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Sesquiterpenoids of *Torilis japonica* fruit

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Abstract

From the methanolic extract of *Torilis japonica* D. C. fruit (Umbelliferae), two eudesmane-type sesquiterpenoids were isolated together with five previously described sesquiterpenoids. From the results of spectral analyses, they were characterized as 4(15)-eudesmene-1β,5α-diol and 4α,15-epoxyeudesmane-1β,6α-diol, respectively. The absolute stereostructures of these sesquiterpenoids were elucidated by the modified Mosher's method. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Torilis japonica*; Umbelliferae; Sesquiterpenoid; 4(15)-Eudesmene-1β,5α-diol; 4α,15-Epoxyeudesmane-1β,6α-diol

1. Introduction

The fresh fruit of *Torilis japonica* D.C. is used as a substitute medicament for "She chuang zi", the fruit of *Cnidium monnieri* CUSSON (Umbelliferae) and which is a principal Chinese medicament prescribed as a stimulant and aphrodisiac, and for renal disorders in Japan. From the methanolic extract of the fruit, torilin (Chikamatu et al., 1969) a guaiane-type sesquiterpenoid angelate was isolated as its main sesquiterpenoid constituent. Along with torilin, a humulene-type, four germacrane-type, two eudesmane-type and four oppositane-type sesquiterpenoids (Itokawa et al., 1983a,b, 1986) were obtained. Furthermore, we reported the presence of nine guaiane-type sesquiterpenoid glycosides (Kitajima et al., 1998). Along with their glycosides, we also obtained three eudesmane-type sesquiterpenoids (**1**–**3**), three guaiane-type sesquiterpenoids (**4**–**6**) and a humulane-type sesquiterpenoid (**7**) in its free form. Among them, **2** and **3** are new, and **4**–**6** are newly isolated as their free forms. In this paper, we discuss the structures of **2** and **3** as well as the absolute stereostructures of **1**–**3**.

2. Results and discussion

The methanolic extract of the fresh fruit was suspended in water and then extracted with ether and ethyl acetate successively. From the ether extract, **1**–**3** and **7** were obtained, while **4**–**6** were isolated from the aqueous layer.

The formulae of the new and newly isolated compounds were obtained from analyses, the accurate mass number of $[M+H]^+$ ion peaks in the high-resolution positive FAB–MS.

Sesquiterpenoid **1** ($C_{15}H_{26}O_2$, mp 129–130 °C, $[\alpha]_D^{23}$ +32°) was identified as 4(15)-eudesmene-1β,6α-diol, by comparison of its ^1H and ^{13}C NMR data with those previously reported (Bohlmann et al., 1983; Gutierrez et al., 1988). It was also reported to occur in racemic form in the stem bark of *Lepidotrichilia volensii*, but the absolute stereostructures of these enantiomers had not been determined (Hoffmann et al., 1978).

Sesquiterpenoid **2** ($C_{15}H_{26}O_2$, mp 108–110 °C, $[\alpha]_D^{23}$ +108°) revealed the presence of one *tert*-methyl, two *sec*-methyls, five methylenes, three methines (one of which was oxygenated), two quaternary carbons (one of which was oxygenated) and one terminal-methylene group, following analysis of the ^1H, ^{13}C and ^{13}C–^1H correlation spectroscopy (COSY) NMR spectral data (Tables 1 and 2). From the analysis of heteronuclear multiple-bond correlation (HMBC) spectral data (correlation: H-1α/C-2, C-14; H-2α/C-1, C-3, C-4, C-10; H-3α/C-1, C-2, C-4, C-5, C-15; H-3β/C-2, C-4, C-15; H-9β/C-7, C-8, C-10, C-14; H_3-12/C-7, C-11, C-13; H_3-13/C-7, C-11, C-12; H_3-14/C-1, C-5, C-9, C-10; H_2-15/C-3, C-4, C-5), **2** was indicated to be an eudesmane-type sesquiterpenoid as **1**, with the position of the double bond and the hydroxyl groups being found to be C-4(15), C-1 and C-5, respectively. As

