PERGAMON

Phytochemistry 62 (2003) 115–120

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Glycosides of 2-*C*-methyl-D-erythritol from the fruits of anise, coriander and cumin

Junichi Kitajima*, Toru Ishikawa, Eiko Fujimatu, Kyoko Kondho, Tomomi Takayanagi

Showa Pharmaceutical University, Higashi-Tamagawagakuen 3, Machida, Tokyo 194-8543, Japan

Received 8 July 2002; received in revised form 5 September 2002

Abstract

Eight glycosides of 2-*C*-methyl-D-erythritol (**1**) were isolated from the fruit of anise, and their structures were clarified as 1-*O*-β-D-glucopyranoside, 3-*O*-β-D-glucopyranoside, 4-*O*-β-D-glucopyranoside, 1-*O*-β-D-fructofuranoside, 3-*O*-β-D-fructofuranoside, 4-*O*-β-D-fructofuranoside, 1-*O*-β-D-(6-*O*-4-hydroxybenzoyl)-glucopyranoside and 1-*O*-β-D-(6-*O*-4-methoxybenzoyl)-glucopyranoside of 2-*C*-methyl-D-erythritol (**2**–**9**), respectively. Furthermore, **2** and **4** were isolated from the fruit of coriander, and **2**, **3** and **4** were isolated from the fruit of cumin. Though the phosphate of **1** was known to be one of the first precursors of isoprenoids in the non-mevalonate pathway, and **1** is considered to be a common constituent in Umbelliferous plants, the glycosides of **1** are found for the first time.

Keywords: 2-*C*-Methyl-D-erythritol glycoside; Anise; *Primpinella anisum* L.; Coriander; *Coriandrum sativum* L.; Cumin; *Cuminum cyminum* L; Umbelliferae

1. Introduction

2-*C*-Methyl-D-erythritol 4-phosphate, as well as 2-*C*-methyl-D-erythritol (**1**) in some organisms, are an important biosynthetic precursors of isoprenoids in the non-mevalonate pathway (Rohmer et al., 1996; Eisenreich et al., 1997; Disch et al., 1998; Fontana et al., 2001). Indeed, **1** is metabolized by wild-type *Escherichia coli* and supports the growth of mutants lacking 1-deoxy-D-xylulose 5-phosphate (DXP) synthase and/or the isomeroreductase (Duvold et al., 1997; Charon et al., 2000). Though **1** is a common constituent in Umbelliferous plants (Kitajima et al., 1998), no reports have been published about its glycosides. In this study, the water-soluble portion of 70% aq. methanol extracts of some Umbelliferous fruits were investigated, resulting in the isolation of eight glycosides of **1** from the fruit of anise (*Pimpinella anisum* L.), coriander (*Coriandrum sativum* L.) and cumin (*Cuminum cyminum* L.), respectively. These fruts have been used as popular spices and medicine in Europe since the Middle Ages. In this paper, the isolation and characterization of these glycosides are discussed.

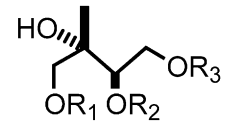
2. Results and discussion

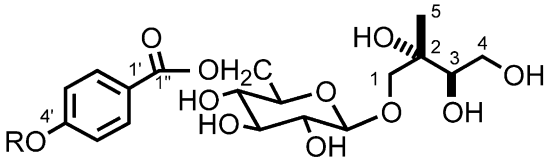
Commercial anise was extracted with 70% aq. of methanol, and the concentrated methanol extract was suspended in water and successively extracted with ether and ethyl acetate. The aqueous layer was applied to an Amberlite XAD-II to give water and methanol eluate fractions. Both fractions were then subjected to Sephadex LH-20 chromographs, followed by a combination of silica gel, Lobar RP-8 column chromatography and HPLC procedures [Carbohydrate Analysis (Waters) and Symmetryprep C_{18} 7 μm (Waters) were used as columns] to isolate **1** and its glycosides (**2**–**7**) from the water eluate fraction, and two acyl derivatives of **2** (**8** and **9**) from the methanol eluate fraction (yields from the methanolic extract: **1**, 0.042%; **2**, 0.050%; **3**, 0.037%; **4**, 0.009%; **5**, 0.026%; **6**, 0.007%; **7**, 0.012%; **8**. 0.006%; **9**, 0.001%).

* Corresponding author. Tel.: +81-427-21-1577; fax: +81-427-21-1588.

