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ABSOLUTE CONFIGURATION OF OCTANOL DERIVATIVES IN APPLE FRUITS

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Key Word Index—*Malus sylvestris*; Rosaceae; apple fruit; enantiodifferentiation; 5(*Z*)-octene-1,3-diol; ethyl 3-hydroxyoctanoate; ethyl 5(*Z*)-3-hydroxyoctenoate.

Abstract—In extracts obtained by liquid-liquid extraction and enzymatic hydrolysis from five apple cultivars (Renao; Bedan; Peau de Chien; Noel des Champs; Red Delicious), chiral evaluation of free and glycosidically-bound octane-1,3-diol and 5(*Z*)-octene-1,3-diol, as well as ethyl 3-hydroxyoctanoate and ethyl 5(*Z*)-3-hydroxy-octenoate, was performed by multidimensional gas chromatography (MDGC), combining a polar achiral column (DB-Wax) with a chiral main column (2,3-di-*O*-acetyl-6-*O*-*tert.* butyldimethylsilyl-β-cyclodextrin/OV 1701). Comparison of retention times of synthesized optically-enriched reference compounds with isolated diols and hydroxyesters, revealed the (*R*)-configuration for the free diols in cvs. Renao, Bedan, Peau de Chien and Noel des Champs and the (*R*)-configuration for the bound diols in cvs Bedan, Peau de Chien and Noel des Champs, exhibiting enantiomeric excesses (ees) greater than 99%. (*R*)-hydroxyesters (ee > 99%) were detected in cvs. Noel des Champs and Red Delicious. Copyright © 1996 Elsevier Science Ltd

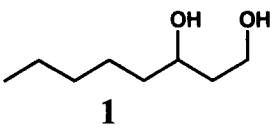
INTRODUCTION

In 1973, octane-1,3-diol **1** was identified for the first time as a natural apple constituent [1]. Some years later, the cultivar-dependent occurrence of this antifungal β-glycol was described [2]. Further octanol derivatives, such as 5(*Z*)-octene-1,3-diol have also been reported, in part, as constituents in Kogyoku apples [3], Rheinischer Bohnapfel, Purpurroter Cousinot and Börtlinger Weinapfel [4]. More recently, 3-hydroxy-octyl β-D-glucoside has been identified as a bound form of the 1,3-diol and chiral evaluation after derivatization with Mosher's reagent revealed the occurrence of optically pure (*R*)-(+)-octane-1,3-diol **1** in Jonathan apples [5].

Although **1** and its corresponding 5 (*Z*)-unsaturated isomer **2** are considered to be intermediates of fatty acid metabolism originating from 3-hydroxy acids [6], nothing is known about the enantiomeric distribution of **2**–**4** in apples. The present paper is concerned with the determination of the absolute configuration of free and glycosidically bound **1** and **2**, as well as their potential metabolites, ethyl 3-hydroxyoctanoate **3** and ethyl 5(*Z*)-3-hydroxyoctenoate **4**, in five apple cultivars.

RESULTS AND DISCUSSION

In concentrates from apple juices from cvs Renao, Bedan, Peau de Chien and Noel des Champs, octane-1,3-diol **1** and 5(*Z*)-octene-1,3-diol **2** were obtained by solvent extraction and identified by HRGC and HRGC-mass spectrometry. The identification of glycosidically bound **1** and **2** was performed after complete removal of free diols and subsequent enzymatic hydrolysis using β-glucosidase. Table 1 gives the amounts of free and bound forms of **1** and **2**. Similar diol concentrations



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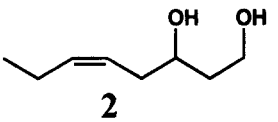

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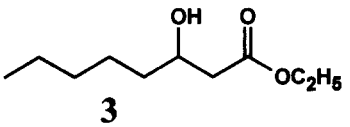

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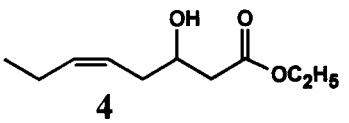