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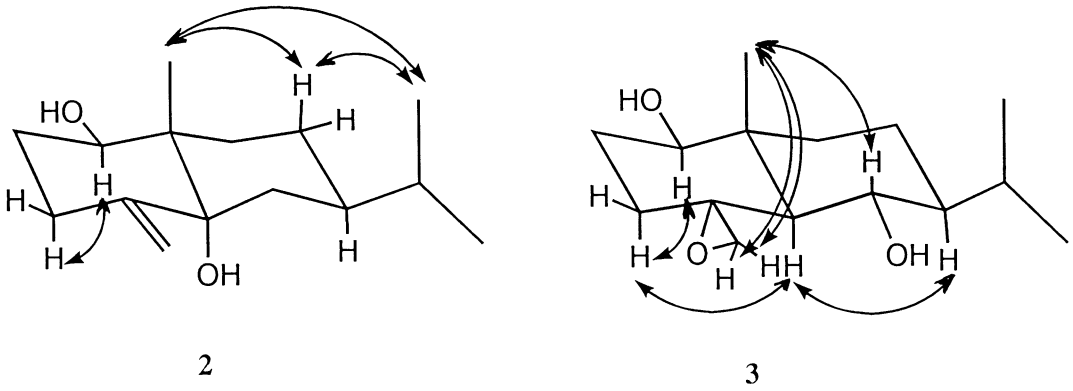

2 3

Fig. 1. Important NOE correlation for compounds **2** and **3**.

Table 1
^{1}H NMR spectral data for compounds **1**–**3**[a]

	1	**2**	**3**
H-1	3.42 (*dd*, *J* = 11.5, 4.5 Hz, α-H)	4.05 (*dd*, *J* = 11.0, 5.0 Hz, α-H)	3.41 (*dd*, *J* = 11.5, 5.0 Hz, α-H)
H-2	1.86 (*dddd*, *J* = 12.5, 5.0, 5.0, 4.5 Hz, α-H) 1.54 (*m*, β-H)	1.83 (*dddd*, *J* = 13.0, 5.0, 5.0, 2.0 Hz, α-H) 1.56 (*dddd*, *J* = 13.0, 13.0, 11.0, 5.0 Hz, β-H)	1.92 (*dddd*, *J* = 13.0, 5.0, 4.5, 2.5 Hz, α-H) 1.53 (*m*, β-H)
H-3	2.07 (*br ddd*, *J* = 13.5, 12.5, 5.0 Hz, α-H) 2.33 (*ddd*, *J* = 13.5, 5.0, 3.5 Hz, β-H)	2.70 (*ddddd*, *J* = 13.0, 13.0, 5.0, 1.5, 1.5 Hz, α-H) 2.15 (*br ddd*, *J* = 13.0, 5.0, 2.0 Hz, β-H)	2.08 (*dddd*, *J* = 13.5, 13.5, 5.0, 2.0 Hz, α-H) 1.32 (*br ddd*, *J* = 13.5, 5.0, 2.5 Hz, β-H)
H-5	1.75 (*d*, *J* = 10.0 Hz, α-H)		1.65 (*d*, *J* = 9.5 Hz, α-H)
H-6	3.72 (*dd*, *J* = 10.0, 10.0 Hz, β-H)	1.58 (*dd*, *J* = 13.5, 4.0 Hz, α-H) 1.54 (*ddd*, *J* = 13.5, 13.5 Hz, β-H)	3.43 (*dd*, *J* = 9.5, 9.5 Hz, β-H)
H-7	1.30 (*m*, α-H)	1.59 (*m*, α-H)	1.24 (*m*, α-H)
H-8	1.54 (*m*, α-H) 1.23 (*br ddd*, *J* = 12.0, 12.0, 3.0 Hz, β-H)	1.53 (*m*, α-H) 1.24 (*br ddd*, *J* = 13.0,13.0, 4.5 Hz, β-H)	1.52 (*m*, α-H) 1.21 (*m*, β-H)
H-9	1.17 (*ddd*, *J* = 13.0, 12.0, 3.0 Hz, α-H) 1.93 (*br dd*, *J* = 13.0, 3.0 Hz, β-H)	1.64 (*ddd*, *J* = 13.0, 13.0, 4.5 Hz, α-H) 1.70 (*br dd*, *J* = 13.0, 4.0 Hz, β-H)	1.16 (*br dd*, *J* = 13.5, 13.0 Hz, α-H) 1.87 (*ddd*, *J* = 13.5, 2.5, 2.5 Hz, β-H)
H-11	2.25 (*m*)	1.48 (*m*)	2.23 (*m*)
H_3-12	0.87 (*s*)	0.909 (*s*)	0.81 (*s*)
H_3-13	0.96 (*s*)	0.912 (*s*)	0.91 (*s*)
H_3-14	0.71 (*s*)	0.76 (*s*)	0.87 (*s*)
H_2-15	4.75, 5.02 (each *br d*, *J* = 1.0 Hz)	4.75, 4.85 (each *dd*, *J* = 1.5, 1.5 Hz)	3.22 (*dd*, *J* = 3.5, 2.0 Hz) 2.78 (*d*, *J* = 3.5 Hz)

