Cadinane-Type Sesquiterpenes from the Roots of *Taiwania cryptomerioides* HAYATA

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Five new cadinane-type sesquiterpenes, 15-acetoxy-T-muurolol (1), isokhusinodiol (2), cadin-10(14)-ene-4β,5α-diol (3), cadinane-4β,5α,10β-triol (4), and muurolane-4β,5β,10β-triol (5), together with five known compounds, T-cadinol (6), T-muurolol (7), α-cadinol (8), δ-cadinol (9), and khusinodiol (10), were isolated from the roots of *Taiwania cryptomerioides*. The structure of the new constituents were elucidated through chemical and spectral studies.

Key words *Taiwania cryptomerioides*; 15-acetoxy-T-muurolol; isokhusinodiol; cadin-10(14)-ene-4β,5α-diol; cadinane-4β,5α,10β-triol; muurolane-4β,5β,10β-triol

Taiwania cryptomerioides HAYATA (Taiwania, Taxodiaceae), an edemic plant, is an important building material. This plant occurs as one genus and one species in Taiwan. Due to the large amount of essential oil, the plant has antifungal and decay-resistant characteristics. We have previously investigated the chemical components of the heartwood[1—3] and bark[4—8] of this plant.

α-Cadinol, a major component of the heartwood of *T. cryptomerioides*, shows selectivity for human colon tumor cell lines.[9] In our previous reports of the search for biologically active substance, it was also found that lignans and cadinane-type compounds from this plant have significant cytotoxicity against three human tumor cell lines.[10] We also discovered α-cadinol with the highest activity against *Coriolus versicolor* (white-rot fungi) and *Laetiporus sulphurens* (brown-rot fungi) (100% antifungal indices at 100 ppm).[11] Since there were no chemical studies on the root of this plant or on cadinane derivatives showing biological activities to the best of our knowledge, we were encouraged to study the chemical constituents of its root. We report here five new cadinane-type derivatives, 15-acetoxy-T-muurolol (**1**), isokhusinodiol (**2**), cadin-10(14)-ene-4β,5α-diol (**3**), cadinane-4β,5α,10β-triol (**4**), and muurolane-4β,5β,10β-triol (**5**), together with five known components, T-cadinol (**6**),[12] T-muurolol (**7**),[12] α-cadinol (**8**),[12] δ-cadinol (**9**),[12] and khusinodiol (**10**).[13]

Compound **1** was isolated as an amorphous substance and showed a molecular ion at *m/z* (M^+ 280.2043) for $C_{17}H_{28}O_3$. The IR spectrum of **1** showed bonds attributable to an acetoxyl group (1738, 1240, 1032 cm^{-1}), a hydroxyl group (3441 cm^{-1}), and a trisubstituted olefinic group (1674 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) shows signals for an isopropyl group at δ 0.83, 0.87 (3H each, d, J=6.8 Hz) and 1.91 (1H, m, H-11), a three-proton singlet at δ 1.19 for a methyl attached to a quaternary carbon bearing an hydroxyl group, a trisubstituted olefinic proton at δ 5.88 (1H, d, J=6.0 Hz), an oxymethylene group at δ 4.43 (s) linked between the acetoxyl and olefinic groups, and an acetoxyl group at δ 2.05 (s). The ^{1}H–^{1}H correlated spectroscopy (COSY) of **1** displayed the connectivity of H-5 to H-6, which was also coupled to H-1 (δ 1.57, m) and H-7 (δ 1.26, m). The ^{13}C-NMR spectrum (Table 1) exhibited 17 carbon signals for an acetoxyl, three CH_3, five CH_2, two olefinic carbons, four CH, and one C. After removal of acetoxyl carbons, it can be proposed to be a cadinane-type sesquiterpene spectrum. By comparison of the ^{1}H- and ^{13}C-NMR data of **1** with 15-acetoxy-α-cadinol (**11**)[12] and 15-acetoxy-T-cadinol (**12**),[14] the structure of **1** was different from the above two cadinol derivatives. The *trans*-fused **11** and **12** were revealed from the presence of a broad singlet for the olefinic proton H-5. H-5 in **1** appears as a doublet coupling (J=6.0 Hz) with H-6, providing further evidence for the *cis*-fused muurolene derivative.[1,3] The signals of H_3-14 in T-muurolol and δ-cadinol are at δ 1.16 and 1.27, respectively.[12] In compound **1**, H_3-14 appeared at δ 1.17, similar to T-muurolol in an α-equatorial orientation. The further evidence is that H_3-14 exhibited a nuclear Overhauser enhancement and exchange spectroscopy (NOESY) (see structure **13**) correlation with H-2. This evidence confirmed that the methyl group is in an α-equatorial orientation. Thus, based on the above evidence, the structure of **1** was established to be 15-acetoxy-T-muurolol.

