Phytochemistry, Vol. 31, No. 9, pp. 3105–3107, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

A 4-HYDROXY-β-IONONE DISACCHARIDE GLYCOSIDE FROM RASPBERRY FRUITS

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(*Received* 6 *January* 1992)

Key Word Index—*Rubus idaeus*; Rosaceae; raspberry fruits; (4*S*)-4-hydroxy-β-ionone 4-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside.

Abstract—From a methanolic extract of raspberry fruits, the 4-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside of (4*S*)-4-hydroxy-β-ionone was isolated by adsorption chromatography on XAD-2, followed by liquid chromatography on Sephadex LH-20, RP-18 and silica gel as well as by reverse-phase HPLC. The structure of the norcarotenoid glycoside was determined by ^{1}H and ^{13}C NMR spectroscopy and DCI-mass spectral analysis. The absolute configuration of the aglycone was established to be (4*S*).

INTRODUCTION

Continuing our research on glycosidically bound volatiles in raspberry fruit [1–6], we now report on the first isolation of a 4-hydroxy-β-ionone glycoside.

RESULTS AND DISCUSSION

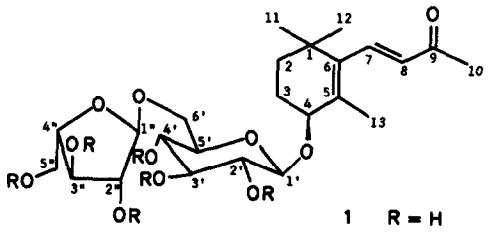
A methanolic extract from raspberry fruits was subjected to adsorption chromatography on Amberlite XAD-2 resin. The methanol eluate was prefractionated by LC on Sephadex LH-20 and MPLC on RP-18. Subsequent flash chromatography on silica gel as well as prep. HPLC on RP-select 8 phase led to the isolation of pure compound **1**.

The DCI-mass spectrum of **1** exhibited a base peak at *m/z* 520 $[M+NH_4]^+$ and a fragment at *m/z* 503 $[M+H]^+$ indicating that it is a disaccharide glycoside. The fragments at *m/z* 388 [(aglycone ≈ hexose) + NH_4]$^+$ and 371 [(aglycone ≈ hexose) + H]$^+$ resulted from the loss of a pentose unit, whereas the fragment at *m/z* 312 [(M − aglycone) + NH_4]$^+$ corresponded to a disaccharide moiety These data suggested the presence of a disaccharide chain with a hexose directly linked to the aglycone moiety and a pentose as a terminal unit.

Beside the aglycone moiety, the ^{13}C NMR spectrum of **1** (Table 1) revealed the presence of six signals derived from a glucopyranosyl [2] and five signals from an arabinofuranosyl moiety [7]. In the ^{1}H NMR spectrum of the acetylated glycoside **1a** (Table 2), the anomeric proton signal of the arabinofuranose moiety appeared as a singlet at δ5.14 and was therefore deduced to be α [8]. On the other hand, the β-configuration of the anomeric centre of the glucopyranose moiety was suggested by the coupling constant (8.0 Hz) of the anomeric proton at δ 4.37.

In comparison with the peracetylated glucose residue [2], the signals of H-6′ in the ^{1}H NMR spectrum of **1a** (Table 2) appeared at higher field, suggesting that the arabinose moiety was attached to C-6′ of glucose. In addition, the ^{13}C NMR spectrum of **1** revealed a significant downfield shift of C-6′ as compared to unsubstituted glucopyranosides [2], whereas the carbon signal of C-5′ was shifted slightly upfield. Thus, the linkage of the sugars was confirmed to be arabinofuranose(1→6)glucopyranose. Appropriate carbon glycosylation shifts were observed for the resonances of C-4 (downfield shift), C-3 and C-5 (upfield shift) of free 4-hydroxy-β-ionone (**2**) compared to the glycosidically bound form of **2** (Table 1).

The absolute configuration at C-4 of the aglycone was established according to the method of Helmchen [9, 10] correlating stereochemistry of chiral secondary alcohols



1 R = H
1a R = Ac

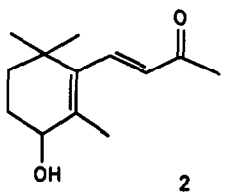


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Table 1. ^{13}C NMR data of compounds 1 and 2 (CD_3OD; 50 MHz)

