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TWO DIASTEREOMERIC 3-OXO-α-IONOL β-D-GLUCOSIDES FROM RASPBERRY FRUIT*

ANNI PABST, DENIS BARRON,† ETIENNE SÉMON‡ and PETER SCHREIER§

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, 8700 Würzburg, Germany; †Laboratoire de Pharmacognosie, Université Joseph Fourier-Grenoble I, UFR de Pharmacie, 38706 La Tronche, France; ‡Laboratoire de Recherche sur les Arômes, INRA, BP 1540, 21034 Dijon Cédex, France

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Key Word Index—*Rubus idaeus*; Rosaceae; raspberry fruit; 3-oxo-α-ionol glucosides; 4-[3′-(β-D-glucopyranosyloxy)-1′-butenyl]-3,5,5-trimethyl-2-cyclohexen-1-one.

Abstract—From a methanolic extract of raspberry fruit two 3′-*O*-β-D-glucopyranosides of diastereomeric 4-(3′-hydroxy-1′-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one (3-oxo-α-ionol) were isolated by adsorption chromatography on XAD-2 followed by LC on Sephadex LH-20, MPLC on RP-18, and flash chromatography on silica gel as well as by HPLC on diol and RP-18 phases. Their structures were determined by ^{1}H and ^{13}C NMR spectroscopy. The absolute configurations of the diastereomeric aglycones were established as (6*R*,9*R*) and (6*R*,9*S*), respectively, by CD correlation as well as ^{1}H NMR analysis of their corresponding (*R*)-(−)-α-phenylpropionic acid esters.

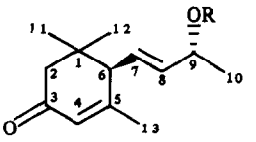
INTRODUCTION

Naturally occurring norisoprenoids have mostly been identified as tobacco constitutents [1]. A number of these compounds have also been detected in grapes and wine [2], quince fruit [3] and *Osmanthus absolute* [4]. Recent studies about the enzymatic hydrolysis of raspberry pulp [5] have demonstrated the abundant occurrence of glycosidically bound norisoprenoids, among others 3-oxo-α-ionol, in this fruit. This paper deals with the isolation and structural elucidation of two diastereomeric 3-oxo-α-ionol glucosides from raspberry fruit.

RESULTS AND DISCUSSION

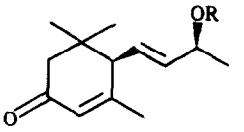
The extract obtained by adsorption chromatography of raspberry juice on XAD-2 and subsequent methanol elution was further fractionated by liquid chromatography on Sephadex LH-20, RP-18 and silica gel. Further purification by HPLC both on diol and RP-18 phases yielded two fractions which upon enzymatic hydrolysis liberated 3-oxo-α-ionol, suggesting the presence of two different conjugated forms of the latter compound.

DCI-MS of isolated glycosides **1** and **3** revealed similar fragmentation patterns. The presence of peaks at *m/z* 388 $[M+NH_4]^+$ and 371 $[M+H]^+$ demonstrated that both compounds were monoglycosides. In the ^{13}C NMR spectra of **1** and **3** (Table 1) the signals due to the carbohydrate moiety were identical and assigned to originate from a glucose residue [6]. The β-configuration of the anomeric centres was deduced from the coupling constants (Table 2) of the anomeric protons. Thus, compounds **1** and **3** were both 3-oxo-α-ionol β-D-glucosides.



	R
1	β-D-Glc
1a	β-D-Glc(Ac)$_4$
2	H

(6*R*,9*R*)



	R
3	β-D-Glc
4	H

(6*R*,9*S*)

The presence of two asymmetric centres in 3-oxo-α-ionol suggested that the aglycones of **1** and **3** might be diastereoisomers. In fact, NMR analysis of the aglycone moieties of glycosides **1** and **3** (Tables 1 and 2) revealed several significant chemical shift differences of signals near the C-9-asymmetric centre (positions 7, 8, 9 and 10). One of the most obvious differences consisted of the inversion of the chemical shift of the olefinic protons 7 and 8 between the two glycosides (Table 2).

