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4-OXO-β-IONOL AND LINALOOL GLYCOSIDES FROM RASPBERRY FRUITS

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Key Word Index—*Rubus idaeus*; Rosaceae; raspberry fruit; 4-oxo-β-ionol β-D-glucopyranoside; 3-[3′-(β-D-glucopyranosyloxy)-1′-butenyl]-2,4,4-trimethyl-2-cyclohexen-1-one; linalool disaccharide glycoside; 1-ethenyl-1,5-dimethyl-4-hexenyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside.

Abstract—From a methanolic extract of raspberry fruits, the β-D-glucopyranoside of 3-(3′-hydroxy-1′-butenyl)-2,4,4-trimethyl-2-cyclohexen-1-one (4-oxo-β-ionol) as well as the α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside of (*S*)-(+)-1-ethenyl-1,5-dimethyl-4-hexene [(*S*)-(+)-linalool] were isolated by adsorption chromatography on XAD-2 resin followed by liquid chromatography on Sephadex LH-20, RP-18 and silica gel as well as by HPLC on diol, RP-18 and RP-select 8 phases, respectively. Identifications were based on results of ^{1}H and ^{13}C NMR spectroscopy and DCI-mass spectral analysis. Absolute configuration of bound linalool was determined by direct chiral analysis using MDGC-mass spectrometry after enzymatic hydrolysis of the glycoside.

INTRODUCTION

Enzymatic hydrolysis studies on raspberry fruit pulp have demonstrated the abundant occurrence of raspberry aroma compounds as glycosylated conjugates [1]. Subsequent work on these flavour 'precursors' led to the isolation of β-D-glucosides of 4-(4′-hydroxyphenyl)-butan-2-one ('raspberry ketone'), 4-(3′,4′-dihydroxyphenyl)butan-2-one [2] and two diastereomeric 3-oxo-α-ionols [3] together with α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosides of α-ionol [4] and 4-hydroxy-β-ionone [5]. The α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside of linalool has also been identified [6]. This paper deals with the isolation and characterization of 4-oxo-β-ionol β-D-glucopyranoside and linalool α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside from raspberry fruits.

RESULTS AND DISCUSSION

A methanolic extract from raspberry fruits (*Rubus idaeus*, cv. Héritage) was subjected to XAD-2 adsorption chromatography. The methanol eluate was prefractionated by gel filtration on Sephadex LH-20. Further fractionation by reverse-phase (RP-18) medium pressure chromatography allowed the separation of the glycosides according to the nature of the aglycone [7]. 4-Oxo-β-ionol glycoside (**1**) eluted in 50% aqueous methanol, while the linalool disaccharide glycoside **3** was present in the 70% aqueous methanol fraction. Further fractionation of both compounds was carried out by flash CC on silica gel. Final purification of **1** was achieved by HPLC on diol and RP-18 phases, while glucoside **3** was purified by HPLC using a RP-select 8 phase.

Results of DCI-mass spectral analysis of compound **1** indicated that a 4-oxo-β-ionol monoglycoside was present. The ^{13}C NMR spectrum of **1** (Table 1) revealed six signals due to a glucose residue [2]. The chemical shift of the anomeric carbon of glucose at 102.5 ppm was consistent with a β-D-glucose linked to a secondary alcohol [8]. The β-configuration of the anomeric centre of glucose was confirmed by the coupling constants in the ^{1}H NMR spectra (Table 2) of both **1** and its acetylated derivative **1a** ($J = 7.7$ and 7.9 Hz, respectively). Therefore, compound **1** was identified as 4-oxo-β-ionol β-D-glucopyranoside.

The DCI-mass spectrum of compound **3** revealed a pseudomolecular ion at m/z 466 $[M+NH_4]^+$, thus indicating the presence of a linalool disaccharide glycoside. The fragmentation pattern (see Experimental) was found to be nearly identical to that of the previously reported linalool *O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**4**) [6], suggesting that **3** is structurally similar to glycoside **4**. However, ^{1}H NMR spectra of the acetylated derivatives **3a** and **4a** (Table 2) displayed significant differences. Evaluation of the ^{13}C NMR data of **3** demonstrated the presence of an α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside moiety [4]. The configurations of the anomeric centres were confirmed by the chemical shifts and the coupling constants of the anomeric protons in the acetylated compound **3a** [4]. In addition, the chemical shift of the anomeric proton at 99.5 ppm was in accordance with a β-D-glucose linked to a tertiary alcohol [8]. The absolute configuration of the glycosidically-bound linalool was established after enzymatic hydrolysis of **3** by direct chiral analysis of the liberated aglycone using MDGC-mass spectrometry [9]. Thus, compound **3** was deduced to be *S*-(+)-linalool *O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside.

