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AN α-IONOL DISACCHARIDE GLYCOSIDE FROM RASPBERRY FRUIT

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Key Word Index—*Rubus idaeus*; Rosaceae; raspberry fruit; (6*R*,9*R*)-α-ionol 9-*O*-α-L-arabinofuranosyl- (1→6)-β-D-glucopyranoside.

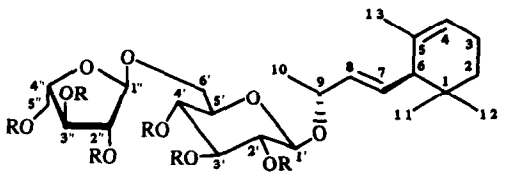
Abstract—From a methanolic extract of raspberry fruit the 9-*O*-α-L-arabinofuranosyl- (1→6)-β-D-glucopyranoside of (6*R*,9*R*)-α-ionol was isolated. Its structure was established on the basis of ^{1}H and ^{13}C NMR spectroscopy. The absolute configuration of the aglycone was determined by direct chiral analysis using MDGC-mass spectrometry as well as ^{1}H NMR analysis of its (*R*)-(−)-α-phenylpropionic acid ester.

INTRODUCTION

As part of a study on glycosidically bound volatiles in raspberry fruit [1–3], we now report the isolation of an α-ionol disaccharide glycoside. α-Ionol is structurally related to α-ionone, one of the most important aroma constituents of raspberry fruit [4].

RESULTS AND DISCUSSION

A crude glycosidic mixture obtained from raspberry fruit (cv. Héritage) by adsorption chromatography on Amberlite XAD resin followed by methanol elution was prefractionated by LC on Sephadex LH-20 and MPLC on RP-18. Further purification of **1** was achieved by flash chromatography on silica gel followed by HPLC on RP-select 8 phase.



	R
1	H
1a	Ac

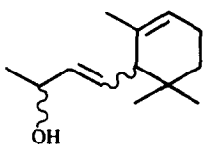


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Table 1. ^{13}C NMR data of compounds **1** and **2** (CD_3OD: 50 MHz)

C	1	2 (racemate)*
Aglycone		
1	32.9	32.9
2	32.5	32.6
3	24.0	24.1
4	121.9	121.8/121.7
5	135.3	135.4/135.3
6	55.4	55.2
7	133.8	131.4/131.4
8	135.1	137.4/137.3
9	76.6	69.1
10	21.4	24.1
11[a]	28.4	28.1
12[a]	27.4	27.5
13	23.2	23.3
Glucose		
1′	102.3	
2′	75.3	
3′	78.2[b]	
4′	71.8	
5′	78.0[b]	
6′	67.8	
Arabinose		
1″	109.7	
2″	83.1	
3″	79.0	
4″	86.1	
5″	63.1	

*Assignments were based on a ^{1}H–^{13}C COSY experiment.
[a,b]Assignments may be reversed.

The DCI-mass spectrum of **1** exhibited a base peak at *m/z* 506 $[M+NH_4]^+$ indicating that **1** was an α-ionol disaccharide glycoside. Analysis of the ^{13}C NMR data of **1** (Table 1) suggested the presence of a glucopyranosyl [2]

and an arabinofuranosyl moiety [5]. The interglycosidic linkage between the terminal arabinose and the inner glucose unit was assigned to be at C-6′, since ^{13}C NMR revealed a downfield shift of C-6′ by *ca* 5 ppm, while the other glucose carbon signals were nearly unchanged, as compared with unsubstituted β-D-glucopyranosides [2]. On the other hand, the linkage of arabinose to position 6 of glucose was confirmed by the fact that the signals of H-6′ in **1a** (Table 2) appeared at higher field in comparison with unsubstituted acetylated glucose [2]. Concerning the aglycone moiety, the downfield shift observed for the C-9 resonance and the upfield shifts experienced by the C-