[a] Solvent: $CDCl_3$.

nuclear Overhauser effect (NOE) interactions between the proton signals of H-1α and H-3α, H-8β and H_3-12, H-8β and H_3-14, and H_3-12 and H_3-14 were observed in its nuclear Overhauser and exchange spectroscopy (NOESY) spectrum (Fig. 1), and from the downfield shift of H-1α (by 0.63 ppm) and H-7α (by 0.29 ppm) signal to those in **1**, the configuration at the C-1, C-5 hydroxyl and C-7 isopropyl group should be β, α and β, respectively. Therefore, **2** was characterized as 4(15)-eudesmane-1β, 5α-diol.

Sesquiterpenoid **3** ($C_{15}H_{26}O_3$, mp 160–162 °C, $[\alpha]_D^{23}$ −34°) was also indicated to be an eudesmane-type sesquiterpenoid having oxygenated functions at C-1, C-4, C-6 and C-15 by analysis of the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) and the results of an HMBC experiment (correlation: H-1α/C-5, C-9, C-14; H-2α/C-1, C-3, C-4; H-3α/C-2, C-4, C-15; H-5/C-1, C-4, C-6, C-7, C-10, C-14, C-15; H-7/C-5, C-6; H-9β/C-5, C-7, C-8, C-10; H-11/C-7, C-8, C-12, C-13; H_3-12/C-7, C-11, C-

Table 2
^{13}C NMR spectral data for compounds **1**–**3**[a]

	1	**2**	**3**
C-1	79.02	73.13	78.05
C-2	31.91	30.58	29.32
C-3	35.10	29.79	33.27
C-4	146.24	150.63	61.61
C-5	55.87	76.18	46.77
C-6	67.01	34.32	67.65
C-7	49.32	38.28	49.65
C-8	18.14	23.68	17.96
C-9	36.28	29.95	36.82
C-10	41.69	42.26	41.92
C-11	25.99	32.82	25.01
C-12	16.18	19.72	15.91
C-13	21.11	20.01	20.99
C-14	11.60	12.73	12.14
C-15	107.82	108.64	51.58

[a] Solvent: $CDCl_3$.

13; H_3-13/C-7, C-11, C-12; H_3-14/C-1, C-5, C-9, C-10; H_2-15/C-3, C-4, C-5). The unsaturation degree of **3** and the chemical shifts of C-4 (δ 61.61) and C-15 (δ 51.58) [^{13}C NMR spectrum of the sesquiterpenoid ajugarin-V showed epoxy carbon peaks at δ 66.1 (C-4) and δ 52.3 (C-15) (Kubo et al., 1983)] showed the presence of an epoxy linkage between C-4 and C-15. As NOE interactions between H-1α and H-3α, H-3α and H-5α, H-5α and H-7α, H-6β and H_3-14, and H_3-14 and H_2-15 were observed in its NOESY spectrum (Fig. 1), the configuration at the C-1, C-6 hydroxyl, C-7 isopropyl and C-4 epoxy group should be β, α, β and α, respectively. From these facts, 3 was characterized as 4α,15-epoxy-eudesmane-1β,6α-diol. We then focused on the absolute structures of **1**–**3**.