4-Oxo-β-ionol is not a common naturally occurring volatile constituent. *Osmanthus* absolute [10] and quince

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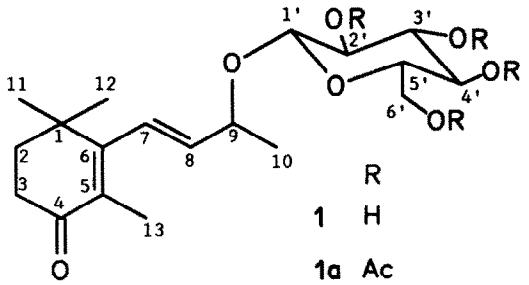

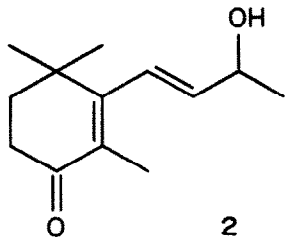

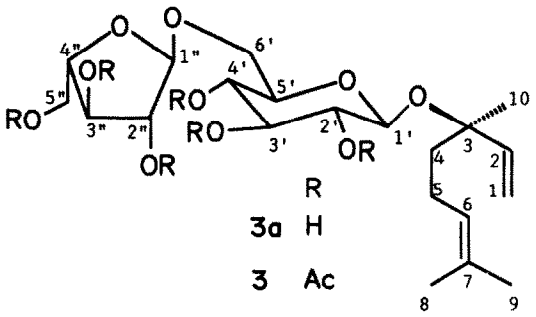

Table 1. ^{13}C NMR data for compounds **1–3**

C	**1** CD_3OD (100 MHz)	**2*** CD_3OD (50 MHz)	**3** CD_3OD (50 MHz)
Aglycone			
1	36.4	36.6	115.9
2	38.1	38.3	144.4
3	34.9	35.1	81.5
4	n.d.	201.6	42.7
5	130.5	130.6	23.7
6	163.5	163.8	125.7
7	127.3	125.8	132.1
8	140.2	142.4	25.8
9	77.0[a]	69.0	17.7
10	20.8	23.6	23.3
11 }	27.5	27.6	—
12 }		27.6	—
13	13.5	13.5	—
Glucose			
1	102.5		99.5
2	75.1		75.2
3	77.9[a]		78.2
4	71.3		72.1
5	77.8[a]		76.3
6	62.4		68.2
Arabinose			
1			110.0
2			83.2
3			79.0
4			86.0
5			63.1

*Assignments were based on results of a ^{13}C–^{1}H COSY experiment.

[a]Assignments may be reversed.

fruit [11] are the only known natural sources for this compound. However, its occurrence in conjugated form has been demonstrated in wine [12] and passion fruit [13] from the results of enzymatic hydrolysis studies. This is the first report on the isolation and structural elucidation of 4-oxo-β-ionol β-D-glucopyranoside. Compound **3** has previously been characterized in grapes and wines [14].

EXPERIMENTAL

General. TLC was carried out on silica gel F_{254} aluminium sheets. Flash CC was performed on silica gel 60 (0.032–0.063 mm) and MPLC on a LiChroprep RP-18 column (40–63 μm, 310 × 25 mm). For HPLC the following columns were used: LiChrosorb Diol (5 μm, 250 × 16 mm), Nucleosil 120 C18 (7 μm, 250 × 16 mm), LiChrosorb RP-select 8 (10 μm, 250 × 10 mm) and LiChrospher Si 60 (5 μm, 250 × 16 mm). HRGC and HRGC-MS were carried out using a CP-Wax-58-CB WCOT column (30 m × 0.32 mm i.d., df = 0.22 μm). MDGC-MS was performed using a DB-5/Lipodex C column coupled as recently described [9]. NMR spectra were measured at 400 (^{1}H) and 100 (^{13}C) MHz or at 200 (^{1}H) and 50 (^{13}C) MHz using TMS as ref. DCI-MS spectra of compounds **1** and **3** were recorded at 70 eV with NH_3 as reagent gas, scanning from *m/z* 100 to 600 with source pres. 0.4 mbar, source temp. 90°.