C	1	2*
Aglycone		
1	35.4	35.5
2	35.9	36.0
3	27.7	29.3
4	76.7	70.4
5	134.5	136.1
6	141.0	140.1
7	144.6	144.6
8	134.1	133.9
9	201.1	200.9
10	27.2	27.2
11/12	29.3	29.2
	27.9	28.1
13	19.1	18.8
Glucose		
1′	106.5	—
2′	75.5	—
3′	79.0	—
4′	72.1	—
5′	78.1	—
6′	66.3	—
Arabinose		
1″	109.9	—
2″	83.2	—
3″	81.1	—
4″	86.0	—
5″	63.1	—

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

with ^1H NMR spectroscopic behaviour of their diastereomeric esters prepared from optically pure α-phenylpropionic acid. In this way, diastereomeric esters of racemic reference 4-hydroxy-β-ionone were prepared using (*R*)-(−)-α-phenylpropionic acid and subsequently separated by HPLC. Comparison of the ^1H NMR data of the separated esters **I** and **II** (numbering refers to the order of elution in HPLC) (Table 3) showed a significant upfield shift for the resonance of Me-13 in ester **I** to which the (4*R*)-configuration was assigned. Accordingly, due to the upfield shift for the resonances of CH_2-2 and CH_2-3 in ester **II**, the (4*S*)-configuration was deduced. After enzymatic hydrolysis of glycoside **1**, the liberated aglycone was esterified in the same way with (*R*)-(−)-α-phenylpropionic acid and the resulting ester co-chromatographed with the reference phenylpropionic acid esters **I** and **II** using analytical HPLC on silica gel with diode array detection. The α-phenylpropionic acid ester prepared from the glycosidically bound 4-hydroxy-β-ionone and the reference ester **II** showing identical chromatographic properties; the aglycone of glycoside **1** was thus assigned the (4*S*)-configuration.

EXPERIMENTAL

General. ^1H NMR were recorded at 400 and 200 MHz, ^{13}C NMR at 50 MHz; chemical shifts are given in δ with TMS as int. standard. The DCI-MS of **1** was recorded at 70 eV with NH_3 as reagent gas, scanning from *m/z* 100 to 600; source pressure 0.4 mbar; source temp. 90°.

Extraction and isolation of **1**. Lyophilized *Rubus idaeus* fruits (cv. Héritage) (10.4 kg fr. wt) were extracted × 2 with MeOH–H_2O (4:1); the pH was previously adjusted to 7 with NaOH to prevent hydrolysis of the glycosides. After evapn of MeOH, the aq. residue was dil. with 1 l H_2O and stirred with polyvinylpolypyrrolidone (PVPP) overnight for removal of pigments. After centrifugation, the extract was applied to a column Amberlite XAD-2 resin. The column was washed with H_2O and the glycosidic compounds subsequently eluted with MeOH. The MeOH eluate was prefractionated by LC on Sephadex LH-20 using H_2O and a linear gradient of MeOH in H_2O. The norcarotenoid glycoside was present in the H_2O fraction which was subsequently subjected to MPLC on a LiChroprep RP-18 column (40–63 μm; 310 × 25 mm; Merck) using a linear gradient from H_2O to MeOH. The 4-hydroxy-β-ionone glycoside eluted with 50% aq. MeOH. Further fractionation was carried out by flash CC (silica gel 60; 0.032–0.063 mm; $CHCl_3$–MeOH–H_2O 80:20:1). Final purification of **1** was achieved by semi-prep. HPLC on a LiChrosorb RP-select 8-column (10 μm; 250 × 10 mm; Merck) using H_2O–MeCN (17:3) at 5 ml min^{-1} with UV detection at 270 nm. DCI-MS of **1** *m/z* (rel. int.) 520 [M + NH_4^+] (100), 503 [M + H]$^+$ (47), 388 [(aglycone ≈ hexose) + NH_4]$^+$ (30), 371 [(aglycone ≈ hexose) + H]$^+$ (39), 330 [(hexose ≈ pentose) + NH_4]$^+$ (9), 312 [(M − aglycone) + NH_4]$^+$ (22), 226 [aglycone + NH_4]$^+$ (26), 208 [(aglycone − H_2O) + NH_4]$^+$ (53), 191 [(aglycone − H_2O) + H]$^+$ (97), 168 [pentose + NH_4]$^+$ (11), 150 [(pentose − H_2O) + NH_4]$^+$ (30).

Identification of the 4-hydroxy-β-ionone glycoside in chromatographic fractions. Based on enzymatic hydrolysis of an aliquot in 0.2 M Pi buffer (pH 5) using Rohapect D5L pectinase (Röhm). Liberated aglycones were extracted with Et_2O and analysed by HRGC-MS (Chrompack CP-Wax-58-CB 30 m × 0.25 mm i.d., df = 0.22 μm).

