and two FIDs at 250°. A J&W DB-Wax-fused-silica capillary column (30 m × 0.25 mm i.d., df = 0.25 μm) was used for the preseparation of volatiles. Sepn of enantiomers of **1b** and **2b** was achieved in the second oven using a fused-silica capillary column coated with *heptakis*-2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin/OV 1701 (30 m × 0.25 mm i.d., df = 0.25 μm) [8]. The columns were connected by a Live-T-interface; cuts of 0.3 s were carried out. Ethyl-3-hydroxybutanoate (**1b**) and butyl-3-hydroxybutanoate (**2b**) were sepd in the same run with Oven 1, 80° to 200° at 10° min^{-1}, Oven 2 60° for 25 min, then to 200° at 1° min^{-1}. Enantiomeric sepn of octane-1,3-diol (**4**) was performed in Oven 2 using a fused-silica capillary column coated with 2,3-di-*O*-acetyl-6-*O*-tert-butyldimethylsilyl-β-cyclodextrin/OV 1701 (25 m × 0.25 mm i.d., df = 0.15 μm), Oven 1 60° to 240° at 10° min^{-1}, Oven 2 80° for 20 min, then to 200° at 2° min^{-1} [15]. Evaluation of the elution order of enantiomers was achieved using ref. compounds with known enantiomeric ratios and determined to be (*S*) before (*R*). HPLC-APCIMS/MS: for **1**, **2** and **3** HPLC on an Eurospher 100 C-18 column (Knauer; 5 μm; 100 × 2 mm) with a linear 5 mM NH_4Ac–MeCN gradient (0–100% MeCN in 20 min) was used; for **1a**, **2a** and **3a** loop injection (2 μl) with 5 mM NH_4AC–MeCN (1:1), flow rate 200 μl min^{-1}, was carried out. Corona current was set to 5 μA (4–4.6 kV), temp. of heated inlet capillary to 160°, vaporizer to 300°. N_2 served both as sheath (50 psi) and auxiliary gas (10 ml min^{-1}). Positive ions were detected scanning from 50 to 550 mu with a scan duration of 1 s and a dwell time of 2 ms. MS/MS expts were performed at a collision pressure of 1.8 mTorr Ar and collision offset C_{off} from −10 to −15 eV. Electron-multiplier voltage was set to 1200 V in scan mode and 1800 V for MS/MS expts. ^{1}H/^{13}C NMR spectra were recorded in $CDCl_3$ at 250/62.5 MHz, 400/100 Mhz or 600/150 MHz, respectively.

Plant material. Mountain papaya (*Carica pubescens* Lenne et Koch, syn. *C. candamarcensis*) fruits were obtained from a local market in Bogotá, Colombia, in 1994.

Extraction and isolation of compounds **1a**, **2a** *and* **3a**. Fresh mountain papaya (2 kg) was deseeded and homogenized with 1 l of 0.2 M Pi buffer pH 7 containing 0.2 M glucono-δ-lactone. After centrifugation (30 min, 3000 *g*), the supernatant was subjected to LC on Amberlite-XAD. After washing the column with 5 l distilled H_2O, glycosides were eluted with 1 l MeOH. The eluate was concd *in vacuo*, extracted with Et_2O to remove volatiles and fractionated by MLCCC. MLCCC frs were pooled after enzymic hydrolysis. Portions (5%) of frs 4 and 5 were acetylated using Ac_2O–pyridine at room temp. for 24 hr. The reaction was stopped by addition of MeOH and the supernatant extracted with Et_2O. The peracetylated compounds were analysed by HRGC and HRGC-MS.

Localization of **1**, **2** *and* **3** *during isolation steps.* (i) Enzymic hydrolysis of the MeOH isolate was carried out at 37° for 12 hr using a β-glucosidase (emulsin, Serva). Liberated aglycones were extracted with Et_2O and analysed by HRGC-MS. (ii) Pooled MLCCC frs 4 and 5 were submitted to HPLC-APCI-MS/MS analyses. MLCCC fr 4 *m/z*: (**1**) 312 $[M+NH_4]^+$, 295 $[M+H]^+$, 133 [aglycone + H]$^+$. MLCCC fr 5 *m/z*: (**2**) 340 $[M+NH_4]^+$, 323 $[M+H]^+$, 161 [aglycone + H]$^+$, (**3**) 324 $[M+NH_4]^+$, 307 $[M+H]^+$, 145 [aglycone + H]$^+$.

Preparation of reference compounds. (i) *Butyl-3-hydroxybutanoate* (**2b**) [16]. A suspension of 2 g ethyl-3-hydroxybutanoate (0.015 mol), 2.6 g *n*-BuOH (0.05 mol) and 20 g porcine pancreatic extract (EC 3.1.1.3, Sigma) in 200 ml hexane was stirred for 3 days at room temp. The reaction mixt. was filtered through Celite (acid-washed, Sigma) and concd. The residue was chromatographed on silica gel using a pentane–Et_2O gradient to yield butyl 3-hydroxybutanoate **2b** (395 mg, 2.46 mmol, 17%). RI (DB-Wax) 1678. EIMS *m/z* (rel. int.): 43 (100), 45 (70), 56 (48), 57 (36), 60 (23), 71 (7), 87 (50), 89 (17), 105 (3), 145 (4). MDGC (ee%): (*R*)-30. NMR: Tables 1 and 2.

(ii) *Butyl* (S)-3-*hydroxybutanoate*. Synthesized analogously from ethyl (*S*)-3-hydroxybutanoate.