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Table 1. Amounts of free and glycosidically bound octane-1,3-diol (**1**) and 5(Z)-octene-1,3-diol (**2**) in four apple cultivars (mg kg^{-1} fresh weight)

Compound	Renao	Bedan	Peau de Chien	Noel des Champs
Free form				
Octane-1,3-diol (**1**)	0.1	20.0	58.0	26.2
5(Z)-Octene-1,3-diol (**2**)	0.05	5.8	14.4	6.7
Glycosidically-bound form				
Octane-1,3-diol (**1**)	n.d.*	2.4	5.3	4.7
5(Z)-Octene-1,3-diol (**2**)	n.d.*	1.3	2.2	4.7

*Not detected.

have been found previously in apple cvs Rheinischer Bohnapfel and Purpurroter Cousinot [4].

Chiral evaluation of free and glycosidically-bound **1** from apple cs Renao, Bedan, Peau de Chien and Noel des Champs was performed after derivatization of the isolated diol with Mosher's reagent using HRGC analysis. In all experiments, HRGC analysis of *R*-(+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) derivatives [7] revealed only one peak exhibiting the presence of optically pure **1** in apple fruit. Comparison of the retention index with those of synthesized MTPA derivatives of (*R*)-, as well as (*S*)-enriched **1**, led to the assignment of the (*R*)-configuration for free and bound **1** in apple fruit. (*R*)-enriched **1** (encantiomeric excess (ee) = 56%) was synthesized by yeast reduction of ethyl 3-oxooctanoate to (*R*)-ethyl 3-hydroxyoctanoate **3** followed by reduction with $LiAlH_4$. Modified Sharpless-oxidation [8, 9] starting with 2(*E*)-octen-1-ol followed by reduction with $LiAlH_4$ yielded (*S*)-enriched **1** (ee = 87%). Free and glycosidically-bound **1** has already been identified as optically pure (*R*)-(+)-enantiomer in apples cv. Jonathan [5].

For the first time, enantiomeric separation of **1**, without derivatization, was achieved by MDGC using 2,3-di-*O*-acetyl-6-*O*-*tert.* butyldimethylsilyl-β-cyclodextrin/OV 1701 as the chiral column. MDGC analyses confirmed the (*R*)-configuration for free and glycosidically-bound **1** in apples cvs Renao, Bedan, Peau de Chien and Noel des Champs.

The separation of enantiomers of **2** was also realized by MDGC. In all experiments MDGC analysis of free and glycosidically-bound **2** exhibited only one peak indicating the occurrence of the same pure enantiomer (ee > 99%) in the apple cultivars under study. We obtained $[\alpha]_D^{25} = -14.1°$. In order to evaluate the absolute configuration, free **2** isolated from apple cv Peau de Chien was purified by preparative argentation chromatography. Complete removal of **1** was confirmed by HRGC analysis. Reduction using Pd on charcoal and hydrogen yielded **1**. Since chiral evaluation of formed **1** revealed the (*R*)-configuration (ee > 99%), configuration of the starting material 5(*Z*)-octene-1,3-diol was assigned as (*R*).

Ethyl 3-hydroxyoctanoate **3** and ethyl 5(*Z*)-3-hydroxyoctenoate **4** have already been described as natural constituents of apples cv Red Delicious [10]. We now report for the first time the occurrence of both 3-hydroxy esters in apples cv Noel des Champs. Chiral evaluation was again achieved using MDGC. Reduction of ethyl 3-oxooctanoate using $NaBH_4$ yielded racemic **3**, while (*R*)-enriched **3** (ee = 67%) was prepared by yeast reduction of ethyl 3-oxooctanoate [11]. The (*R*)-configuration of the synthesized 3-hydroxy ester was confirmed by MDGC after reduction to **1** with $LiAlH_4$. Comparison of the retention index of synthesized (*R*)-enriched and racemic reference compound with that of the isolated ethyl ester from apple cvs Red Delicious and Noel des Champs established the occurrence of optically pure (*R*)-**3** (ee > 99%) in both apple cultivars.