Extraction and isolation of compounds. Plant material and part of the isolation procedure has been described previously [2]. The 50% aq. MeOH eluate of the RP-18 MPLC prep. column containing **1** was further fractionated by flash CC on silica gel ($CHCl_3$–MeOH–H_2O, 80:20:1). Final purification of glycoside **1** was achieved by prep. HPLC on a diol column using *n*-hexane–*n*-BuOH–MeOH–H_2O (65:25:9:1) as mobile phase (flow rate 8 ml min^{-1}, UV detection 270 nm) and subsequent prep. HPLC on RP-18 by eluting with MeCN–H_2O (13:17, flow rate 5 ml min^{-1}, UV detection 270 nm). The linalool glycoside **3** eluted in 70% aq. MeOH from the RP-18 MPLC prep. column. This fr. was submitted to flash CC ($CHCl_3$–MeOH–H_2O,

Table 2. ^{1}H NMR data for compounds **1**, **1a**, **2**, **3**, **3a** and **4a**

H	**1** CD_3OD (400 MHz)	**1a** C_6D_6 (400 MHz)	**2** CD_3OD (200 MHz)	**3*** CD_3OD (200 MHz)	**3a*** C_6D_6 (400 MHz)	**4a*** C_6D_6 (200 MHz)
Aglycone						
1	—	—	—	5.20 *d* (10.8) 5.22 *d* (17.9)	*ca* 5.1–5.2 *m*	5.11 *dd* (10.9; 1.1) 5.14 *dd* (17.8; 1.2)
2	1.85 *t* (7.0)	1.42 *t* (7.0)	1.85 *t*† (7.1)	5.91 *dd* (17.8; 10.9)	5.77 *dd* (17.6; 10.8)	5.77 *dd* (17.7; 10.9)
3	2.48 *t* (7.0)	2.33 *t* (7.0)	2.48 *t*† (7.0)	—	—	—
5	—	—	—	2.0–2.1 *m*	2.0–2.2 *m*	2.1–2.2 *m*
6	—	—	—	5.08 *t* (7.0)	5.0–5.2 *m*	5.0–5.2 *m*
7	6.31 *d* (16.3)	6.07 *d* (16.0)	6.26 *d* (16.2)	—	—	—
8	5.77 *dd* (16.3; 6.3)	5.48 *dd* (16.1; 5.6)	5.70 *dd* (16.2; 5.6)	1.65 *s*	1.65 *s*	1.65 *s*
9	4.52 *m*	4.0–4.1 *m*	4.38 *m*	1.58 *s*	1.59 *s*	1.59 *s*
10	1.35 *d* (6.4)	1.08 *d* (6.4)	1.29 *d* (6.5)	1.36 *s*	1.34 *s*	1.39 *s*
11, 12	1.17 *s* 1.18 *s*	0.86 *s* 0.87 *s*	1.17 *s* (6H)	—	—	—
13	1.78 *s*	1.99 *s*	1.78 *s*	—	—	—
Glucose						
1	4.39 *d* (7.7)	4.32 *d* (7.9)		4.34 *d* (7.6)	4.53 *d* (7.8)	4.52 *d* (7.6)
2	*ca* 3.30–3.60	5.43 *t* (9.6)			5.31 *t* (9.6)	5.34 *t* (7.7)
3		5.29 *t*[a] (8.8)			5.39 *t* (9.7)	5.39 *t* (8.7)
4		5.26 *t*[a] (9.5)			5.16 *t* (10.0)	5.09 *dd* (10.0; 8.9)
5	*m*	3.25 *m*		*ca* 3.10–4.00	3.25–3.29 *m*	3.37 *ddd* (10.6; 7.0; 1.9)
6	3.66 *dd* (11.7; 5.0) 3.81 *dd* (11.7; 2.2)	4.0–4.1 *m*			3.73 *m* 3.44 *m*	3.46 *dd* (10.6; 7.0) 3.84 *dd* (10.6; 1.9)
Arabinose						
1	—	—			5.12 *s*	4.28 *d* (6.7)
2	—	—		*m*	5.42 *d* (1.3)	5.55 *dd* (9.1; 6.7)
3	—	—			5.21 *d* (5.0)	5.16 *dd* (9.1; 3.5)
4	—	—			4.43 *m*	5.23 *td* (3.5; 1.9)
5	—	—			4.29 *dd* (11.6; 5.5) 4.52 *dd* (11.8; 3.9)	2.93 *dd* (12.8; 1.8) 3.67 *dd* (12.8; 3.5)
Acetyls		1.67–1.74			1.58–1.74	1.58–1.81

*Assignments based on results of a ^{1}H–^{1}H COSY experiment.