1-*Hydroxyoctan*-3-*one* (**3b**) [17]. (i) 1-*Acetoxyoctan*-3-*one*. A suspension of BF_3–Et_2O (0.24 ml), MeOH (0.24 ml), 0.12 mg of HgO (0.05 mmol), some crystals of TCA and 2 g HoAc was kept at 50–55°. After 10 min, 4 g of 2-octin-1-ol (31.7 mmol) in 2 g of HoAc were added dropwise and the mixt. stirred for 2 hr. The suspension was neutralized with Na_2CO_3 and extracted × 2 with 50 ml Et_2O. After drying (Na_2SO_4) and concentrating, the yield of 1-acetoxyoctan-3-one was calculated from HRGC and HRGC-MS analysis to be *ca* 70%. RI (DB-Wax) 1913. EIMS *m/z* (rel. int.): 43 (100), 55 (41), 70 (39), 71 (16), 99 (13), 115 (2), 130 (7), 144 (1), 187 (1).

(ii) 1-*Hydroxyoctan*-3-*one* (**3b**). The residue was taken up in 50 ml 1M NaOH and 20 ml THF and refluxed for 2 hr. The reaction mixt. was neutralized, extracted with Et_2O and dried (Na_2SO_4). Subsequently, the concd mixt. was purified by LC on silica gel using Et_2O to afford 1-hydroxyoctan-3-one (**3b**, 411 mg, 9%). RI (DB-Wax) 1897. EIMS *m/z* (rel. int.): 41 (20), 43 (100), 45 (17), 55 (16), 58 (10), 70 (15), 71 (17), 73 (27), 83 (1), 88 (20), 99 (15), 101 (3), 144 (1). CIMS *m/z* (rel. int.): (NH_3) 179 $[M+NH_4+NH_3]^+$ (100), 162 $[M+NH_4]^+$ (30); (*i*-butane) 145 $[M+H]^+$ (100), 127 $[M+H-H_2O]^+$. HRGC-FTIR cm^{-1}. 3610, 2966, 2943, 1724, 1466, 1362, 1072. NMR: Tables 1 and 2.

Octane-1,3-*diol* (**4**) [18]. Baker's yeast (0.5 g) was stirred in a soln containing 1 g sucrose and 20 ml H_2O at 30°. After 1 day, 10 mg **3b** was introduced. After 2 days at 30°, the fermentation mixt. was filtered through celite (acid-washed, Sigma) and the aq. phase extracted × 2 with 30 ml Et_2O. The combined organic layers were dried (Na_2SO_4), concd and analysed. MDGC (ee%): (*R*)-16. Yield was calculated to be *ca* 5% from HRGC. MS and linear R_i were identical with published data [15].

Synthesis of β-D-*glucosides.* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucosides.* Glucosides (**1a**–**3a**) were synthesized under modified Koenigs-Knorr conditions [19]. To 3 mmol of the corresponding alcohol in 10 ml anhydrous CH_2Cl_2, 500 mg Drierite and 1 mmol Ag_2O were added and the mixt. stirred in the dark at room temp. for 30 min. The corresponding 1 mmol α-D-acetobromoglucose in 10 ml anhydrous CH_2Cl_2 was added dropwise. After stirring the mixt. in the dark at room temp. for 3 days, it was filtered through celite (acid-washed, Sigma), concd and purified by LC on silica gel using pentane–EtOAc (2:1). Yield of purified compound was 18, 6, and 11% for **1a**, **2a** and **3a**, respectively.

Ethyl-3-O-(*tetra*-O-*acetyl*-β-D-*glucopyransosyl*) *butanoate* (**1a**). $C_{20}H_{30}O_{12}$, M_r 462. RI (DB-5) 2397. EIMS *m/z* (rel. int.): 43 (100), 45 (7), 69 (6), 73 (11), 81 (8), 87 (3), 98 (6), 109 (3), 115 (17), 127 (1), 140 (2), 145 (3), 157 (39), 161 (2), 169 (3), 200 (1): HPLC-APCIMS/MS *m/z*: 480 $[M+NH_4]^+$, 331 $[M-\text{aglycone}+H]^+$, 271 $[331-HAc]^+$, 228 $[271-CH_2{=}C{=}O]^+$, 211 $[271-HAc]^+$, 169 $[211-CH_2{=}C{=}O]^+$, 109 $[169-HAc]^+$. NMR: Tables 1 and 2.

Butyl 3-O-(*tetra*-O-*acetyl*-β-D-*glucopyranosyl*) *butanoate* (**2a**). $C_{22}H_{34}O_{12}$, M_r 490. RI (DB-5) 2531. EIMS *m/z* (rel. int.): 41 (11), 43 (100), 44 (16), 45 (9), 57 (8), 69 (7), 73 (2), 81 (7), 87 (14), 98 (6), 109 (3), 112 (4), 115 (5), 127 (2), 140 (3), 143 (5), 157 (3), 169 (3), 189 (2), 200 (1). HPLC-APCIMS/MS *m/z*: 508 $[M+NH_4]^+$, 331, 271, 211, 169. NMR: Tables 1 and 2.

3-*Oxo-octyl-tetra*-O-*acetyl*-β-D-*glucopyranoside* (**3a**). $C_{22}H_{34}O_{11}$, M_r 474. RI (DB-5) 2610. EIMS *m/z* (rel. int.): 43 (100), 55 (7), 57 (2), 69 (4), 70 (5), 71 (4), 81 (6), 97 (3), 98 (4), 99 (4), 109 (3), 115 (4), 127 (5), 145 (2), 157 (2), 169 (3), 200 (1). HPLC-APCIMS/MS

m/z: 492 $[M+NH_4]^+$, 331, 271, 211, 169. NMR: Tables 1 and 2.

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