In addition to **3**, the 5(*Z*)-unsaturated analogue **4** was identified in apples cvs Red Delicious and Noel des Champs, and analysed for its enantiomeric distribution. Racemic **4** was synthesized by Reformatzky reaction starting with 3(*Z*)-hexenal and ethyl 1-bromoacetate, while (*S*)-enriched **4** (ee = 25%) was obtained after esterification of racemic **4** with dodecanoic acid catalysed by porcine pancrease lipase. The (*S*)-configuration of remaining **4** was confirmed by reduction to **3** using Pd on charcoal under a hydrogen atmosphere followed by MDGC analysis. Pure (*R*)-enantiomer of **3** (ee > 99%) was identified in extracts isolated from apples cvs Red Delicious and Noel des Champs.

For the first time, the absolute configuration of the octane-1,3-diol metabolites, **2–4**, was evaluated and the co-occurrence of the 3-hydroxy esters, **3** and **4**, and the 1,3-diols, **1** and **2**, was demonstrated in the apple cv Noel des Champs. Although **1** and **2** are generally considered to be intermediates of fatty acid metabolism, their biosynthesis is discussed controversially. Two catabolic (β-oxidation or lipoxygenase reaction) [6] and an anabolic pathway are assumed [5]. The detection of optically pure (*R*)-enantiomers for **1–4** as natural ingredients of the investigated apple cultivars strongly supports the assumption of a close biogenetic relation between 3-hydroxy esters and 1,3-diols, but cannot unambiguously prove the existence of either one of the postulated biogenetic pathways. Feeding experiments using radioactively labelled fatty acids are under way in order to elucidate the biological formation of 1,3-diols.

EXPERIMENAL

General. EIMS was determined at 70 eV by HRGCMS, scanning from *m/z* 41 to 499 with total ion current monitoring. HRGC and HRGCMS were carried out using a fused silica WCOT column (30 m ×

0.25 mm, df = 0.25 μm) coated with DB-Wax 20 M. Split injection (1:20) was used (1 μl). The column was prog. from 50° for 3 min, then to 240° at 4° min^{-1}. FID temp. 300°; carrier gas He 3 ml min^{-1}. MTPA derivatives were separated on a fused silica WCOT column (30 m × 0.25 mm, df = 0.25 μm) coated with DB5. The column was prog. at 2° min^{-1} from 140° to 300°. Linear R_i and MS data were compared with those of synthesized ref. compounds. MDGC analyses were carried out with a double oven gas chromatograph fitted with a split injector (1:28) at 250° and two FIDs at 250°. A J&W DB-Wax 20 M fused silica capillary column (25 m × 0.25 mm, df = 0.25 μm) was used in the first oven for the preseparation of volatiles. Separation of enantiomers of **1–4** was achieved in the second oven using a fused silica capillary column coated with 2,3-di-*O*-acetyl-6-*O*-*tert.* butyldimethylsilyl-β-cyclodextrin/OV 1701 (25 m × 0.25 mm, df = 0.15 μm). The column in oven 1 was connected by a Live-T-interface to the column in oven 2. *Octane*-1,3-*diol* (**1**), oven 1, 60° to 240° at 10° min^{-1}; oven 2, 80° for 20 min then to 200° at 2° min^{-1}, cut 21.65 min to 21.95 min. 5(Z)-*Octene*-1,3-*diol* (**2**) oven 1, 60° to 240° at 10° min^{-1}; oven 2, 80° for 20 min then to 200° at 2° min^{-1}, cut 22.25 min to 22.55 min. *Ethyl* 3-*hydroxy octanoate* (**3**), oven 1, 60° to 240° at 10° min^{-1}; oven 2, 80° for 20 min then to 200° at 2° min^{-1}, cut 18.25 min to 18.55 min. *Ethyl* 5(Z)-3-*hydroxy octenoate* (**4**), 60° to 240° at 10° min^{-1}; oven 2, 80° for 15 min then to 200° at 1° min^{-1}, cut 19.0 min to 19.30 min. Enantiomeric separation of **3** was also performed in oven 2 using a fused silica capillary column coated with 2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin/OV 1701 (25 m × 0.25 mm, df = 0.15 μm). *Ethyl* 3-*hydroxyoctanoate* **3**, oven 1, 80° to 240° at 5° min^{-1}; oven 2, 80° for 25 min then to 200° at 1° min^{-1}, cut 25.0 min to 25.3 min. Evaluation of elution order of enantiomers was achieved using synthesized reference compounds with known enantiomeric ratios. Optical rotation values were measured at 25° at 546 and 435 nm, and converted to the D-line of Na. Argentation chromatography was performed on silica gel 60 after impregnation of the adsorbent by spraying with a 20% soln of $AgNO_3$ in 50% EtOH and drying for 1 hr at 80°.