High-resolution mass spectroscopy (HR-EI-MS) revealed isokhusinodiol (**2**) to be a sesquiterpene with the formula $C_{15}H_{26}O_2$ [M^+ *m/z* 238.1935]. The IR spectrum of **2** displayed peaks for an hydroxyl (3407 cm^{-1}) and olefinic (1646 cm^{-1}) group. The ^{1}H-NMR signals (Table 1) of an isopropyl [δ 0.75 and 0.90 (3H each, d, J=7.2 Hz) and 2.09 (1H, m)], a singlet of methyl (δ 1.46, s) attached to the quarternary carbon bearing a hydroxyl group, a broad methyl (δ 1.67) linked on the olefinic group, and a trisubstituted olefinic proton (δ 5.47, br s) suggested it was a cadinane-type sesquiterpene. Fifteen ^{13}C-NMR signals (Table 1) appeared for four CH_3, three CH_2, six CH (including one oxygenated carbon at δ 70.9 and one olefinic carbon at δ 123.5), and two C (one olefinic carbon at δ 132.0 and one oxygenated carbon at 71.2). These data indicated that a secondary alcohol is located at C-2, C-3, C-8, or C-9 in compound **2**. Compound **2** is a *trans*-fused cadinol because the olefinic proton is br s,[1,3,9] and the carbinyl proton at δ 4.06 with triple doublets (J=9.6, 9.4, 5.6 Hz) can exclude the location of the hydroxyl group at C-3 and C-9. Based on the analysis of the proton-detected heteronuclear multiple-bond correlation (HMBC) spectrum, the signal at δ 1.20 was assigned to be H-1 which coupled with a carbinyl proton (J=9.6 Hz) and with H-6 (J=10.6 Hz). This evidence supported the *trans*-fused ring cadinol, and the hydroxyl group positioned at C-2 in the α-

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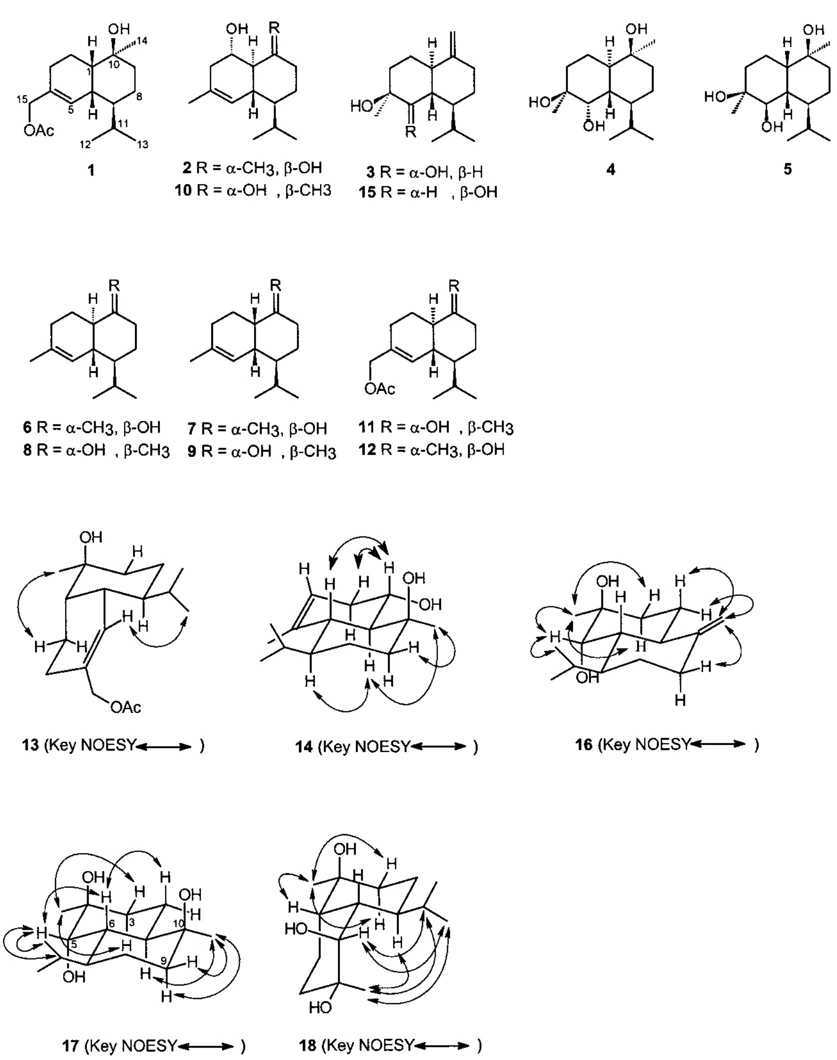