The absolute configuration at C-9 was established according to the method of Helmchen [7, 8], which has been developed for the determination of the absolute configuration of chiral secondary alcohols. It is based on the derivatization of the alcohol in the form of an ester with an optically pure α-phenylpropionic acid. The phenyl group of the acid moiety induces chemical shift differences between diasteromeric esters that are directly

*Dedicated to Professor W. Jennings on the occasion of his seventieth birthday.

§Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR data of compounds **1**, **2**, **3** and **4**

C	**1** CD_3OD (100 MHz)	**2** CD_3OD (50 MHz)	**3** CD_3OD (100 MHz)	**4*** CD_3OD (50 MHz)
Aglycone				
1	37.1	37.1	37.2	37.1
2	†	48.4	†	48.4
3	202.0	202.0	n.d.	202.0
4	126.2	126.1	126.2	126.1
5	165.8	165.9	165.6	166.0
6	56.8	56.7	56.9	56.7
7	128.8	127.1	131.1	127.3
8	138.3	140.2	137.1	140.3
9	77.0	68.7	74.7	68.8
10	21.0	23.8	22.2	23.8‡
11§	27 6	27.3	27.4	27.3
12§	28.1	28.1	28.0	28.1
13	23.8	23.7‡	23.9	23.7‡
Glucose				
1′	102.5		101.3	
2′	75.3		75.0	
3′	78.1‡		78.4‡	
4′	71.6		71.8	
5′	78.0‡		78.2‡	
6′	62.7		62.9	

*Assignments were based on a ^1H–^{13}C COSY experiment.
†Overlapped by MeOH signal.
‡§Signals may be reversed.

correlated to the absolute configuration of the alcohol moiety. In this way, diastereomeric esters of racemic reference 3-oxo-α-ionol were prepared using (*R*)-(−)-α-phenylpropionic acid and subsequently separated by HPLC. ^1H NMR (Table 3) analysis of the four resulting diastereomeric esters revealed nearly identical chemical shifts for diastereomers **I** and **II**, and **III** and **IV**, respectively (numbering of the diastereomeric esters refers to the order of elution in HPLC). Based on the upfield shift of the 10-Me the absolute configuration at C-9 was assigned to be (*S*) in the diastereomeric esters **III** and **IV**. Accordingly, C-9 in **I** and **II** was deduced to be (*R*).

Stereochemistry at C-6 was established on the basis of CD correlation with literature data. The optically pure 3-oxo-α-ionols were released from the above mentioned α-phenylpropionic acid esters by enzymatic hydrolysis using an esterase from hog liver. CD spectra of each of the four isomers were recorded after purification by flash chromatography. 3-Oxo-α-ionols liberated from **II** and **III** exhibiting a positive maximum at 243 nm were deduced to be (6*R*) after comparison with reported data from 3-oxo-α-ionone [9]. Similarly, 3-oxo-α-ionol resulting from **I** and **IV** showed a negative maximum at 243 nm; thus the (6*S*) configuration was assigned. The influence of the chiral centre at C-9 in 3-oxo-α-ionol on the shape of the CD spectra was shown to be negligible. After oxidation of 3-oxo-α-ionol from **III** to the corresponding optically pure 3-oxo-α-ionone [10], both the alcohol and the corresponding diketone exhibited positive maxima at 243 and 240 nm, respectively.

The absolute configuration of the glycosidically bound 3-oxo-α-ionols was determined after enzymatic hydrolysis of compounds **1** and **3**, subsequent derivatization of the liberated aglycones with (*R*)-(−)-α-phenylpropionic acid, and by co-chromatography with reference phenylpropionic acid esters **I**–**IV**. Thus, glycoside **1** was assigned to possess the (6*R*,9*R*) and glycoside **3** the (6*R*,9*S*) configuration, respectively.

