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IDENTIFICATION OF 3-HYDROXYOCTYL β-D-GLUCOSIDE AND ABSOLUTE CONFIGURATION OF FREE AND BOUND OCTANE-1,3-DIOL IN APPLE FRUIT

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Key Word Index— *Malus sylvestris*; Rosaceae; apple; fruit; 3-hydroxyoctyl β-D-glucoside; free and bound (*R*)-(+)-octane-1,3-diol.

Abstract—In an extract obtained from neutralized apple juice, cv. Jonathan, by LC separation on Amberlite XAD resin using ethyl acetate elution, 3-hydroxyoctyl β-D-glucoside was identified by HRGC, HRGC-MS and HRGC-FTIR after per-*O*-methylation. Chiral evaluation of free and glucosidically bound octane-1,3-diol was performed by HRGC after derivatization with Mosher's reagent revealing the occurrence of optically pure diol in apple fruit. By means of comparison of optical rotation of the 1,3-diacetoxy derivative with previously published data, (*R*)-(+)-configuration was evaluated.

INTRODUCTION

The first report on the natural occurrence of octane-1,3-diol in apple fruit was presented by Brulé [1], who also showed that its concentration increased after thermal treatment of the juice. This antifungal β-glycol [2] has been found later in several apple cultivars, in part, as one of the major constituents of the juice [3, 4]. The present paper concerns the identification of its glucosidically bound form and the evaluation of the absolute configuration of free and bound octane-1,3-diol in apple fruit, cv. Jonathan.

RESULTS AND DISCUSSION

In a concentrate from apple juice, cv. Jonathan, obtained by solvent extraction and subsequent liquid chromatographic preparation on silica gel, octane-1,3-diol was identified by HRGC, HRGC-MS and HRGC-FTIR. Quantitative HRGC revealed an amount of 10 mg/kg fruit.

The identification of bound octane-1,3-diol was performed after separation of a glycosidic fraction from neutralized apple juice using adsorption chromatography on Amberlite XAD resin according to Gunata *et al.* [5]. Half of the ethyl acetate eluate was subjected to acidic (pH 1.0) and enzymatic treatment with emulsin, respectively. In both experiments, octane-1,3-diol was identified by HRGC, HRGC-MS and HRGC-FTIR as major product of hydrolysis. The other half of the eluate was per-*O*-methylated [6]. After subsequent HPLC separation on silica gel, HRGC, HRGC-MS and HRGC-FTIR revealed the occurrence of per-*O*-methylated 3-hydroxyoctyl β-D-glucoside. 3-Hydroxyoctyl β-D-glucoside has not been found in nature as yet.

Chiral evaluation of free and glucosidically bound octane-1,3-diol from apple fruit was performed by HRGC after derivatization of the isolated diol with Mosher's reagent [7]. In both experiments, HRGC of MTPA* derivatives revealed only one peak exhibiting the presence of optically pure octane-1,3-diol in apple fruit. In order to evaluate its absolute configuration, octane-1,3-diol isolated from apple fruit was acetylated, since the optical rotation values of both acetylated enantiomers of octane-1,3-diol were reported [8]. We obtained $[\alpha]_D^{25} = +7.1°$ for octane-1,3-diol and $[\alpha]_D^{25} = -12.7°$ for 1,3-diacetoxy-octane. Comparison of optical rotation of the 1, 3-diacetoxy derivative with that of previously published results [8] led to evaluation of (*R*)-(+) configuration for both free and bound octane-1,3-diol.

The biogenetic formation of octane-1,3-diol can be understood by reduction of 3-hydroxyoctanoic acid, and the subsequent glucosidation should be regarded as generation of a transport form of the diol [9]. 3-Hydroxyacids and their derivatives are major constituents in various fruits [10]. They are intermediates of fatty acid metabolism, in which opposite enantiomers are formed in course of catabolic and anabolic pathways. During β-oxidation (*S*)-(+)-3-hydroxyacids are generated by hydration of (*E*)-2-enoyl-CoA, whereas the reduction of 3-ketoacyl-ACP, an intermediate of *de novo* synthesis, leads to the (*R*)-configurated derivative (Fig. 1) [10]. Octane-1,3-diol from apple fruit has the same absolute configuration (*R*) as the 3-hydroxy fatty acids which are formed by *de novo* synthesis. This shows that the diol is likely synthesized via the anabolic pathway of fatty acid synthesis. Due to the same absolute configuration of free and glucosidically bound diol, the optical purity of free octane-1,3-diol is not caused by separation of the other enantiomer via glucosidation. There is no information available about the occurrence of 3-ketooctan-1-ol in apple juice, while oct-1-en-3-ol was tentatively identified in this fruit [11]. These data suggest that the formation of the chiral center of octane-1,3-diol is probably performed biogenetically before the glucosidation step.