E-mail address: kitajima@ac.shoyaku.ac.jp (J. Kitajima).

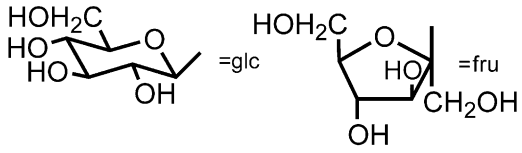
0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00438-7





1: $R_1=R_2=R_3=H$
2: R_1=glc, $R_2=R_3=H$ **5**: R_1=fru, $R_2=R_3=H$
3: $R_1=R_3=H$, R_2=glc **6**: $R_1=R_3=H$, R_2=fru
4: $R_1=R_2=H$, R_3=glc **7**: $R_1=R_2=H$, R_3=fru

8: R=H **9**: $R=CH_3$



Hemiterpenoid **1** ($[\alpha]_D^{21}+15^\circ$) was identified as 2-*C*-methyl-D-erythritol. Glycosides **2** ($[\alpha]_D^{21}-15^\circ$), **3** ($[\alpha]_D^{21}-13^\circ$) and **4** ($[\alpha]_D^{21}-8^\circ$) were obtained as colorless syrups, and their molecular formulae were deduced as $C_{11}H_{22}O_9$ from the accurate mass number of $[M+H]^+$ ion peaks in the high-resolution positive FAB-MS (299.1341 for **2**, 299.1339 for **3** and 299.1325 for **4**). The glycosides were hydrolyzed with β-glucosidase and, from the hydrolyzed mixtures, **1** and D-glucose were obtained. From the ^1H and ^{13}C NMR data obtained (Tables 1 and 2), **2**, **3** and **4** appeared to be β-D-glucopyranosides of **1**. The positions of the β-glucosyl units of **2**, **3** and **4** were proved to be C-1, C-3 and C-4, respectively, from the analysis of the HMBC spectra which showed a cross-peak between glucosyl H-1/C-1 in **2**, between glucosyl H-1/C-3 in **4**, and between glucosyl H-1/C-4 in **4**, respectively. Consequently, glycosides **2**, **3** and **4** were identified as 2-*C*-methyl-D-erythritol 1-*O*-β-D-glucopyranoside, 2-*C*-methyl-D-erythritol 3-*O*-β-D-glucopyranoside and 2-*C*-methyl-D-erythritol 4-*O*-β-D-glucopyranoside, respectively.

Table 1
^1H NMR chemical shifts of **1**–**9** (in pyridine-*d*5, 500 MHz)

	1	**2**	**3**	**4**
H-1a	4.13 *d* (10.5)	4.02 *d* (10.5)	3.89 *d* (11.0)	4.09 *d* (11.0)
1b	4.24 *d* (10.5)	4.61 *d* (10.5)	4.42 *d* (11.0)	4.19 *d* (11.0)
H-3	4.49 *dd* (4.0, 7.0)	4.52 *dd* (4.0, 8.0)	4.67 *dd* (3.0, 8.5)	4.63 *dd* (3.0, 8.5)
H-4a	4.32 *dd* (7.0, 10.5)	4.29 *dd* (8.0, 11.0)	4.39 *dd* (8.5, 11.5)	4.28 *dd* (8.5, 10.5)
4b	4.54 *dd* (4.0, 10.5)	4.52 *dd* (4.0, 11.0)	4.55 *dd* (3.0, 11.5)	4.94 *dd* (3.0, 10.5)
H_3–5	1.66 *s*	1.56 *s*	1.45 *s*	1.60 *s*
Glc-1		5.02 *d* (8.0)	5.29 *d* (8.0)	5.04 *d* (8.0)
	5	**6**	**7**	
H-1a	4.12 *d* (9.5)	4.18 *d* (11.0)	4.09 *d* (11.0)	
1b	4.54 *d* (9.5)	4.23 *d* (11.0)	4.18 *d* (11.0)	
H-3	4.55 *dd* (4.0, 7.0)	4.88 *dd* (3.5, 5.0)	4.54 *dd* (3.0, 7.0)	
H-4a	4.27 *dd* (7.0, 11.0)	4.31 *dd* (5.0, 12.0)	4.41 *dd* (7.0, 10.0)	
4b	4.48 *dd* (4.0, 11.0)	4.45 *dd* (3.5, 12.0)	4.83 *dd* (3.0, 10.0)	
H_3–5	1.53 *s*	1.61 *s*	1.60 *s*	
	8	**9**		
H-1a	4.10 *d* (11.0)	4.12 *d* (10.5)		
1b	4.60 *d* (11.0)	4.59 *d* (10.5)		
H-3	4.47 *dd* (4.0, 7.0)	4.47 *dd* (4.0, 7.0)		
H-4a	4.28 *dd* (7.0, 11.0)	4.28 *dd* (7.0, 10.5)		
4b	4.50 *dd* (4.0, 11.0)	4.50 *dd* (4.0, 10.5)		
H_3–5	1.56 *s*	1.58 *s*		
Glc-1	5.04 *d* (8.0)	5.04 *d* (8.0)		
H-2′,6′	8.25 *d* (8.5)	8.22 *d* (8.5)		
H-3′,5′	7.12 *d* (8.5)	6.93 *d* (8.5)		
OCH_3		3.66 *s*		