Norisoprenoid compounds such as 3-oxo-α-ionol are regarded to be degradation products of carotenoids [11]. The fact that carotenoids like lutein [9] and compounds **1** and **3** possess the same configuration at C-6 might confirm the relationships between these constituents. Glycoside **1** has previously been identified in *Polystichum tripteron* [12], citrus spp. [13, 14] and *Chamaecyparis obtusa* [15]. However, comparison of the NMR data of the glycoside isolated from the two latter sources with our results suggests that the configuration of the aglycone might be (6*R*,9*S*) and not (6*R*,9*R*) as reported. Another 3-oxo-α-ionol glucoside has been isolated from *Gentiana pneumonanthe* [16], but the stereochemistry has not been evaluated. Comparison of NMR data seems to indicate that the glycoside could be identical to compound **3**. Finally, a β-D-glucoside of 3-oxo-α-ionol has been identified in leaves of *Nicotiana rustica* [17].

EXPERIMENTAL

General. TLC was carried out on silica gel F254 aluminium sheets (Merck) using vanillin–sulphuric acid detection. Flash chromatography was performed on silica gel 60 (0.032–0.063 mm) and MPLC on a LiChroprep RP-18 column (Merck; 40–63 μm; 310 × 25 mm). For prep. HPLC the following columns were used: LiChrosorb Diol (Knauer; 5 μm; 250 × 16 mm), Nucleosil 120 C18 (Knauer; 7 μm; 250 × 16 mm) and LiChrospher Si 60 (Knauer; 5 μm; 250 × 16 mm). Analytical HPLC was performed on LiChrospher Si 60 (Knauer; 5 μm; 250 × 4 mm) using diode array detection. HRGC and HRGC-MS were carried out using a CP-Wax-58-CB WCOT column (Chrompack; 30 m × 0.32 mm i.d.; df = 0.22 μm). The column was programmed from 40 to 220° at 3° min^{-1}, and held at the upper temp. for 30 min. FID temp. was 250°. Injection was split-splitless (split ratio 1:9). Carrier gas was H_2 with a flow rate of 4 ml min^{-1}. NMR spectra were measured either at 400 (^1H) and 100 MHz (^{13}C) or at 200 (^1H) and 50 MHz (^{13}C) using TMS as ref. DCI mass spectra of compounds **1** and **3** were 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°. CD spectra were recorded in EtOH.

Extraction and isolation of compounds **1** *and* **3**. Plant material and part of the isolation procedure were described previously [6]. The 50% aq. MeOH eluate of the RP-18 medium pressure prep. column was further fractionated by flash chromatography ($CHCl_3$–MeOH–H_2O, 80:20:1). Final purification of the glycosides was achieved by prep. HPLC on a diol column using *n*-hexane–*n*-BuOH–MeOH–H_2O (65:25:9:1) as solvent (flow rate 8 ml min^{-1}; UV detection 240 nm) and subsequent prep. HPLC on RP-18 by eluting with MeCN–H_2O (3:17; flow rate 5 ml min^{-1}; UV detection 240 nm).

Localization of the 3-oxo-α-ionol glycosides in the fractions resulting from chromatographic separations. This was performed by hydrolysis of an aliquot of separated fractions in 0.2 M citrate–Pi buffer pH 5 using Rohapect D5L pectinase (Röhm) at 35° overnight. The liberated aglycones were extracted with Et_2O and analysed by HRGC-MS.