The absolute configuration at the C-1 position in **1**–**3** was determined by means of the modified Mosher's method (Ohtani et al., 1991). Treatment of **1**–**3** with (−)-(*S*)- and (+)-(*R*)-2-methoxy-2-trifluoromethyl acetate acid (MTPA) chloride gave the 1-*O*-ester of (−)-(*S*)-MTPA (**1a**, **2a** and **3a**) and (+)-(*R*)-MTPA (**1b**, **2b** and **3b**), respectively. Signals due to protons on C-1 to C-3 and C-14 of **1a**, **2a** and **3a** appeared at a lower field than those of **1b**, **2b** and **3b**, while the proton signals due to the C-8, C-9, C-12 and C-13 of **1a**, **2a** and **3a** were observed at a higher field than those of **1b**, **2b** and **3b** (Fig. 2). Though the H_3-14 signals of **1a**, **2a** and **3a** were observed at a slightly lower field than those of **1b**, **2b** and **3b**, these shifts were considered to be the result of the conformation, in which the phenyl group of (−)-(*S*)-MTPA esters (**1a**, **2a** and **3a**) come closer to the 10-methyl group than that of (+)-(*R*)-MTPA esters (**1b**, **2b** and **3b**). Thererfore, the absolute configuration at C-1 of **1**–**3** was concluded to be *R*. Consequently, the absolute structures of **1**–**3** could described as shown in Fig. 2.

Sesquiterpenoid **4** ($C_{15}H_{24}O_3$, mp 180–182 °C, $[\alpha]_D^{21}$ −16°), **5** ($C_{15}H_{26}O_4$, mp 213–215 °C, $[\alpha]_D^{21}$ +10°) and **6** ($C_{15}H_{26}O_3$, amorphous powder, $[\alpha]_D^{21}$ +12°) were identified as torilolone, 2α,7,8β-trihydroxykessane and 2α,7-dihydroxykessane, respectively, by direct comparison with authentic samples which were obtained by the alkaline hydrolysis of torilin (Chikamatu et al., 1969) or enzymatic hydrolysis of their glycosides (Kitajima et al., 1998).

Sesquiterpenoid **7** ($C_{15}H_{24}O_2$, mp 170–172 °C, $[\alpha]_D^{21}$ +1°) was identified as 2,6-dihydroxyhumula-3(12),7(13),9(*E*)-triene by comparison of the ^{1}H and ^{13}C NMR spectral data with those of published values (Takeda et al., 1990). From the result of optical rotation, **7** was suggested to be an equilibrium mixture of (2*R*,6*S*)- and (2*S*,6*R*)-forms ($[\alpha]_D$ +74.3° and $[\alpha]_D$ −83.5°), this previously being a step for the same compound obtained from the red alga *Laurencia lobata* (Takeda et al., 1990).

3. Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. 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