δ in ppm from TMS [coupling constants (*J*) in Hertz are given in parentheses].

Glycosides **5** ($[\alpha]_D^{22}-28°$), **6** ($[\alpha]_D^{22}-15°$) and **7** ($[\alpha]_D^{22}-19°$) was obtained as colorless syrups, whose molecular formulae of $C_{11}H_{22}O_9$ was consistent with the accurate mass number of $[M+H]^+$ ion peaks in the high-resolution positive FAB-MS of **5**, **6** and **7** (299.1331 for **5**, 299.1326 for **6** and 299.1331 for **7**). All gave **1** and D-fructose by enzymatic hydrolysis and, from their ^{13}C NMR spectral data (Table 2), could be considered β-D-fructofuranosides of **1**.[1] The position of attachment of the fructosyl units was found to be C-1 for **5**, C-3 for **6**, and C-4 for **7** from HMBC correlations between the signals of glucosyl H-1 and C-1 carbon of **5**, glucosyl H-1 and C-3 carbon in **6**, and glucosyl H-1 and C-4 carbon in **7**, respectively. Therefore, glycosides **5**, **6** and **7** were determined to be 2-*C*-methyl-D-erythritol 1-*O*-β-D-fructofuranoside, 2-*C*-methyl-D-erythritol 3-*O*-β-D-fructofuranoside and 2-*C*-methyl-D-erythritol 4-*O*-β-D-fructofuranoside, respectively.

Glycoside **8** ($[\alpha]_D^{22}-12°$) was obtained as an amorphous powder, and showed $[M+K]^+$, $[M+Na]^+$ and $[M+H]^+$ ion peaks at *m*/*z* 457, 441 and 419 in the positive FAB-MS, respectively. Its molecular formula was determined to be $C_{18}H_{26}O_{11}$ from the high-resolution positive FAB-MS (457.1125 $[M+K]^+$). Since **8** was deacylated to **2** by heating in a water bath with 5% NH_4OH–MeOH for 2 h, it was considered to be an acyl derivative of **2**. The IR spectrum of **8** showed absorptions due to hydroxyl (3550 cm^{-1}), ester carbonyl (1710 cm^{-1}) and phenyl (1600, 1420 cm^{-1}) groups, and the ^1H- and ^{13}C NMR spectral data (Tables 1 and 2) showed signals of a *p*-hydroxybenzoyl group in addition to resonaces due to **2**. So, **8** was a *p*-hydroxybenzoate of **2**. The position of the *p*-hydroxybenzoyl moiety was found to be at C-6 of glucose from the analysis of the HMBC spectrum, which showed a cross-peak between the glucosyl H_2-6 and the carbonyl carbon of the acyl unit. Therefore, **8** was 2-*C*-methyl-D-erythritol 1-*O*-β-D-(6-*O*-4-hydroxybenzoyl)-glucopyranoside.

Glycoside **9** ($[\alpha]_D^{22}-13°$) was obtained as an amorphous powder, and its positive FAB–MS spectrum showed $[M+K]^+$, $[M+Na]^+$ and $[M+H]^+$ ion peaks at *m*/*z* 471, 455 and 433. Its molecular formula was determined as $C_{19}H_{28}O_{11}$ by the high-resolution positive FAB-MS (471.1269 $[M+K]^+$). As the ^1H and ^{13}C NMR spectra of **9** (Tables 1 and 2) showed good similarity with those of **8**, except for one more methoxyl proton and a carbon signal, **9** seemed to be a monomethlyether of **8**. The cross-peak between the methoxyl group protons and the C-4 carbon of the benzene ring was observed together with the cross peaks between the glucosyl H_2-6 and carbonyl carbon, and the glucosyl H-1 and C-1 carbon in the HMBC spectrum, which suggested that the methoxyl group was located at C-4′ of the benzene ring. Thus, **9** was identified as 2-*C*-methyl-D-erythritol 1-*O*-β-D-(6-*O*-4-methoxybenzoyl)-glucopyranoside.