Acetylation of **1**. Compound **1** was acetylated using standard Ac_2O–pyridine procedure at room temp. for 2 days in the dark. Purification of **1a** was carried out by prep. HPLC on silica gel

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

H	**1** CD_3OD (200 MHz)	**1a** C_6D_6 (400 MHz)	**2** CD_3OD (200 MHz)	**3** CD_3OD (400 MHz)	**4*** CD_3OD (200 MHz)
Aglycone					
2α	2.03 *d* (16.7)	2.09 *d* (16.2)	2.04 *d* (16.8)	2.14 *d* (16.7)	2.03 *d* (16.8)
2β	2.43 *d* (16.7)	2.30 *d* (16.6)	2.40 *d* (16.8)	2.56 *d* (16.7)	2.41 *d* (16.8)
4	5.87 *br s*	5.92 *br s*	5.87 *br s*	5.97 *br s*	5.87 *br s*
6	2.67 *d* (8.4)	2.10 *d* (10.0)	2.66 *d* (8.5)	2.78 *d* (9.3)	2.65 *d* (8.3)
7	5.63 *dd* (15.5; 8.4)	5.29 *dd* (15.3; 9.0)	5.56 *dd* (15.3; 8.5)	5.84 *dd* (15.4; 9.4)	5.56 *dd* (15.5; 8.3)
8	5.78 *dd* (15.3; 6.0)	5.40 *dd* (15.5; 5.9)	5.70 *dd* (15.3; 5.4)	5.67 *dd* (15.4; 7.4)	5.69 *dd* (15.2; 5.5)
9	4.39–4.45 *m*	4.00 *m*	4.26 *m*	4.56 *m*	4.26 *m*
10	1.28 *d* (6.3)	1.10 *d* (6.4)	1.23 *d* (6.4)	1.37 *d* (6.3)	1.23 *d* (6.4)
11†	1.02 *s*	0.84 *s*	1.02 *s*	1.11 *s*	1.02 *s*
12†	1.00 *s*	0.78 *s*	0.99 *s*	1.07 *s*	0.97 *s*
13	1.93 *d* (1.1)	1.48 *s*	1.93 *d* (0.8)	2.06 *d* (1.1)	1.95 *d* (1.1)
Glucose					
1′	4.34 *d* (7.6)	4.34 *d* (8.0)		4.37 *d* (7.7)	
2′		5.43 *t* (9.5)			
3′	*ca* 3.10–3.60 *m*	5.27 *dd*‡ (9.6; 7.1)		*ca* 3.10–3.60 *m*	
4′		5.26 *t*‡ (9.8)			
5′		3.25 *ddd* (10.1; 3.9; 2.2)			
6′α	3.81 *dd* (11.7; 2.0)	4.31 *dd* (12.5; 4.1)		3.93 *dd* (11.7; 2.3)	
6′β	3.64 *dd* (11.7; 4.9)	4.10 *dd* (12.3; 2.2)		3.71 *dd* (11.9; 6.1)	
Acetyls		1.67 *s*			
		1.68 *s*			
		1.71 *s*			
		1.73 *s*			

*Assignments were based on a ^{1}H–^{1}H COSY experiment.
†‡Assignments may be reversed.

Table 3. ^{1}H NMR data of the 3-oxo-α-ionol moiety in the optically pure diastereomeric (*R*)-(−)-α-phenylpropionic acid esters **I**–**IV** (200 MHz; $CDCl_3$; δ in ppm; TMS)

H	**I** (6*S*,9*R*)	**II** (6*R*,9*R*)	**III** (6*R*,9*S*)	**IV** (6*S*,9*S*)
2a	1.92 *d* (16.6)	1.97 *d* (16.6)	2.03 *d* (16.7)	2.05 *d* (16.7)
2b	2.04 *d* (16.7)	2.13 *d* (16.6)	2.21 *d* (16.7)	2.28 *d* (16.7)
4	5.83 *br s*	5.82 *br s*	5.89 *br s*	5.88 *br s*
6	2.36 *d* (8.5)	2.36 *d* (8.9)	2.47 *d* (7.6)	2.47 *d* (7.7)
7/8/9	5.22–5.49 *m*	5.18–5.49 *m*	5.30–5.55 *m*	5.29–5.55 *m*
10	1.30 *d* (6.4)	1.30 *d* (6.4)	1.24 *d* (6.5)	1.24 *d* (6.5)
11/12	0.77 *s*	0.77 *s*	0.89 *s*	0.89 *s*
	0.94 *s*	0.93 *s*	1.00 *s*	1.00 *s*
13	1.72 *d* (0.9)	1.72 *d* (0.8)	1.83 *d* (1.1)	1.83 *d* (0.9)

using *n*-hexane–EtOAc, 1:3 (flow rate 8 ml min^{-1}; UV detection 240 nm).

