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BIOSYNTHESIS OF OCTANE-1,3-DIOL IN APPLE FRUIT

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Key Word Index—*Malus sylvestris*; Rosaceae; apple fruit; octane-1,3-diol; 3-hydroxy-octyl-β-D-glucopyranoside; hexanoic acid; octanoic acid; linoleic acid; biosynthesis.

Abstract—The biosynthesis of octane-1,3-diol and 3-hydroxy-octyl-β-D-glucopyranoside was investigated by administering [1-^{14}C]hexanoic acid, [1-^{14}C]octanoic acid and [U-^{14}C]linoleic acid into intact ripe apple fruits cv. Peau de Chien. After a storage period of 2 months at 4°, the metabolites were isolated by solid phase extraction and analysed by HPLC and TLC. The fatty acids were converted to octane-1,3-diol with incorporation rates of 3.9, 3.4 and 16.2% and to 3-hydroxy-octyl-β-D-glucopyranoside with transformation rates of 0.9, 0.4 and 3.0% for hexanoic, octanoic and linoleic acid, respectively. No other major metabolites were detected. Enzymatic hydrolysis of the solid residues released additional amounts of [^{14}C] octane-1,3-diol.

INTRODUCTION

Several apple cultivars, e.g. Jonathan, Peau de Chien and Rheinischer Bohnapfel, accumulate high concentrations of octane-1,3-diol (**1**), 5(*Z*)-octene-1,3-diol and their respective glucosylated forms during storage [1–3]. Both diols exhibit antimicrobial effects against bacteria and yeasts but are harmless to humans [4, 5]. Recent analyses revealed the presence of enantiomerically pure *R*-(+)-octane-1,3-diol (**1**), *R*-(−)-5(*Z*) octene-1,3-diol, *R*-3-hydroxy-octyl-β-D-glucopyranoside (**2**) and *R*-5(*Z*)-3-hydroxy-octenyl-β-D-glucopyranoside in the cvs Jonathan and Peau de Chien [1, 2]. Both diols are considered to be intermediates of fatty acid metabolism and their biosynthesis may be explained by at least three possible pathways: (i) generation via fatty acid synthesis (*de novo*) [1], (ii) a catabolic route of formation (β-oxidation) [6] or (iii) a lipoxygenase-like reaction [6]. In the present work, we present the first evidence that both octane-1,3-diol (**1**) and its glucosylated form are derived primarily from linoleic acid.

RESULTS AND DISCUSSION

Based on the three hypotheses for the biosynthesis of octane-1,3-diol (**1**) and 3-hydroxy-octyl-β-D-glucopyranoside (**2**) [1-^{14}C]hexanoic acid (**3**), [1-^{14}C]octanoic acid (**4**) and [4-^{14}C]linoleic acid (**5**) were injected subepidermally into ripe apple fruits. The fruits were stored for 2 months at 4°, extracted with water and the extract subjected to solid phase extraction. Table 1 shows the ^{14}C recovery data for the different fractions as a percentage of the applied radioactivity. Moderate portions of the applied radioactivity (13–15%) were extracted after the application of **3** and **4**. In contrast, 42% of the applied ^{14}C was recovered in the aqueous extract obtained after incubation with **5**.

Analyses of the extractable radioactive residues were carried out by HPLC and TLC. One single radioactively labelled compound was detected in the diethyl ether extracts obtained after the administration of **3**–**5**. The metabolite of each incubation experiment was separated by TLC followed by HPLC. HRGC mass spectrometric-analysis of the purified ^{14}C-labelled compound revealed the presence of a pure substance identified as **1** from its R_t and mass spectrum.

The methanol extracts obtained after the application of **3**–**5** contained one major ^{14}C-labelled metabolite corresponding with *ca* 30% of the injected radioactivity and several minor compounds (< 10%). The principal metabolite showed the same TLC behaviour as **2**. Hydrolyses of the methanol extracts using a pectinolytic enzyme preparation followed by diethyl ether extraction yielded **1**. As only the major ^{14}C-labelled metabolite disappeared from the radiotrace of the HPLC separation, **2** accounted for *ca* 30% of the ^{14}C present in the methanol extracts (Table 1).