Table 2
^{13}C NMR chemical shifts of **1**–**9** (in pyridine-*d*5, 125 MHz)

	1	2	3	4	5	6	7	8	9
C-1	68.88	77.13	68.23	68.58	68.25	67.45	68.87	77.25	77.33
C-2	74.71	74.53	74.09	74.04	74.38	74.84	74.40	74.42	74.47
C-3	76.07	75.31	85.46	75.54	75.26	79.76	74.92	75.24	75.36
C-4	63.97	63.81	62.97	72.62	63.70	63.68	64.48	63.72	63.78
C-5	20.71	20.01	20.13	20.43	20.13	22.24	20.72	20.02	20.13
Glc-1		105.96	106.57	105.33				105.99	106.03
Glc-2		75.09	75.78	75.29				75.15	75.20
Glc-3		78.64	78.70	78.37				78.33	78.39
Glc-4		71.68	72.13	71.43				71.44	71.57
Glc-5		78.55	78.50	78.37				75.56	75.56
Glc-6		62.69	63.08	62.47				64.72	65.00
Fru-1					63.11	64.82	62.86		
Fru-2					105.30	106.55	105.70		
Fru-3					79.24	79.69	79.65		
Fru-4					76.17	76.02	76.85		
Fru-5					83.67	83.86	84.08		
Fru-6					62.78	62.26	63.81		
C-1′								121.53	123.21
C-2′,6′								132.49	132.14
C-3′,5′								116.03	114.14
C-4′								165.53	163.80
C-″								166.72	166.49
OCH3									55.41

δ in ppm from TMS.

Examination of the 70% aq. methanol extracts of the fruit of coriander and cumin was undertaken in the same way as described for anise. Compound **1**, and glycosides **2** and **4** were isolated from the fruit of coriander (yields from the methanolic extract: **1**, 0.12%; **2**, 0.007%; **4**, 0.30%), and glycosides **2**, **3** and **4** were isolated from the fruit of cumin together with **1** (yields from the methanolic extract: **1**, 0.017%; **2**, 0.022%; **3**, 0.007%; **4**, 0.020%). It therefore appears that 2-*C*-methyl-D-erythritol glycosides are fairly common constituents in Umbelliferous plants like 2-*C*-methyl-D-erythritol (**1**).

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. FAB-MS were recorded with a Jeol HX-110 spectrometer using glycerol as matrix. IR spectra were obtained with a JASCO A-103 IR spectrophotometer. ^1H and ^{13}C NMR spectra were taken on a Jeol A-500 spectrometer with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ values. ^1H–^{13}C COSY and HMBC

[1] ^{13}C NMR chemical shifts (in pyridine-d_5; δ) of methyl β-D-fructofuranoside were assigned as follows [C-1 (64.19), C-2 (105.56), C-3 (78.98), C-4 (77.08), C-5 (83.75), C-6 (62.62), OCH_3 (49.65)].

spectra were obtained with the usual pulse sequence, and data processing was performed with standard Jeol software. CC was carried out under TLC monitoring using Kieselgel 60 (70–230 mesh, Merck), Sephadex LH-20 (25–100 μm, Pharmacia), Lobar RP-8 column (Merck) and Amberlite XAD-II (Organo). TLC was performed on silica gel (Merck 5721) and spots were detected with *p*-anisaldehyde-H_2SO_4 reagent. HPLC separation was carried out with Carbohydrate Analysis [Waters; column size, 3.9 × 300 mm; CHA] and Symmetryprep C_{18} 7 μm [Waters; column size, 7.8 × 300 mm; ODS]. No acetoxyl group had been detected by the NMR spectral analysis of the materials prior to acetylation.