Fruits. Fresh, ripe apple fruits of cv Red Delicious were purchased from the local market. Cultivars Renao, Bedan, Peau de Chien and Noel des Champs were kindly provided by Pernod Ricard, France.

Isolation of free diols and esters. Apples (200 g) (cvs Renao, Bedan, Peau de Chien and Noel des Champs) were sliced, the cores removed, and the slices blended in 250 ml of Pi buffer (0.2 M, pH 7). After centrifugation and filtration to remove suspended matter, the clear juice (400 ml) was extracted ×3 with 100 ml portions of Et_2O. Organic layers were combined dried (Na_2SO_4) and concd to a final vol. of 1 ml. Phenol (10 mg) was added as int. standard.

Isolation of bound diols. After removal of free volatiles, the juice was adjusted to pH 4.6 using 0.5 M HCl, 200 mg almond glucosidase (Sigma) added and the mixt. incubated for 3 days at 35°. The soln was then extracted ×3 with 100 ml portions of Et_2O. Organic layers were combined, dried (Na_2SO_4) and concd to a final vol. of 1 ml. Phenol (10 ml) was added as int. standard.

Separation of diols by argentation chromatography. Aliquots of extracts obtained by Et_2O extraction of apple juices, as well as extraction after hydrolysis, were applied as bands onto $AgNO_3$-impregnated TLC plates. EtOAc was used as mobile phase. After development, plates were stored under sunlight until dark bands were visible. Compounds were recovered from the adsorbent by elution with Et_2O.

Isolation of esters. In order to increase the concentration of Et esters, apples (400 g) (cv Red Delicious) were stored for 3 days in an atmosphere satd with EtOH [10]. Subsequently, apples were cut into small pieces, cores removed and the pieces homogenized in 750 ml of MeOH using a blender. After centrifugation and filtration to remove suspended matter, the clear juice (1 l) was extracted ×3 with 300 ml of pentane-Et_2O (1:1). Extracts were combined, dried (Na_2SO_4) and concd to a final vol. of 1 ml.

Derivatization with Mosher's Reagent. Aliquots of extracts (100 μl) were concd to dryness in a stream of N_2, dissolved in 5 μl of pyridine and 5 μl of Mosher's Reagent (MTPA-Cl) [7] added. The reaction was done overnight. After addition of 50 μl of MeOH, the soln was analysed by HRGC.

Catalytic reduction of 5(Z)-*octene*-1,3-*diol* (**2**). Isolated **2** (1 mg) was dissolved in 1 ml of MeOH and 5 mg of Pd (5% on charcoal) added. The flask was evacuated, flooded with H_2 and stored overnight, while applying an excess pres. of H_2. After filtration the soln was concd in a stream of N_2.