Table 2. ^{1}H NMR data of compounds **1**, **1a** and **2**

	1	**1a***	**2** (racemate)*	
H	CD_3OD (200 MHz)	C_6D_6 (400 MHz)	CD_3OD (200 MHz)	C_6D_6 (200 MHz)
Aglycone				
2α/3β	0.90–1.21 *m*	1.05–1.15 *m*	1.10–1.25 *m*	1.04–1.16 *m*
2β	1.42–1.50 *m*	1.40–1.55 *m*	1.40–1.50 *m*	1.35–1.55 *m*
3β	1.99 *br s*	1.97 *br s*	2.00 *br s*	1.94 *br s*
4	5.37–5.55 *m*	5.38–5.41 *m*	5.38–5.55 *m*	5.30–5.58 *m*
6	2.12 *d*	2.12 *d*	2.09 *d*	2.04 *d*
	(7.3)	(8.6)	(7.7)	(8.0)
7	5.37–5.55 *m*	5.45 *dd*	5.38–5.55 *m*	5.30–5.58 *m*
		(15.3; 8.8)		
8		5.55 dd		
		(15.4; 6.4)		
9	†	4.15 *m*	4.22 *m*	4.20 *m*
10	1.25 *d*	1.17 *d*	1.21 *d*	1.23 *d*, 1.22 *d*
	(6.2)	(6.4)	(6.3)	(6.3), (6.4)
11[a]	0.89 *s*	0.95 *s*	0.90 *s* (6H)	0.90 *s*, 0.89 *s*
12[a]	0.84 *s*	0.90 *s*	0.85 *s*, 0.82 *s*	0.86 *s*, 0.83 *s*
13	1.55 *m*	1.65 *d*	1.60 *m*	1.65 *d*, 1.62 *d*
		(1.5)		(1.7), (1.7)
Glucose				
1′	4.31 *d*	4.44 *d*		
	(7.7)	(7.7)		
2′		5.28 *dd*		
		(9.6; 7.9)		
3′		5.46 *t*		
		(9.6)		
4′	*ca*	5.23 *t*		
		(9.9)		
5′		3.49 *m*		
6′α		3.84 *dd*		
		(10.7; 2.0)		
6′β	3.10–	3.53 *dd*		
		(10.8; 5.4)		
Arabinose				
1″		5.15 *br s*		
2″		5.40 *d*		
		(1.7)		
3″	4.10	5.22 *dd*		
		(5.3; 1.7)		
4″		4.43 *dt*		
		(5.4; 3.6)		
5″α	*m*	4.52 *dd*		
		(11.8; 3.7)		
5″β		4.29 *dd*		
		(11.8; 5.4)		
Acetyl-		1.59 *s*; 1.67 *s*		
		1.68 *s*; 1.69 *s*		
		1.70 *s*; 1.73 *s*		

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

†Overlapped by sugar signals.

[a]Interchangeable values.

8 and C-10 resonances, when compared with the corresponding signals in free α-ionol (Table 1), confirmed the attachment of the sugar chain at C-9. The configurations of the anomeric centres of the sugar moieties were derived from the analysis of the ^{1}H NMR spectrum of **1a** (Table 2). The anomeric proton signal of the arabinofuranose moiety appeared as a singlet at 5.15 ppm and was therefore deduced to be α [6], while the β-configuration of glucose was derived by the chemical shift and the coupling constant (7.7 Hz) of the anomeric proton at 4.44 ppm.

In order to determine the stereochemistry at C-6 of the aglycone moiety, **1** was enzymatically hydrolysed and subsequently oxidized using a two-phase system [7]. Direct chiral analysis using MDGC-mass spectrometry showed the resulting optically pure α-ionone to possess (6*R*)-configuration [8]. Stereochemistry at C-9 was established using the method of Helmchen [9, 10] correlating absolute configuration of chiral secondary alcohols with ^{1}H NMR spectroscopic behaviour of their diastereomeric esters prepared from an optically pure α-phenylpropionic acid. ^{1}H NMR analysis of diastereomeric esters prepared from reference racemic α-ionol and (*R*)-(−)-α-phenylpropionic acid revealed two split doublets for the 10-Me. The doublets at δ1.06/1.07 were assigned to the (9*S*) diastereomers and, accordingly, the doublets observed at δ1.14/1.15 were deduced to result from the (9*R*) diastereomers. The area under these resonances showed the expected 1:1 ratio. Enrichment of this sample with an ester accordingly prepared from **1** after enzymatic hydrolysis resulted in a significant enhancement of the signal at δ1.14, corresponding to the (9*R*) diastereomer.

This is the first report on the isolation and structural elucidation of (6*R*,9*R*)-α-ionol 9-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside. Similar absolute configuration at C-6 of α-ionol and carotenoids like α-carotene [11] agrees with the common hypothesis that norisoprenoid compounds are derived from carotenoids [12].

EXPERIMENTAL

General. NMR spectra were measured either at 400 (^{1}H) or at 200 (^{1}H) and 50 (^{13}C) MHz using TMS as ref. DCI mass spectrum of compound **1** was recorded at 70 eV with NH_3 as reagent gas, scanning from 100 to 600; source pressure 0.4 mbar; source temp. 90°. MDGC was performed using DB-5/CP-Cyclodextrin-B-2,3,6-M-19 column coupling as recently described [13].