3.2. Extraction and separation

Commercial anise (the fruit of *Pimpinella anisum* L.; purchased from Asaoka Spices Ltd., Lot. No. anise 99012001; 2.0 kg) was extracted with 70% of MeOH (5 l × 4) at room temp. for 2 weeks. After evaporation of the solvent in vacuo the residue (346.7 g) was successively partitioned into ether–water and EtOAC–water. Removal of the solvent from each phase gave the ether (145.3 g), EtOAC (7.5 g) and aq. (193.9 g) extracts. The aqueous extract was applied to Amberlite XAD-II (H_2O–MeOH) to give the water eluate (141.8 g) and MeOH eluate (52.1 g) fraction. The MeOH eluate fraction was subjected to Sephadex LH-20 (MeOH as eluent) to give six fractions (frs. A–F). Fraction B (40.9 g) was applied to silica gel [$CHCl_3$–MeOH–H_2O (17:3:0.2→4:1:0.1→15:5:0.4→7:3:0.5)→MeOH] to give thirteen fractions (frs. B_1–B_{13}). Fraction B_9 (1.81 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give eighteen fractions (frs. B_{9-1}–B_{9-18}), and fr. B_{9-12} was subjected to HPLC [CHA, MeCN–H_2O (14:1); ODS, MeCN–H_2O (7:33)] to give **9** (4 mg). Fraction B_{10} (7.29 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give ten fractions (frs. B_{10-1}–B_{10-10}) and fr. B_{10-4} was subjected to HPLC [CHA, MeCN–H_2O (14:1), ODS; MeCN–H_2O (3:37)] to give **8** (20 mg). An aliquot of the water eluate fraction (50.4 g) was subjected to Sephadex LH-20 chromatography (MeOH) to give three fractions (frs. G–I). Fraction H (41.55 g) was subjected to silica gel chromatography [$CHCl_3$–MeOH–H_2O (4:1:0.1→15:5:0.4→7:3:0.5→6:4:0.5→1:1:0.1)→MeOH] to give 20 fractions (frs. H_1–H_{20}). Fraction H_{10} (0.98 g) was subjected to a Lobar RP-8 column (H_2O) and HPLC [CHA, MeCN–H_2O (97:3)] to give **1** (52 mg). Fraction H_{13} (4.68 g) was subjected to a Lobar RP-8 column (H_2O) to give six fractions (frs. H_{13-1}–H_{13-6}). Fraction H_{13-3} was subjected to HPLC [CHA, MeCN–H_2O (14:1)] to give a glycosyl fraction. The resulting glycosyl fraction was acetylated with Ac_2O and pyridine, and the acetylated fraction was subjected to HPLC [ODS, MeCN–H_2O (13:7)] to give three components. These three components were deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h. Then, **6** (8 mg), **7** (15 mg) and **4** (11 mg) were obtained after being subjected to Sephadex LH-20 (MeOH) CC. Fraction H_{13-4} was subjected to HPLC [CHA, MeCN–H_2O (14:1)] to give **3** (46 mg), **5** (32 mg) and **2** (62 mg).

Commercial coriander (the fruits of *Coriandrum sativum* L.; purchased from Asaoka Spices Ltd., Lot. No. coriander 99012001; 7.0 kg) was extracted with 70% aq. of MeOH (10 l × 4) at room temp. for 2 weeks. After evaporation of the solvent, the residue (363.6 g) was treated in the same way described for anise to give the ether (73.1 g), EtOAC (12.4 g) and aqueous (278.1 g) extracts. The aqueous extract was applied to Amberlite XAD-II (H_2O–MeOH) to give water eluate (238.1 g) and MeOH (40.0 g) fractions. An aliquot of the water eluate fraction (45.0 g) was subjected to Sephadex LH-20 [MeOH–H_2O (4:1)] to give three fractions (frs. H–J). Fraction I (38.72 g) was applied to silica gel [$CHCl_3$–MeOH–H_2O (4:1:0.1→7:3:0.5→6:4:0.5)→MeOH] to give 24 fractions (frs. I_1–I_{24}). Fraction I_9 (3.14 g) was subjected to a Lobar RP-8 column (H_2O) and HPLC [CHA, MeCN–H_2O (49:1)] to give **1** (90 mg). Fraction I_{17} (0.68 g) was subjected to a Lobar RP-8 column (H_2O) and HPLC [CHA, MeCN–H_2O (14:1)] to give **4** (202 mg) and **2** (5 mg).