Prepn of reference compounds. Ethyl 3-*oxooctanoate.* Synthesized according to ref. [12] yielding 41 g (72%) of Et 3-oxooctanoate (123° at 17 Torr). R_i 1816. EIMS *m/z* (rel. int.): 43 (100), 71 (31), 41 (25), 99 (24), 88 (22), 55 (18), 130 (18), 84 (14), 69 (11), 56 (10). IR and ^1H NMR in accordance with previously published data [13–15]. *Racemic ethyl* 3-*hydroxyoctanoate* (**3**). Within 5 min, 1.9 g of $NaBH_4$ (0.05 mol) was added to a soln containing 18.6 g of Et 3-oxooctanoate (0.1 mol) and 50 ml of dry EtOH. The solvent was removed, 50 ml of H_2O added and the mixt. extracted ×3 with 50 ml of Et_2O. Combined organic layers were washed with brine and dried (Na_2SO_4). Fractionated distillation yielded 6.8 g of Et 3-hydroxyoctanoate **3** (36%) (130° at 20 Torr). R_i 1881. EIMS *m/z* (rel. int.): 43 (100), 117 (82), 71 (75), 55 (50), 89 (36), 41 (35), 88 (35), 60 (25), 61 (19), 75 (18). MS and linear R_i in accordance with previously published data [16]. *Racemic octane*-1,3-*diol* (**1**). A soln of 15.0 g Et 3-hydroxyoctanoate **3** (0.08 mol) and 20 ml of abs Et_2O was carefully added to a suspension of 1.5 g $LiAlH_4$ in 50 ml of abs Et_2O. The soln was refluxed for an additional hr, cooled to 0° and ice–H_2O added until the formation of H_2 had stopped. H_2SO_4 (10%) was