*R-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride.

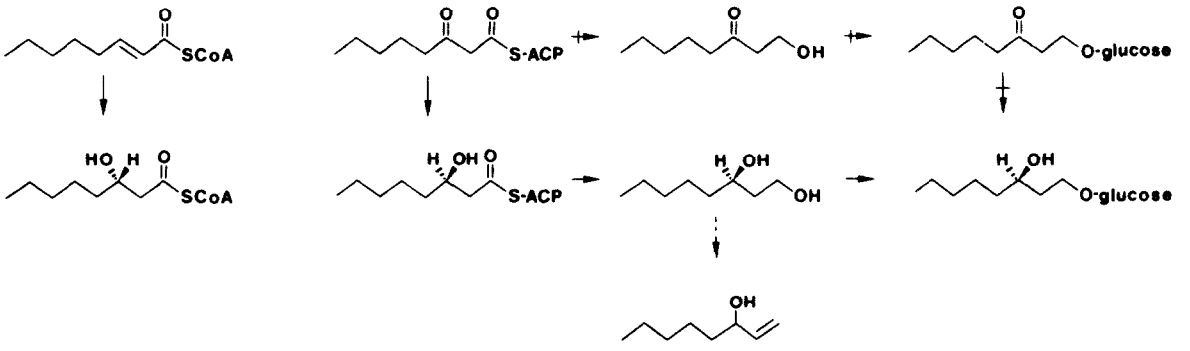
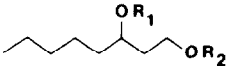


Fig. 1. Formation of (*S*)-(+)-3-hydroxyacids (*β*-oxidation), (*R*)-(−)-3-hydroxyacids (*de novo* synthesis) and possible glucosidation step of octane-1, 3-diol.



1 R_1=R_2=H
2 R_1=R_2=Ac
3 R_1=R_2=Me
4 R_1=R_2=TMS
5 R_1=H, R_2=*β*-D-glucose
6 R_1=H, R_2=2,3,4,6-tetra-*O*-acetyl-*β*-D-glucose
7 R_1=Me, R_2=2,3,4,6-tetra-*O*-methyl-*β*-D-glucose
8 R_1=TMS, R_2=2,3,4,6,-tetra-*O*-TMS-*β*-D-glucose

EXPERIMENTAL

EI- and CIMS were determined at 70 eV by HRGC-MS, scanning from *m/z* 41 to 499 with total ion current monitoring. HRGC, HRGC-MS and HRGC-FTIR were carried out using a fused silica WCOT column (30 m × 0.259 mm, df = 0.25 *μ*m) coated with DB 5. Split injection (1:50) was used (1 *μ*l). The column was programmed at 5°/min from 60 to 300° for the separation of compounds **1–4** and the glucoside derivatives **6–8** and 2°/min from 140 to 300° for the separation of the MTPA derivatives. FID temp. 300°; carrier gas He 2 ml/min. Light pipe and transfer lines were held at 200°; vapour phase spectra were recorded from 700 to 4000 cm^{-1} with 1 cm/sec. Linear R_f, MS and FTIR data were compared with those of synthesized reference compounds. NMR spectra were measured at 200 MHz in CD_3–CO–CD_3. For HRGC, HRGC-MS and HRGC-FTIR the samples were per-*O*-methylated as described [6]. Optical rotation values were measured at 25° at 546 and 435 nm and were converted to D-line of sodium.

Fruits. Fresh, ripe apple fruits (*Malus sylvestris* Mill cv. Jonathan) were obtained from the local market.

Isolation of octane-1,3-diol. After removing the seeds of 4 kg apples and cutting into small pieces, the fruits were submerged in 400 ml 0.2 M Pi buffer (pH 7), homogenized with a Braun blender for 30 sec and centrifuged (4000 *g*, 0°, 30 min). The supernatant was filled in a liquid–liquid extractor and continuously extracted with 500 ml Et_2O for 48 hr. After drying the organic layer over Na_2SO_4 and concng *in vacuo* the extract was passed through a chromatographic column filled with silica gel 60 (Merck 0.06–0.1 mm particle diameter). Pentane–Et_2O mixtures with increasing polarity were used for separating the extract. The octane-1,3-diol was identified in the pure ether eluates.