Extraction and isolation of **1**. Plant material and part of the isolation procedure were described previously [2]. The 80% aq. MeOH eluate of the RP-18 medium pressure column was further subjected to flash chromatography on silica gel (0.032–0.063 mm) eluting with $CHCl_3$–MeOH–H_2O, 85:15:1. Subsequent semi-prep. HPLC on a LiChrosorb RP-select 8 column (10 μm; 250 × 10 mm; Merck, Darmstadt; UV detection: 200 nm) using H_2O–MeCN (4 ml min^{-1}) yielded pure glycoside **1**. DCI-MS *m/z* (rel. int.) 506 $[M+NH_4]^+$ (100), 374 [(α-ionol ≈ glucose) + NH_4]$^+$ (17), 212 [α-ionol + NH_4]$^+$ (5), 177 [(α-ionol-H_2O) + H]$^+$ (11).

Identification of the α-ionol glycoside in chromatographic fractions. Based on enzymatic hydrolysis of an aliquot in 0.2 M phosphate buffer, pH 5, using Rohapect D5L pectinase (Röhm) followed by Et_2O extraction of the released aglycones and subsequent HRGC-MS analysis (Chrompack CP-Wax-58-CB 30 m × 0.25 mm i.d., df = 0.22 μm).

Acetylation of **1**. Performed using standard Ac_2O–pyridine procedure at room temp. for 2 days in the dark. Purification of **1a** was achieved by prep. HPLC on silica gel (LiChrospher Si 60; 5 μm; 250 × 16 mm; Knauer, Berlin; *n*-hexane-*iso*-PrOH 60/40; UV detection: 200 nm).

α-Ionol. Synthesized by $LiAlH_4$ reduction from (±)-α-ionone and subsequently purified by LC on silica gel (*n*-hexane–EtOAc 8:2). GC-MS 70 eV, *m/z* (rel. int.): 138 (32), 123 (11), 95 (100), 93 (13), 91 (15), 79 (16), 43 (45), 41 (15).

Preparation of (R)-(−)-*α-phenylpropionic acid esters of racemic α-ionol.* A 3 equiv. portion of (*R*)-(−)-α-phenylpropionic acid was converted to the corresponding acid chloride with 7 equiv. oxalyl chloride (10 min; 55°). Excess oxalyl chloride was removed by azeotropic distillation with three 5 ml portions of CCl_4. One equiv. of racemic α-ionol dissolved in CCl_4 was added and allowed to react for 3 days at 55°. The reaction mixture was diluted with H_2O, extracted with Et_2O and the organic layer dried over anhydrous Na_2SO_4. Purification of the resulting diastereomeric esters was carried out by LC on silica gel (*n*-hexane–EtOAc 9:1). ^{1}H NMR (400 MHz, C_6D_6) of the α-ionol moiety: δ0.72, 0.74, 0.79, 0.83, 0.85, 0.88, 0.89 (7*s*, H-11, H-12); [1.06 (*d*, *J* = 6.3 Hz), 1.07 (*d*, *J* = 6.3 Hz), 1.14 (*d*, *J* = 6.5 Hz), 1.15 (*d*, *J* = 6.5 Hz) H-10]; 1.10–1.50 (*m*, H-2α,β, H-3α); [1.51 (*d*, *J* = 1.5 Hz), 1.53 (*d*, *J* = 1.8 Hz), 1.58 (*d*, *J* = 1.5 Hz), 1.62 (*d*, *J* = 1.9 Hz) H-13]; 1.91 (*br s*, H-3β); 5.18–5.53 (*m*, H-4, H-7, H-8, H-9).

(R)-(−)-*α-Phenylpropionic acid ester of glycosidically bound α-ionol.* Prepared accordingly after enzymatic hydrolysis of **1** and extraction of the liberated aglycone with Et_2O. ^{1}H NMR signals enhanced after addition of this sample to the above mixture of (*R*)-(−)-α-phenylpropionic acid esters of racemic α-ionol: δ1.14 (*d*, *J* = 6.5 Hz, H-10); 0.74, 0.85 (2*s*, H-11, H-12); 1.51 (*d*, *J* = 1.5 Hz, H-13).

Oxidation of glycosidically bound α-ionol. Compound **1** was enzymatically hydrolysed and the liberated aglycone extracted with Et_2O. A solution (0.8 ml) containing sodium dichromate (1 g), H_2O (5 ml) and H_2SO_4 (1.36 g) was added to the Et_2O layer and stirred for 1 hr at room temp. The mixture was diluted with H_2O, extracted with Et_2O and subsequently purified by LC on silica gel eluting with Et_2O.

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