Commercial cumin (the fruits of *Cuminum cyminum* L.; purchased from Asaoka Spices Ltd., Lot. No. cumin 99012001; 2.0 kg) was extracted with 70% aq. MeOH (10 l × 4) at room temp. for 2 weeks. After evaporation of the solvent, the residue (281.1 g) was treated in the same way described for anise to give the ether (121.8 g), EtOAC (7.0 g) and aqueous (152.3 g) extracts. The aqueous extract was chromatographed over Amberlite XAD-II (H_2O–MeOH) to give water eluate (87.1 g) and MeOH eluate (65.2 g) fractions. A part of the water eluate fraction (51.4 g) was subjected to Sephadex LH-20 [MeOH–H_2O (4:1)] to give three fractions (frs. E–G). Fraction F (46.56 g) was applied to silica gel [$CHCl_3$–MeOH–H_2O (4:1:0.1→7:3:0.5→6:4:0.5→1:1:0.1)→MeOH] to give 21 fractions (frs. F_1–F_{21}). Fraction F_6 (0.16 g) was passed through a RP-8 column (H_2O) to give a glycosyl fraction. The so-obtained glycosyl fraction was acetylated with Ac_2O and pyridine, and the acetylated fraction was subjected to HPLC [ODS, MeOH–H_2O (2:3)] to give two components. Both components were deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h. Then, **1** (18 mg) and glycerol (9 mg) were obtained after being subjected to Sephadex LH-20 (MeOH) CC. Fraction F_{12} (0.68 g) was subjected to a Lobar RP-8 column (H_2O) and HPLC [CHA, MeCN–H_2O (9:1)] to give **3** (7 mg), and fr. F_{14} (2.30 g) was subjected to a Lobar RP-8 column (H_2O) and HPLC [CHA, MeCN–H_2O (14:1)] to give **4** (22 mg) and **2** (19 mg).

Hemiterpenoid **1** was identified as 2-*C*-methyl-D-erythritol by comparison with an authentic sample.

3.2.1. 2-C-Methyl-D-erythritol 1-O-β-D-glucopyranoside (2)

A colorless syrup, $[\alpha]_D^{21}-15^\circ$ ($c=1.2$, MeOH). Positive FAB–MS m/z: 597 $[2M+H]^+$, 321 $[M+Na]^+$, 299.1341 $[M+H]^+$ (base, calc. for $C_{11}H_{23}O_9$; 299.1342), 137 $[M-C_6H_{10}O_5+H]^+$. For ^1H NMR (Pyridine-d_5, 500 MHz) and ^{13}C–NMR (pyridine-d_5, 125 MHz) see Tables 1 and 2. HMBC correlations: H_2-1/C-2, C-3, C-5, Glc C-1; H-3/C-1, C-2, C-4, C-5; H_2-4/C-2, C-3; H_3-5/C-1, C-2, C-3; Glc H-1/C-1.

3.2.2. Enzymatic hydrolysis of 2

A mixture of **2** (12 mg) and β-glucosidase (5 mg, TOYOBO CO., Lot. 93240) in water (5 ml) was shaken in a water bath at 37 °C for 14 days. The mixture was evaporated in vacuo to dryness and the residue was applied to silica gel [$CHCl_3$–MeOH–H_2O (7:3:0.5 and 1:1:0.1)] to afford **1** (3 mg) and a sugar fraction. The sugar fraction was passed through Sephadex LH-20 (MeOH) to give a syrup, and HPLC [carbohydrate analysis (waters), detector. JASCO RI-930 detector and JASCO OR-990 chiral detector, solv. MeCN–H_2O (17:3), 2 ml/min; t_R 4.5 min (same retention time as D-glucose)] was consistent with the presence of D-glucose.

3.2.3. 2-C-Methyl-D-erythritol 3-O-β-D-glucopyranoside (3)

Colorless syrup, $[\alpha]_D^{21}-13^\circ$ ($c=1.6$, MeOH). Positive FAB–MS m/z: 321 $[M+Na]^+$, 299.1339 $[M+H]^+$ (base, calc. for $C_{11}H_{23}O_9$; 299.1342), 137 $[M-C_6H_{10}O_5+H]^+$. For ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) see Tables 1 and 2. HMBC correlations: H_2-1/C-2, C-3, C-5; H-3/C-1, C-2, C-4, C-5, Glc C-1; H-4a/C-2, C-3; H-4b/C-2; H_3-5/C-1, C-2, C-3; Glc H-1/C-3.

3.3. Enzymatic hydrolysis of 3

A mixture of **3** (12 mg) and β-glucosidase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 14 days. The mixture was treated in the same way as described for **2** to afford **1** (5 mg) and a sugar fraction. In the sugar fraction the presence of D-glucose was revealed as after hydrolysis of **2**.