Up to 25% of the initial ^{14}C remained in the solid residues (Table 1). Therefore, enzymatic hydrolyses were performed in order to release further radioactivity. Extraction with diethyl ether recovered additional amounts of **1**, which was identified by TLC

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Table 1. Efficiency of incorporation of radioactive precursors and radioactive yield of octane-1,3-diol

	[1-^{14}C]Hexanoic acid*		[1-^{14}C]Octanoic acid*		[U-^{14}C]Linoleic acid*	
	Recovery†	Octane-1,3-diol†	Recovery	Octane-1,3-diol	Recovery	Octane-1,3-diol
XAD flow through	6.5	—	10.0	—	15.0	—
Et_2O extract	3.9	3.9	3.4	3.4	16.2	16.2
MeOH extract	2.4	0.9	1.4	0.4	10.4	3.0
Non-extractable residue	5.9	0.4	6.5	0.2	25.4	1.4
CO_2‡	81.3	—	78.7	—	33.0	—
Sum	—	5.2	—	4.0	—	20.6

* Applied radioactivity 2.0 E+7 dpm, 2.3 E+7 dpm and 9.4 E+7 dpm for [1-^{14}C]hexanoic acid, [1-^{14}C]octanoic acid and [U-^{14}C]linoleic acid, respectively.

† Percentage of applied radioactivity.

‡ Calculated.

and HPLC (Table 1). In total, 5.2. 4.0 and 20.6% of the applied radioactivity was recovered in **1** (Table 1) after the administration of **3**–**5** to apple fruits, respectively.

Three major hypotheses have been advanced concerning the biogenesis of **1** in apple fruit. Based on the *R*-configuration of the chiral carbon in position three, the first hypothesis concluded the formation of **1** in the course of *de novo* fatty acid synthesis [1]. Two alternative, catabolic routes may also deliver *R*-configurated diols, i.e. β-oxidation or lipoxygenase-induced cleavage of polyunsaturated C_{18} fatty acids [6].

In our study, an unusually high incorporation rate of **5** into **1** (21%) was obtained. A major portion of **5**, corresponding with 46% of the theoretical yield was converted into **1**. Thus, the present report offers the first evidence for the formation of **1** by a regio- and enantio-selective enzymatic breakdown of **5** (Fig. 1). Several investigations into the chemical breakdown of **5** (autoxidation) have been conducted, but **1** has never been detected. Our results show some homology

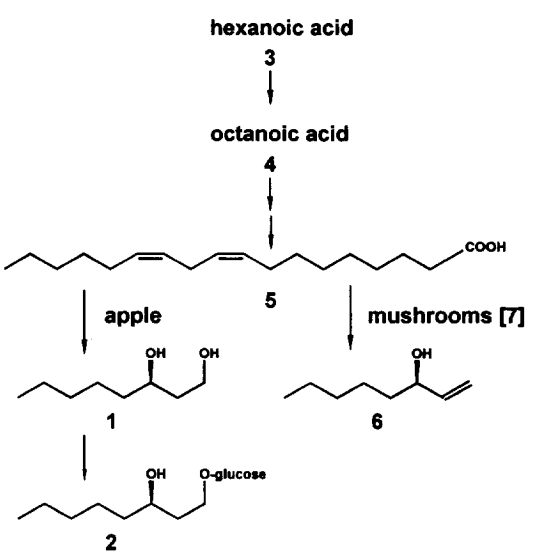

Fig. 1. Formation of octane-1,3-diol (**1**) in apples and 1-octene-3-ol (**6**) in mushrooms.

with the biosynthesis of 1-octene-3-ol (**6**) in mushrooms [7]. In both cases, enantiomerically pure *R*-alcohols [1, 8] are formed originating from the precursor **5**. A hydroperoxide lyase which cleaves a 10-hydroperoxy-*trans*-8,*cis*-12-octadecadienoic acid to **6** and 10-oxo-*trans*-8-decenoic acid has been detected in mushrooms [9]. In the present study, however, there was no indication for a C_{10} fragment in apple fruits. Although the biosynthesis of 5(*Z*)-octene-1,3-diol is assumed to be related to the biogenesis of **1** [6], no major ^{14}C-labelled compounds were detected in addition to **1** and its glucosylated form **2**.