XAD conditioning. 100 g Amberlite XAD-2 (SERVA 3.0–1.0 mm particle diameter) was successively extracted for 24 hr in a Soxhlet apparatus with 250 ml pentane, 250 ml EtOAc and 250 ml MeOH. The last slurry was filled into a chromatographic column (100 × 2.5 cm) conditioned by passing 2.0 l distilled water (20 ml/min).

Isolation of bound forms of octane-1,3-diol. After removing the seeds of 1.2 kg apples and cutting into small pieces, the fruits were submerged in 1 l 0.2 M Pi buffer (pH 7.0, containing 0.2 M glucono-*δ*-lactone as glycosidase inhibitor), homogenized with a Braun blender for 30 sec and centrifuged (4000 *g*; 0°; 30 min). The supernatant was passed through a conditioned XAD column. After washing the column with 1.5 l distilled water and 0.5 l pentane the bound forms of octane-1,3-diol were eluted with 750 ml EtOAc. The EtOAc extract was concd *in vacuo* followed by dissolving the residue in 100 ml 0.2 M Pi buffer (pH 5) and extracted continuously with 100 ml ether in a liquid–liquid extractor to separate the volatiles.

Acid hydrolysis. A part of the purified glycosidic extract was acidified to pH 1 and hydrolysed using a modified Likens–Nickerson-apparatus [12]. The alcohols remaining in the aq. soln after hydrolysis were extracted × 3 with 100 ml ether each and identified by means of HRGC and HRGC-MS.

Enzymatic hydrolysis. A part of the purified glycosidic extract was removed from the diethyl ether by decanting the organic layer. Ten mg emulsin (Boehringer) were added to the aqueous phase and incubated at 40° for 24 hr. Finally, the aglycons were isolated by continuous extraction of the enzymatic hydrolysate with 100 ml Et_2O. The obtained alcohols were identified in the organic phase by means of HRGC and HRGC-MS.

Synthesis of octane-1,3-diol (**1**): 0.3 M hept-1-ene, 0.2 M HCOH (40% aq. soln) and 0.04 M H_2SO_4 (35% soln in acetic acid) were refluxed for 24 hr at 130–135°. After neutralizing the mixture with NaOH, it was filtered through glass wool, evapd *in vacuo* and the residue extracted five times with 200 ml Et_2O. The crude product was purified by LC on silica gel using pentane–Et_2O mixtures with increasing polarity. Octane-1,3-diol (**1**). R_f, EIMS and FTIR cf. Table 1.

*Synthesis of 3-hydroxyoctyl β-*D-glucoside (**5**). 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucoside. 3-Hydroxyoctyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucoside (**6**) was synthesized under the following modified Koenigs–Knorr conditions [13]. To 5.5 mM of octane-1,3-diol in 50 ml CH_2Cl_2 1 g drierite and 3.4 mM Ag_2O were added and the mixture stirred in the dark at room temp. for 30 min. Then 3.2 mM *α*-D-acetobromoglucose in 50 ml CH_2Cl_2 were added within 20 min. After stirring the mixture in the dark at room temp. for 3 days, it was filtered through celite, evaporated *in vacuo*, resolved in 90 ml pentane–Et_2O (2:1) and extracted with

Table 1. Mass spectra and FTIR data of compounds **1–4**, **6–8**

Compounds	R_t	EIMS *m/z* (%)	FTIR cm^{-1}
1	1268	75 (100), 57 (85), 45 (66), 43 (63), 55 (58), 41 (51), 44 (40), 56 (39), 99 (22), 83 (21)	3664, 3602, 2936, 2885, 1451, 1395, 1311, 1285, 1262, 1063, 1047, 996, 974, 942, 911
2	1241	43 (100), 99 (12), 117 (10), 55 (10), 57 (10), 68 (8), 82 (7), 114 (7), 88 (6), 159 (2)	
3	1176	45 (100), 103 (38), 71 (25), 55 (22), 83 (17), 73 (15), 41 (14), 115 (12), 43 (7), 58 (5)	
4	1261	73 (100), 103 (84), 147 (32), 173 (31), 219 (26), 75 (22), 55 (20), 69 (20), 45 (18), 41 (18)	
6	2651	43 (100), 69 (19), 55 (9), 81 (7), 169 (7), 70 (6), 98 (6), 111 (6), 157 (5), 171 (5)	3649, 3609, 2962, 2943, 2885, 2866, 1772, 1443, 1420, 1373, 1219, 1054, 899
7	2191	88 (100), 45 (37), 101 (33), 71 (22), 143 (21), 69 (13), 75 (13), 203 (13), 55 (11), 41 (10)	2984, 2938, 2868, 2839, 1465, 1374, 1107, 987, 934
8	2589	73 (100), 204 (86), 72 (32), 205 (18), 103 (16), 147 (14), 69 (14), 217 (13), 129 (12), 111 (11)	