Acetylation of **1**. Performed using Ac_2O–pyridine 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) using *n*-hexane–*iso*-PrOH (2:3) at 8 ml min^{-1} with UV detection (270 nm).

Separation of racemic reference 4-hydroxy-β-ionone into its optical isomers via the corresponding (R)-(−)-*α-phenylpropionic acid esters.* A 3 equiv. portion of (*R*)-(−)-α-phenylpropionic acid was converted to the corresponding acid chloride with 7 equivs of oxalyl chloride (10 min; 55°). Excess oxalyl chloride was removed by azeotropic distn with three 5-ml portions of CCl_4. One equiv. of racemic 4-hydroxy-β-ionone dissolved in CCl_4 was added and allowed to react for 3 days at 55°. The reaction mixt. was dild with H_2O, extracted with Et_2O and the organic layer dried (Na_2SO_4). The resulting diastereomeric esters were purified by LC on silica gel (*n*-hexane–EtOAc, 4:1) and subsequently sepd by prep. HPLC on silica gel (LiChrospher Si 60; 5 μm; 250 × 16 mm; Knauer) using *n*-hexane–*iso*-PrOH (49:1) at 5 ml min^{-1} with UV detection (270 nm).

(R)-(−)-*α-Phenylpropionic acid ester of glycosidically bound 4-hydroxy-β-ionone.* Prepd on an analytical scale after enzymatic hydrolysis of **1** and extraction of the liberated aglycone with Et_2O. Assignment of the absolute configuration of the resulting ester was performed by co-chromatography with phenylpropionic acid esters of racemic 4-hydroxy-β-ionone using analyt. HPLC (LiChrospher Si 60; 5 μm; 250 × 4 mm; Knauer) using *n*-hexane–*iso*-PrOH (19.9:0.1) at 1 ml min^{-1} with diode-array detection.

Acknowledgement—We are indebted to F. Fauvelle and J. C. Debouzy (Centre de Recherche du Service de la Santé des

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

	1	**1a***	**2**
H	CD_3OD (200 MHz)	C_6D_6 (400 MHz)	CD_3OD (200 MHz)
Aglycone			
2a,b	1.30–2.10 *m*	1.25–2.10 *m*	1.35–1.80 *m*
3a,b			1.63–1.95 *m*
4	3.98 *m*	3.66 *t* (4.7)[a]	3.95 *m*
7	7.29 *d* (16.4)		7.3 *d* (16.5)
8	6.13 *d* (16.5)	6.06 *d* (16.4)	6.13 *d* (16.5)
10	2.31 *s*	1.90 *s*	2.31 *s*
11/12	1.05 *s* (6H)	0.89 *s*	1.08 *s*
		0.87 *s*	1.05 *s*
13	1.87 *s*	1.72 *s*	1.83 *s*
Glucose			
1′	4.43 *d* (7.6)	4.37 *d* (8.0)	
2′		5.27 *dd* (9.6; 8.0)	
3′		5.44 *t* (9.5)	
4′		5.18 *t* (9.5)	
5′		3.49–3.53 *m*	
6′a		3.78–3.81 *m*	
6′b		3.49–3.53 *m*	
Arabinose	*ca* 3.10–4.10 *m*		
1″		5.14 *s*	
2″		5.43 *d* (2.1)	
3″		5.22 *dd* (5.0; 1.5)	
4″		4.43 *m*	
5″a		4.54 *dd* (11.8; 3.9)	
5″b		4.30 *dd* (11.8; 5.4)	
Acetyl-		1.66 *s* (6H)	
		1.60 *s*/1.68 *s*	
		1.70 *s*/1.72 *s*	

*Assignments based on results of a ^{1}H–^{1}H COSY experiment.
[a]Overlapped by C_6D_6 signal.

Table 3. ^{1}H NMR data of the 4-hydroxy-β-ionone moiety in the optically pure diastereomeric (*R*)-(−)-α-phenylpropionic acid esters **I** and **II** (200 MHz; $CDCl_3$)

H	**I** (4*R*)	**II** (4*S*)
2/3	1.59–1.92 *m*	1.33–1.89 *m*
4	5.18 *m*	5.22 *m*
7	7.12 *d* (16.4)	7.17 *d* (17.5)
8	6.04 *d* (16.4)	6.11 *d* (16.4)
10	2.28 *s*	2.31 *s*
11/12	1.03 *s*/1.07 *s*	1.00 *s*/1.03 *s*
13	1.38 *s*	1.65 *s*

Armées, 38706 La Tronche, France) for recording the ^{1}H–^{1}H COSY spectrum.

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