EXPERIMENTAL

Chemicals. [1-^{14}C]Hexanoic acid; (55 mCi mmol^{-1}) was obtained from Biotrend, [1-^{14}C]octanoic acid (55 mCi mmol^{-1}) and [U-^{14}C]linoleic acid (1045 mCi mmol^{-1}) were obtained from Du Pont. **1** and **2** were synthesized and purified according to refs [1, 2]. XAD-2, a polystyrene adsorbent, was purchased from Aldrich.

General. Aliquots of liquid samples were added to 10 ml of scintillation cocktail (Emulsifier-Safe). Solid samples were combusted in a biological oxidizer. The formed $^{14}CO_2$ was absorbed in 12 ml of the scintillation cocktail, Oxysolve 400. Recoveries of ^{14}C as $^{14}CO_2$ from test combustions fortified with ^{14}C standards, immediately before combustion, were greater than 90%. All measurements were carried out by means of liquid scintillation counting using corrections for chemiluminescence. The HPLC was equipped with a UV and a radioactivity monitor and was fitted with a RP18 column (25 cm × 4.0 mm i.d., particle size 5 μm). The HPLC gradient was conducted in two linear steps at a flow rate of 1 ml min^{-1}, utilizing MeCN and acidic H_2O, adjusted to pH 2.5 with 1 M H_2SO_4. The gradient proceeded from 5 to 80% MeCN in 30 min followed by 80 to 100% MeCN in 10 min and remained at 100% MeCN for an additional 5 min. TLC plates (silica gel; Et_2O–MeOH, 19:1; visualization vanillin–H_2SO_4) were scanned using a radiodetector with Ar–CH_4 (9:1) as counting gas at 1381

V. EIMS were recorded at 70 eV by HRGCMS, scanning from m/z 41–499 with total ion current monitoring, using a fused silica WCOT column (30 m × 0.25 mm, film thickness 0.25 μm) coated with DB 5. The column was programmed at 5° min^{-1} from 60 to 300°; carrier gas He 2 ml min^{-1}.

Fruits. Fresh, ripe apple fruits (cv. Peau de Chien) were kindly provided by Pernod Ricard, France.

Application of fatty acids. EtOH solns (100 μl) of ^{14}C-labelled fatty acids were injected subepidermally with a syringe into two ripe apples (*ca* 160 g in total). Apples were kept for 2 months at 4° connected to a hood.

Solid phase extraction. Apples were cut into small pieces and homogenized with 100 ml of H_2O. After centrifugation (4000 g, 15 min), the solid residues were washed × 3 with 100 ml of H_2O. The supernatants were combined and passed through a conditioned XAD column. After washing the column with 500 ml of H_2O, **1** was eluted with 500 ml of Et_2O and **2** with 500 ml of MeOH. Solid residues were dried at 80° for 24 hr.

Enzymatic hydrolysis. Aliquots of solid residues (1.6–2.7 g) were resuspended in 15 ml of 0.2 M Pi buffer (pH 5.5). The pectinolytic enzyme prepn, Rohapect D5L (150 mg), was added and the suspension incubated at room temp. for 4 days. Liberated radioactively labelled compounds were extracted with Et_2O and analysed by HPLC.

Identity of **1**. Aliquots (*ca* 60 000 dpm) of Et_2O extracts obtained by solid phase extraction were analysed by TLC. Areas possessing radioactivity were scraped off and eluted with 5 ml Et_2O. Et_2O was then removed and the residue redissolved in 100 μl of H_2O and analysed by HPLC. Frs (1 ml) were collected and analysed for radioactivity. Frs containing radioactivity were extracted × 3 with 1 ml of Et_2O and finally analysed by HRGCMS.

Identity of **2**. Aliquots (*ca* 60 000 dpm) of MeOH extracts obtained by solid phase extraction were diluted with 6 ml of H_2O. The pectinolytic enzyme prepn, Rohapect D5L (25 mg), was added and incubated at room temp. for 24 hr. Liberated radioactively labelled compounds were extracted with Et_2O, concd to dryness, redissolved in 1 ml of H_2O and analysed by HPLC. Radioactivity remaining in the aq. phase was also analysed by HPLC.

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