3.3.1. 2-C-Methyl-D-erythritol 4-O-β-D-glucopyranoside (4)

Colorless syrup, $[\alpha]_D^{21}-8^\circ$ ($c=0.9$, MeOH). Positive FAB–MS m/z: 337 $[M+K]^+$, 321 $[M+Na]^+$, 299.1325 $[M+H]^+$ (Calc. for $C_{11}H_{23}O_9$; 299.1342), 137 $[M-C_6H_{10}O_5+H]^+$ (base). For ^1H NMR (Pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) see Tables 1 ans 2. HMBC correlations: H_2-1/C-2, C-3, C-5; H-3/C-1, C-2, C-4, C-5; H_2-4/C-2, C-3, Glc C-1; H_3-5/C-1, C-2, C-3; Glc H-1/C-4.

3.4. Enzymatic hydrolysis of 4

A mixture of **4** (10 mg) and β-glucosidase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 14 days. The mixture was treated in the same way described for **2** to afford **1** (3 mg) and a sugar fraction. In the sugar fraction the presence of D-glucose was revealed as after hydrolysis of **2**.

3.4.1. 2-C-Methyl-D-erythritol 1-O-β-D-fructofuranoside (5)

Colorless syrup, $[\alpha]_D^{22}-28^\circ$ ($c=1.4$, MeOH). Positive FAB–MS m/z: 299.1331 $[M+H]^+$ (base, calc. for $C_{11}H_{23}O_9$; 299.1342), 245 $[M-3H_2O+H]^+$, 137 $[M-C_6H_{10}O_5+H]^+$. For ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) see Tables 1 and 2. HMBC correlations: H_2-1/C-2, C-3, C-5, Fru C-2; H-3/C-1, C-2, C-4, C-5; H-4a/C-2, C-3; H-4b/C-2; H_3-5/C-1, C-2, C-3.

3.5. Enzymatic hydrolysis of 5

A mixture of **5** (14 mg) and β-glucosidase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 30 days. The mixture was treated in the same way described for **2** to afford **1** (4 mg) and a sugar fraction. The sugar fraction was passed through Sephadex LH-20 (MeOH) to give a syrup, and HPLC [carbohydrate analysis (waters), detector: JASCO RI-930 detector and JASCO OR-990 chiral detector, solv.: MeCN–H_2O (9:1), 2 ml/min; t_R 5.5 min (same location as that of D-fructose)] showed the presence of D-fructose.

3.5.1. 2-C-Methyl-D-erythritol 3-O-β-D-fructofuranoside (6)

Colorless syrup, $[\alpha]_D^{22}-15^\circ$ ($c=0.7$, MeOH). Positive FAB–MS m/z: 321 $[M+Na]^+$, 299.1326 $[M+H]^+$ (base, calc. for $C_{11}H_{23}O_9$; 299.1342), 137 $[M-C_6H_{10}O_5+H]^+$. For ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2. HMBC correlations: H_2-1/C-2, C-3, C-5; H-3/C-1, C-2, C-4, C-5, Fru C-2; H_2-4/C-2, C-3; H_3-5/C-1, C-2, C-3.

3.6. Enzymatic hydrolysis of 6

A mixture of **6** (7 mg) and β-glucosidase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 14 days. The mixture was treated in the same way described for **2** to afford **1** (2 mg) and a sugar fraction. In the sugar fraction the presence of D-fructose was revealed as after hydrolysis of **5**.

3.6.1. 2-C-Methyl-D-erythritol 4-O-β-D-fructofuranoside (7)

Colorless syrup, $[\alpha]_D^{22}-19^\circ$ ($c=1.2$, MeOH). Positive FAB–MS m/z: 299.1331 $[M+H]^+$ (base, calc. for

$C_{11}H_{23}O_9$; 299.1342), 137 $[M-C_6H_{10}O_5+H]^+$. For ^1H NMR (pyridine-d_5, 500 MHz) ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2. HMBC correlations: H_2-1/C-2, C-3, C-5; H-3/C-1, C-2, C-4, C-5; H_2-4/C-2, C-3, Fru C-2; H_3-5/C-1, C-2, C-3.

3.7. Enzymatic hydrolysis of 7

A mixture of **7** (7 mg) and β-glucosidase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 14 days. The mixture was treated in the same way described for **2** to afford **1** (2 mg) and a sugar fraction. In the sugar fraction the presence of D-fructose was revealed as after the hydrolysis of **5**.