then added to dissolve solids; layers were separated and the aq. phase extracted ×3 with 50 ml Et_2O. Combined organic layers were washed with brine and dried (Na_2SO_4). Quantitative yield was obtained. R_i 2129. EIMS *m/z* (rel. int.): 75 (100), 57 (80), 45 (68), 43 (48), 55 (47), 41 (41), 56 (38), 83 (16), 72 (15), 99 (15). IR, ^{1}H NMR, MS and linear R_t in accordance with previously published data [4, 5]. (S)-*Octane*-1,3-*diol* (**1**). 2(*E*)-Octenol [2.1 g (16 mmol)] was epoxidized using the Sharpless method [8, 9], employing natural (+)-diethyl tartrate as the chiral pool. The epoxide was treated with 0.86 g $LiAlH_4$ (20 mmol) in 112 ml THF. After 20 min at 0°, 23 ml H_2O was added, followed by 15 ml 2% H_2SO_4. The mixt. was filtered and evapd to dryness. The residue was taken up in EtOH–H_2O (1:3) and treated with 1.03 g $NaIO_4$ (4.8 mmol) in 45 ml H_2O at 0°. After 2.5 hr, excess $NaBH_4$ was added followed by 1 M Pi (pH 7) buffer. The mixt. was stirred until it was homogeneous. EtOH was evapd, the remaining aq. mixt. lyophilized, redissolved in Et_2O and analysed. Yield was calculated to be *ca* 30% by HRGC. Optical rotation, IR, ^{1}H NMR, linear R_t and MS in accordance with previously published data [4, 5, 9]. (R)-*Ethyl* 3-*hydroxyoctanoate* (**3**). Bakers' yeast (10 g) was added to a soln containing 15 g of saccharose and 80 ml of tap H_2O [11]. While stirring, the soln was maintained at 30°. After 1 hr, 1 g Et 3-oxooctanoate (5.4 mmol) was added, shaken carefully and the flask closed by an air-permeable cotton plug. Saccharose (10 g) dissolved in 10 ml of tap H_2O (40°) was added after 1 day and 1 g Et 3-oxooctanoate (5.4 mmol) was introduced after an additional hr. After 2 days at 30°, the fermentation mixt. was filtered through 4 g of Celite. The aq. phase was satd with NaCl and extracted ×3 with 25 ml Et_2O. Combined organic layers were dried (Na_2SO_4), concd and analysed. Yield was calcd to be *ca* 10% by HRGC. MS and linear R_t identical with those previously published [16]. (R)-*Octane*-1,3-*diol* (**1**). To a soln containing 0.53 mg of $LiAlH_4$ (0.014 mmol) and 20 ml abs Et_2O, 5 mg Et 3-hydroxyoctanoate (0.027 mmol) dissolved in 10 ml abs Et_2O was carefully added and refluxed for 1 hr. H_2O was added until the formation of H_2 stopped and solids were dissolved in 10% H_2SO_4. The organic layer was sepd and the aq. phase extracted ×3 with 50 ml Et_2O. Combined organic extracts were washed with brine, dried (Na_2SO_4), concd and analysed. Quantitative yield was obtained. IR, ^{1}H NMR, linear R_t and MS in accordance with previously published data [4, 5, 9]. *Racemic ethyl* 5(Z)-3-*hydroxyoctenoate* **4**. To a cold soln (0°) containing 6 g 3 (Z)-hexenol (0.06 mol) and 200 ml CH_2Cl_2, 9.36 g DMSO (0.12 mol) and 15.36 g P_2O_5 was added successively. After removal of the ice bath, the soln was stirred for 45 min and subsequently cooled again (0°). Freshly dist. Et_3N (21.24 g; 0.209 mol) was introduced within 30 min, while stirring. The reaction was stopped by addition of 200 ml of HCl (10%), the organic layer sepd, washed with 50 ml of HCl (10%) and ×3 with 100 ml of brine. After dying (Na_2SO_4) and concn, HRGC and HRGCMS analysis revealed the formation of 3(Z)-hexenal. In parallel, 10 g ZN–Cu (9:1) alloy was washed with HCl (20%) until formation of H_2 stopped; the acid was removed and the alloy washed with Me_2CO and Et_2O, followed by drying. To a refluxing suspension containing 3.5 g Zn–Cu alloy and 15 ml of benzene–toluene (5:1), a soln of 4 g 3 (Z)-hexenal (0.041 mol) and 7 g Et 1-bromoacetate (0.44 mol) was added under an Ar atmosphere. After complete addition, refluxing was maintained for 30 min. Subsequently, the solution was cooled (0°), H_2SO_4 slowly introduced and the organic layer sepd. The acid aq. phase was extracted ×3 with 50 ml of Et_2O and the combined organic phases washed with $NaHCO_3$ soln and H_2O. After drying (Na_2SO_4) and concn, the product (25%) was purified by flash CC on silica gel using a pentane–Et_2O gradient of increasing polarity. R_i 1941. EIMS *m/z* (rel. int.): 71 (100), 43 (81), 117 (55), 41 (54), 55 (49), 89 (38), 70 (25), 75 (20), 141 (5), 168 (5). MS and linear R_t identical with previously published data [17]. (S)-*Ethyl* 5-(Z)-3-*hydroxyoctenoate* (**4**). To a soln containing 11.6 mg Et 5(Z)-3-hydroxyoctenoate (**4**), 15 mg dodecanoic acid and 1 ml of heptane containing 5 mg porcine pancrease lipase was added and stirred for one week at room temp. After addition of Celite and centrifugation, the supernatant was removed, concd in a stream of N_2 and redissolved in Et_2O. Remaining **4** was analysed by MDGC for its enantiomeric distribution. In order to evaluate absolute configuration, remaining **4** was dissolved in a soln containing MeOH and Pd (5%) on charcoal. The flask was evacuated, flooded with H_2 and stored overnight while applying an excess pres. of H_2. After filtration, the soln was concd in a stream of N_2 and analysed. Yield was calcd to be *ca* 30% by HRGC. MS and linear R_t identical with previously published data [17]. *Racemic* 5-(Z)-*octene*-1,3-*diol* (**2**). Hydrogenation of 2,5-octadiinol using Lindlar catalyst yielded 2(Z), 5(Z)-octadienol [18]. Epoxidation of 2(Z), 5(Z)-octadienol employing (±)-diethyl tartrate was conducted as reported in ref. [19]. Opening of the epoxide ring was performed analogously to the above-mentioned procedure [8]. IR, ^{1}H NMR, linear R_t and MS in accordance with previously published data [4].

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