Table 2. ^1H and ^{13}C NMR spectral data of (**5**)

^{13}C δ*		^1H δ*	
Glucose			
103.9 *d*	C_1	4.19 *d*	H_1
74.6 *d*	C_2	3.10	H_2
77.9 *d*	C_3	3.57	H_3
71.5 *d*	C_4	3.40	H_4
77.4 *d*	C_5	3.19	H_5
62.8 *t*	C_6	3.61, 3.80	H_{6a}, H_{6b}
Octane-1,3-diol			
67.8 *t*	C_7	3.28	H_7
38.0 *t*	C_8	1.58	H_8
69.1 *d*	C_9	3.97	H_9
38.4 *t*	C_{10}	1.36	H_{10}
26.0 *t*	C_{11}	1.26	H_{11}
32.6 *t*	C_{12}	1.22	H_{12}
23.2 *t*	C_{13}	1.22	H_{13}
14.3 *q*	C_{14}	0.85	H_{14}

*200 MHz in CD_3–CO–CD_3; δ in ppm; TMS as internal standard.

90 ml aqueous MeOH (50%). The crude product was purified by LC on silica gel using pentane–EtOAc (3:1) as solvent. Yield of purified compound was 12%. 3-Hydroxyoctyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (**6**) R_t, EIMS and FTIR cf. Table 1.

Deacetylation of compound (**6**). To a solution of 200 mg (**6**) in 20 ml MeOH 20 ml 0.02 M sodium methanolate solution was added. After 12 hr the reaction mixture was neutralized by adding Dowex 50 WX8 (20–50 mesh, H^+ form) and filtered off. The crude product was purified by LC on silica gel using EtOAc–MeOH (9:1) as solvent. Yield of purified compound was 86%. 3-Hydroxyoctyl β-D-glucoside (**5**). ^1H and ^{13}C NMR cf. Table 2.

Derivatization. Per-*O*-methylation of compounds (**5**) and (**1**) was performed by Finne's method [6]. 3-Methoxyoctyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside (**7**). R_t, EIMS and FTIR cf. Table 1. 1,3-Dimethoxy-octane (**3**). R_t, EIMS cf. Table 1.

Derivatization. Per-*O*-trimethylsilylation of compounds (**5**) and (**1**) was performed by dissolving 5 mg (**5**) or (**1**) in 0.8 ml pyridine, adding 0.3 ml Silyl 21 (Machery-Nagel) and subsequent heating at 80° for 20 min. 3-Trimethylsilyl 2,3,4,6-tetra-*O*-(trimethylsilyl)-β-D-glucoside (**8**). R_t EIMS cf. Table 1. 1,3-Di-(trimethylsilyl)-octane (**4**). R_t, EIMS cf. Table 1.

Derivatization with Mosher's reagent [7]. To 1 μl octane-1,3-diol in 4 μl pyridine, 2 μl Mosher's reagent (MTPA-Cl) were added and the mixture stirred. After standing one day at room temp. the solution was diluted with 0.5 ml MeOH and analysed.

Acetylation of isolated octane-1,3-diol. The dried residue obtained from the liquid–liquid extraction of the apple juice was treated with 2 ml Ac_2O–pyridine (2:1) overnight at room temp. After adding of 5 ml H_2O and extracting × 3 with 5 ml CH_2Cl_2 the organic layer was washed with 10 ml satd $NaHCO_3$ solution and 10 ml H_2O. After drying the organic phase over Na_2SO_4 it was analysed by means of HRGC and HRGC-MS. 1,3-Diacetyloctane (**2**). R_t, EIMS cf. Table 1.

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