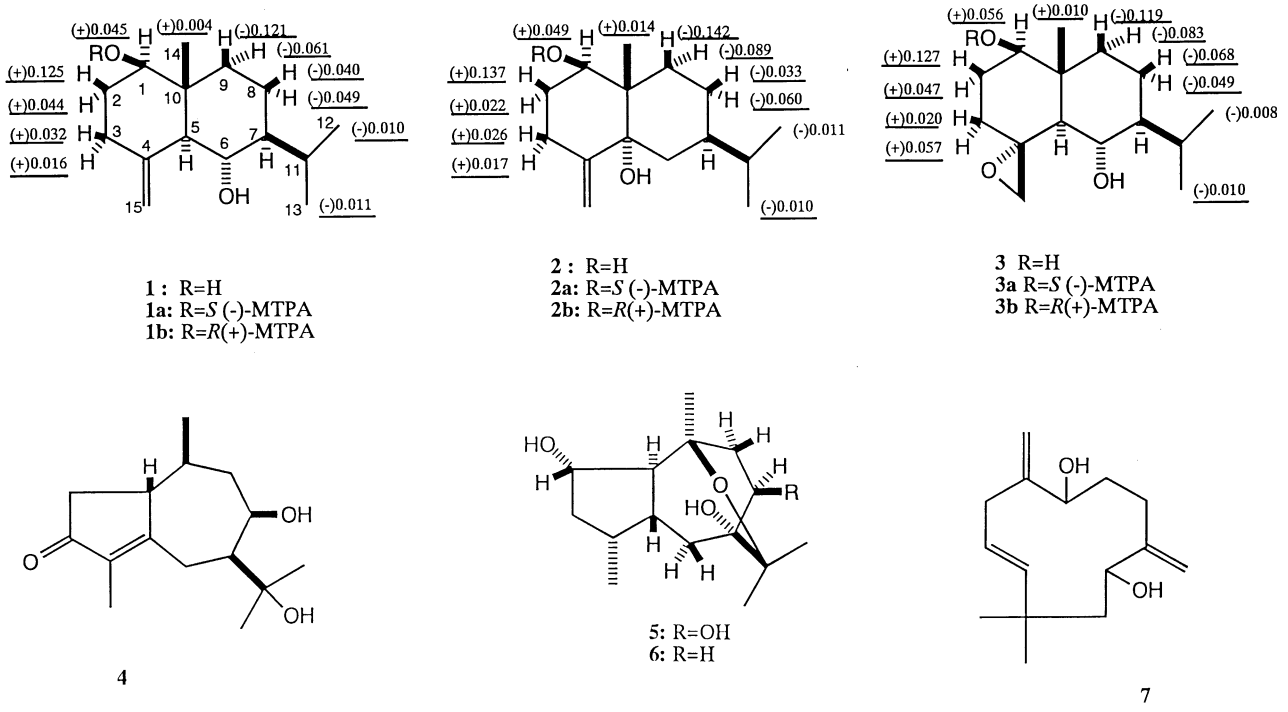

Fig. 2. Sesquiterpene structures with $\Delta\delta$ values in Hz [$=\delta S(-)$-$\delta R(+)$].

obtained with a JASCO A-103 IR spectrophotometer. ^1H and ^{13}C NMR spectra were taken on Jeol JNM GX-270 and A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ value. ^1H–^{13}C COSY, HMBC and NOESY spectra were obtained with the usual pulse sequence, and data processing was performed with standard Jeol software. Column chromatography was carried out under TLC monitoring using Kieselgel 60 (70–230 mesh, Merck), Sephadex LH-20 (25–100 mm, 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 on a JASCO chromatography (980-system) with a JASCO RI-930 detector, and ODS-3251-D [Senshu pak; column size, 8×250 mm] and carbohydrate analysis [Waters; column size, 3.9×300 mm] were used as column.

3.1. Extraction and isolation of sesquiterpenoids

The fruit of *Torilis japonica* D. C. was collected at Machida City, Tokyo, Japan, in July 1992. The fresh fruit (1090 g) was extracted with MeOH (6 l) at room temperature. After evaporation of the solvent, the residue (105.1 g) was suspended with H_2O and successively extracted with Et_2O and EtOAc. Removal of the solvent from each phase gave an ether (73.8 g), ethyl acetate (0.7 g) and an aqueous (30.6 g) residue. The ether extract was subjected to silica gel chromotography [eluted with *n*-hexane:EtOAc = (9:1→7:3→1:1)→ EtOAc→ MeOH] which furnished 13 fractions (frs. E_1–E_{13}). From fr. E_4 (2.72 g), torilin (2.07 g) was obtained by recrystallization from MeOH. Fr. E_5 (4.47 g) was purified by repeated silica gel ($CHCl_3$) and HPLC using ODS column [(CH_3CN–H_2O = 7:13) for **1** and (CH_3CN–H_2O = 9:11) for **2**] to afford **1** (27 mg) and **2** (10 mg). Fr. E_6 (2.39 g) was purified by repeated silica gel ($CHCl_3$–MeOH = 49:1) and Sephadex LH-20 (MeOH) to afford **3** (51 mg) whereas fr. E_7 (1.12 g) was obtained by repeated silica gel ($CHCl_3$–MeOH = 19:1) and an RP-8 column (MeOH-H_2O = 3:2) to give **7** (130 mg). The aqueous residue was next subjected to column chromatography on Amberlite XAD-II (H_2O→MeOH) to afford a water eluate (19.5 g) and a methanol eluated (11.1 g). The methanol eluate was reapplied to a Sephadex LH-20 column, elute with MeOH to furnish five fractions (frs. A_1–A_5). Fr. A_2 (9.3 g) was purified by repeated silica gel ($CHCl_3$–MeOH–H_2O = 4:1:0.1 and 7:3:0.5), Sephadex LH-20 (MeOH) and Lobar RP-8 column (MeOH–H_2O = 1:1) chromatographies. Final purification was completed by HPLC using carbohydrate analysis (CH_3CN–H_2O = 3:2) for **4** (10 mg) and ODS column (CH_3CN–H_2O = 1:4) for **5** (22 mg) and **6** (14 mg).