3.7.1. 2-C-Methyl-D-erythritol 1-O-β-D-(6-O-4-hydroxybenzoyl)glucopyranoside (*8*)

An amorphous powder, $[\alpha]_D^{22}-12^\circ$ ($c=1.6$, MeOH). IRν_{max}^{nujol} cm^{-1}: 3350 (OH), 1710 (ester carbonyl), 1600, 1420 (phenyl). Positive FAB–MS m/z: 457.1125 $[M+K]^+$ (calc. for $C_{18}H_{26}KO_{11}$; 457.1113), 441 $[M+Na]^+$ (base), 419 $[M+H]^+$. For ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2. HMBC correlations: H_2-1/C-2, C-3, C-5, Glc C-1; H-3/C-1, C-2, C-4, C-5; H_2-4/C-2, C-3; H_3-5/C-1, C-2, C-3; Glc H-1/C-1; Glc H_2-6/C-1″; H-2′/C-4′, C-6′, C-1″; H-3′/C-1′, C-4′, C-5′; H-5′/C-1′, C-3′, C-4′; H-6′/C-2′, C-4′, C-1″.

3.8. Alkaline hydrolysis of 8

Glycoside **8** (8 mg) was deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 6 h. **2** (3 mg) was obtained after Sephadex LH-20 (MeOH) CC.

3.8.1. 2-C-Methyl-D-erythritol 1-O-β-D-(6-O-4-methoxybenzoyl)glucopyranoside (*9*)

Amorphous powder, $[\alpha]_D^{22}-13^\circ$ ($c=0.3$, MeOH). Positive FAB–MS m/z: 471.1269 $[M+K]^+$ (calc. for $C_{19}H_{28}KO_{11}$; 471.1269), 455 $[M+Na]^+$, 433 $[M+H]^+$ (base). For ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2. HMBC correlations: H_2-1/C-2, C-3, C-5, Glc C-1; H-3/C-1; C-2, C-4, C-5; H_2-4/C-2, C-3; H_3-5/C-1, C-2, C-3; Glc H-1/C-1; Glc H_2-6/C-1″; H-2′/C-3′, C-4′, C-6′, C-1″; H-3′/C-1′, C-4′, C-5′; H-5′/C-1′, C-3′, C-4′; H-6′/C-2′, C-4′, C-1″, OCH_3/C-4′.

Acknowledgements

The authors thank Mr. Y. Takase and Dr. H. Suzuki of the Analytical Center of this University for NMR and MS measurements.

References

Charon, L., Hoeffler, J.F., Pale-Grosdemange, C., Rohmer, M., 2000. Deuterium-labelled isotopomers of 2-*C*-methyl-D-erythritol as tools for the elucidation of the 2-*C*-methyl-D-erythritol 4-phosphate pathway for isoprenoid biosynthesis. Biochem. J. 346, 737–742.

Disch, A., Schwender, J., Muller, C., Lichtenthaler, H.K., Rohmer, M., 1998. Distribution of the mevalonate and glyceraldehyde phosphate/pyruvate pathways for isoprenoid biosynthesis in unicellular algae and the cyanobacterium *Synechocystis* PCC 6714. Biochem. J. 333, 381–388.

Duvold, T., Cali, P., Bravo, J.M., Rohmer, M., 1997. Incorporation of 2-*C*-methyl-D-erythritol, a putative isoprenoid precursor in the mevalonate-independent pathway, into ubiquinone and menaquinone of *Escherichia coli*. Tetrahedron Lett. 38, 6181–6184.

Eisenreich, W., Sagner, S., Zenk, M.H., Bacher, A., 1997. Monoterpenoid essential oils are not of mevalonoid origin. Tetrahedron Lett. 38, 3889–3892.

Fontana, A., Kelly, M.T., Prasad, J.D., Andersen, R.J., 2001. Evidence for the biosynthesis of squalene *via* the methylerythritol phosphate pathway in *Streptomyces* sp. obtained from a marine sediment. J. Org. Chem. 66, 6202–6206.

Kitajima, J., Suzuki, N., Ishikawa, T., Tanaka, Y., 1998. New hemiterpenoid pentol and monoterpenoid glycoside of *Torillis japonica* fruit, and consideration of the origin of apiose. Chem. Pharm. Bull. 46, 1583–1586.

Rohmer, M., Seemann, M., Horbach, S., Bringer-Meyer, S., Sahm, H., 1996. Glyceraldehyde 3-phosphate and pyruvate as precursors of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis. J. Am. Chem. Soc. 118, 2564–2566.