Separation of racemic reference 3-oxo-α-ionol 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 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 3-oxo-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. The resulting diastereomeric esters were purified by flash chromatography (*n*-hexane–EtOAc, 9:1) and subsequently separated by prep. HPLC on silica gel using *n*-hexane–*iso*-PrOH (49:1) as eluent (flow rate 9 ml min^{-1}; UV detection 240 nm). Each of the separated phenylpropionic acid esters of 3-oxo-α-ionol (**I**–**IV**) was hydrolysed using an esterase from hog liver (Boehringer) in 0.2 M citrate–Pi buffer pH

7 at 37° overnight. Released 3-oxo-α-ionols were extracted with Et_2O and purified by flash chromatography (*n*-hexane–EtOAc, 3:2).

CD (EtOH, nm, Δε) 3-oxo-α-ionol from **I**: 243 (−138), 319 (+6); 3-oxo-α-ionol from **II**: 244 (+138), 319 (−6); 3-oxo-α-ionol from **III**: 244 (+125), 321 (−5); 3-oxo-α-ionol from **IV**: 244 (−130), 323 (+6).

Oxidation of 3-oxo-α-ionol from **III**. To the alcohol (2.5 mg) in Et_2O (2 ml) was added 0.8 ml of a soln containing sodium dichromate (1 g), H_2O (5 ml) and H_2SO_4 (1.36 g). After stirring for 1 hr at room temp. H_2O was added, the mixture extracted with Et_2O and subsequently purified by flash chromatography eluting with Et_2O. Cd (EtOH, nm, rel. int.): 240 (+1040).

(R)-(−)-*α-Phenylpropionic acid esters of glycosidically bound 3-oxo-α-ionol.* These were accordingly prepared in an analytical scale after enzymatic hydrolysis of **1** and **3** and extraction of the liberated aglycones with Et_2O. Assignment of the absolute configuration of the hereby resulting esters was performed by co-chromatography with the phenylpropionic acid esters of reference 3-oxo-α-ionol on analyt. HPLC (LiChrospher Si 60; *n*-hexane–*iso*-PrOH, 99:1; 1 ml min^{-1}) using diode array detection.

(6R,9R)-3-*Oxo-α-ionol β-*D*-glucopyranoside* (**1**). UV λ_{max} nm: 240. DCI-MS *m/z* (rel. int.): 388 $[M+NH_4]^+$ (46), 371 $[M+H]^+$ (100), 226 $[aglycone+NH_4]^+$ (15), 208 $[aglycone-H_2O+NH_4]^+$ (33), 191 $[aglycone-H_2O+H]^+$ (98), 180 $[glucose-H_2O+NH_4]^+$ (8). CD (EtOH, nm, rel. int.): 243 (+207), 327 (−8).

(6R,9S)-3-*Oxo-α-ionol β-*D*-glucopyranoside* (**3**). UV λ_{max} nm: 240. DCI-MS *m/z* (rel. int.): 388 $[M+NH_4]^+$ (59), 371 $[M+H]^+$ (71), 226 $[aglycone+NH_4]^+$ (13), 209 $[aglycone+H]^+$ (13), 208 $[aglycone-H_2O+NH_4]^+$ (16) 191 $[aglycone-H_2O+H]^+$ (100), 180 $[glucose-H_2O+NH_4]^+$ (16). CD (EtOH, nm, rel. int.): 243 (+108), 326 (−4).

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