3.2. 4(15)-Eudesmene-1β,6α-diol (*1*)

Colorless needles (aq. MeOH), mp 129–130 °C, $[\alpha]_D^{23}$ +32° (c = 0.6, MeOH). IR ν_{max}^{nujol} cm^{-1}: 3400 (OH), 1650, 890 (exomethylene). Positive FAB–MS m/z: 239 $[M+H]^+$, 221 $[M-H_2O+H]^+$ (base), 203 $[M-2H_2O+H]^+$.

3.3. 4(15)-Eudesmene-1β,5α-diol (*2*)

Colorless needles (aq. MeOH), mp 108–110 °C, $[\alpha]_D^{23}$ +108° (c = 0.8, MeOH). IR ν_{max}^{nujol} cm^{-1}: 3400 (OH), 1650, 890 (exomethylene). Positive FAB–MS m/z: 239.1969 $[M+H]^+$ (calc. for $C_{15}H_{27}O_2$: 239.1966), 221.1907 $[M-H_2O+H]^+$ (base, calc. for $C_{15}H_{25}O$: 221.1906).

3.4. 4α,15-Epoxyeudesmene-1β,6α-diol (*3*)

Colorless needles (aq. MeOH), mp 160–162 °C, $[\alpha]_D^{23}$ −34° (c = 3.1, MeOH). IR ν_{max}^{nujol} cm^{-1}: 3400 (OH). Positive FAB–MS m/z: 509 $[2M+H]^+$, 277 $[M+Na]^+$, 255.1919 $[M+H]^+$ (base, calc. for $C_{15}H_{27}O_3$: 255.1915), 237 $[M-H_2O+H]^+$, 219 $[M-2H_2O+H]^+$.

3.5. 1-O-MTPA ester of *1*–*3*

Each solution of **1**–**3** (5 mg) in a mixture of $CHCl_3$ and pyridine (each 0.5 ml) was treated with *S*-(+)-MTPA-chloride (5 drops) and the mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with EtOAc, and the EtOAc extract was subjected to silica gel column chromatography (hexane–EtOAc = 4:1) to give **1a**–**3a**. In a similar procedure, **1b**–**3b** were obtained with *R*-(−)-MTPA-chloride.

3.6. Torilolone (*4*)

Colorless needles (aq. MeOH), mp 180–182 °C, $[\alpha]_D^{21}$ −16° (c = 1.4, MeOH). Positive FAB–MS m/z: 505 $[2M+H]^+$, 253.1797 $[M+H]^+$ (base, calc. for $C_{15}H_{25}O_3$: 253.1804), 235 $[M-H_2O+H]^+$.

3.7. Alkaline hydrolysis of Torilin

Torilin (430 mg) was hydrolysed with NaOH (0.37 g) in MeOH (30 ml) and treated in the same way described in the literature (Chikamatu et al., 1969) to afford **1** (29 mg).

3.8. 2α,7,8β-Trihydroxykessane (*5*)

Colorless needles (aq. MeOH), mp 213–215 °C, $[\alpha]_D^{21}$ +10° (c = 0.5, MeOH). Positive FAB–MS m/z: 271.1901 $[M+H]^+$ (Calcd for $C_{15}H_{27}O_4$: 271.1909), 253 $[M-H_2O+H]^+$, 235 $[M-2H_2O+H]^+$ (base), 217 $[M-3H_2O+H]^+$. Negative FAB-MS m/z: 269 $[M-H]^-$ (base).

3.9. 2α,7-Dihydroxykessane (**6**)

Amorphous powder, $[\alpha]_D^{21}$ +12° (c = 0.4, MeOH). Positive FAB–MS m/z: 277 $[M+Na]^+$, 255.1968 $[M+H]^+$ (calc. for $C_{15}H_{27}O_3$: 255.1960), 235 $[M-H_2O+H]^+$ (base). Negative FAB–MS m/z: 253 $[M-H]^-$ (base).

3.10. 3(12),7(13),9(E)-Humulatriene-2,6-diol (7)

Colorless needles (MeOH), mp 170–172 °C, $[\alpha]_D^{21}$ −1° (c = 1.5, MeOH). Positive FAB–MS m/z: 237 $[M+H]^+$, 219 $[M-H_2O+H]^+$ (base).

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