†Assignments based on results of a NOE experiment (irridiation of Me-11,12, at 1.17 ppm).

[a]Assignments may be reversed.

85:15:1). Subsequent semi-prep. HPLC on a RP-select 8 phase ($MeCN-H_2O$, 31:69, 4 ml min^{-1}, UV detection 200 nm) yielded pure compound **3**.

Localization of glycosides in frs resulting from chromatographic sepns was performed by hydrolysis of an aliquot in 0.2 M citrate-Pi buffer pH 5 using Rohapec D5L pectinase at 35° overnight. The liberated aglycones were extracted with Et_2O and analysed by HRGC-MS.

Acetylation of **1** *and* **3**. Compounds **1** and **3** were acetylated using Ac_2O–pyridine at room temp. for 2 days in the dark. Purification of **1a** and **3a** was carried out by prep. HPLC on silica gel using *n*-hexane–EtOAc (1:3, flow rate 10 ml min^{-1}, UV detection 270 nm) for **1a** and *n*-hexane–*iso*PrOH (3:2, flow rate 8 ml min^{-1}, UV detection 200 nm) for **3a**.

*4-Oxo-β-ionol β-*D*-glucopyranoside* (**1**). UV λ_{max} nm (EtOH): 265. DCI-MS *m/z* (rel. int.): 388 $[M+NH_4]^+$ (25), 371 $[M+H]^+$ (38), 226 $[aglycone+NH_4]^+$ (76), 209 $[aglycone+H]^+$ (100), 208 $[(aglycone-H_2O)+NH_4]^+$ (27), 191 $[(aglycone-H_2O)+H]^+$ (63). ^1H and ^{13}C NMR: see Tables 1 and 2.

*S-(+)-Linalool O-α-*L*-arabinofuranosyl-(1→6)-β-*D*-glucopyranoside* (**3**). DCI-MS *m/z* (rel. int.): 466 $[M+NH_4]^+$ (100), 334 $[linalool \approx glucose+NH_4]^+$ (4), 312 $[M-linalool+NH_4]^+$ (4), 154 $[linalool-H_2O+NH_4]^+$ (4), 150 $[arabinose-H_2O+NH_4]^+$ (5), 137 $[linalool-H_2O+H]^+$ (8). ^1H and ^{13}C NMR: see Tables 1 and 2.

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REFERENCES

1. Pabst, A., Barron, D., Etiévant, P. and Schreier, P. (1991) *J. Agric. Food Chem.* **39**, 173.
2. Pabst, A., Barron, D., Adda, J. and Schreier, P. (1990) *Phytochemistry* **29**, 3853.
3. Pabst, A., Barron, D., Sémon, E. and Schreier, P. (1992) *Phytochemistry* **31**, 1649.
4. Pabst, A., Barron, D., Sémon, E. and Schreier, P. (1992) *Phytochemistry* **31**, 2043.
5. Pabst, A., Barron, D., Sémon, E. and Schreier, P. (1992) *Phytochemistry* (in press).
6. Pabst, A., Barron, D., Sémon, E. and Schreier, P. (1991) *Tetrahedron Letters* **32**, 4885.
7. Barron, D. and Pabst, A. (1991) in *Recent Advances in Phytochemistry* (Stafford, H. A., ed.), Vol. 25, *Modern Phytochemical Methods* (Fischer, N. H., Isman, M. B. and Stafford, H. A., eds), p. 33. Plenum Press, New York.
8. Kasai, R., Suzuo, M., Asakawa, J. I. and Osamu, T. (1977) *Tetrahedron Letters* **2**, 175.
9. Bernreuther, A. and Schreier, P. (1991) *Phytochem. Anal.* **2**, 167.
10. Kaiser, R. and Lamparsky, D. (1978) *Helv. Chim. Acta* **61**, 373.
11. Winterhalter, P. and Schreier, P. (1988) *J. Agric. Food Chem.* **36**, 1251.
12. Winterhalter, P., Sefton, M. A. and Williams, P. J. (1990) *J. Agric. Food Chem.* **38**, 1041.
13. Winterhalter, P. (1990) *J. Agric. Food Chem.* **38**, 452.
14. Williams, P. J., Strauss, C. R., Wilson, B. and Massy-Westropp, R. A. (1982) *Phytochemistry* **21**, 2013.