Chart 1

equatorial orientation. H-2 coupled H_2-3 with coupling constants 9.4 and 5.6 Hz, suggesting that it was in a β-axial orientation. On comparison of the ^{1}H- and ^{13}C-NMR data of **2** with khusinodiol (**10**), the only difference is the H-14 chemical shift. The chemical shift of H-14 in **2** showed a downfield shift (δ 1.46) compared with that of H-14 (δ 1.30) in khusinodiol (**10**). The conclusion is that the methyl group is in the β-axial orientation [as α-cadinol (**8**)] exhibited higher field (δ 1.30) than that of α-equatorial (H-14, δ 1.46) [as T-cadinol (**6**)]. The α-equatorial methyl group (H_3-14) exhibited NOESY correlation with H-1 (see structure **14**). The result confirmed the relative configuration. Therefore the structure of **2** is 2α-hydroxy-T-cadinol.

The ^{13}C-NMR spectrum of **3** (Table 1) exhibits 15 carbon signals for a double bond (δ 104.7, 152.9), two oxygenated carbons (C and CH, δ 71.6 and 73.0), three methyl groups, four methylenes, and four methines. The IR spectrum absorption bands at 3435, 3050, 1645, and 889 cm^{-1} are attributable to hydroxyl and terminal methylene groups. The signals at δ 0.73 and 0.93 (3H each, d, J=6.8 Hz, H-12, -13) and 2.02 (1H, m, H-11; COSY correlation with 0.73 and 0.97), together with the MS spectral base peak at m/z 159 $[M-2H_2O, -C_3H_7]^+$, established the presence of an isopropyl and two hydroxyl groups. A methyl singlet at δ 1.27 and a methine singlet at δ 3.55 exhibited attached carbon-bearing hydroxyl groups. Two doublet signals [δ 4.57 and 4.67 (1H each, d, J=1.6 Hz)] could be assigned to the terminal olefinic protons. HR-EI-MS revealed compound **3** to a sesquiterpene with the formula $C_{15}H_{26}O_2$. Three indices of hydrogen deficiency (IHD) were determined from the distortionless enhancement by polarization transfer (DEPT) experiment and HR-EI-MS. The signal at δ 1.27 (H_3-15) had an HMBC correlation with δ 71.6 (C-4) and 73.0 (C-5), and H-5 (δ 3.55) had an HMBC correlation with δ 71.6 (C-4) and 28.0 (C-15). The evidence suggests that it is 1,2-glycol. By the analysis of HMQC and HMBC spectra, the gross structure of **3** was proposed to be cadin-10(14)-ene-4,5-diol or cadin-4(15)-ene-9,10-diol. Based on the comparison of ^{1}H- and ^{13}C-NMR data, compound **3** is similar in structure to **15**.[15] Analysis of the NOESY spectrum of **3**, and the correlation between the carbinyl proton and H-11 (and -12) led to the conclusion that a secondary alcohol is located at C-5. The *trans*-fused cadinane type was revealed from the NOESY correlation between δ 4.57 (H-14) and δ 1.66 (H_2-2) (see structure **16**). A NOESY correlation between H_3-15 and H_2-3 and no correlation of H_3-15/H-6 were observed. The evidence confirmed that H_3-15 was in the α-equatorial orientation. Finally, H-5 was assigned to be in the β-equatorial ori-

Table 1. ^1H- and ^{13}C-NMR Spectral Data of Compounds **1**—**5** (400 MHz and 100 MHz in $CDCl_3$)

No.	**1**		**2**		**3**		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.57 m	45.9	1.20 dd (10.6, 9.6)	54.0	1.94 m	38.0	1.29 m	41.8	1.78 m	42.3
2	1.62 m 2.10 m	20.4	4.06 ddd (9.6, 9.4, 5.6)	70.9	1.56 m 1.66 m	23.9	1.51 m 1.61 m	20.2	1.36 m 1.60 m	19.8
3	2.08 m	27.1	2.09 m 2.26 dd (16.6, 5.6)	41.5	1.52 m 1.71 m	32.4	1.48 m 1.70 m	32.4	1.48 m	34.7
4		132.4		132.0		71.6		71.3		72.2
5	5.88 d (6.0)	129.9	5.47 br s	123.5	3.55 s	73.0	3.58 d (2.3)	73.0	3.78 br s	72.3
6	2.37 m	34.4	1.97 m	38.3	1.55 m	44.2	1.80 ddd (11.2, 11.2, 2.3)	37.4	2.03 m	42.0
7	1.26 m	43.7	1.30 m	45.4	1.52 m	42.5	1.31 m	42.4	1.89 dddd (12.0, 11.6, 3.6, 3.2)	38.0
8	1.37 m 1.54 m	19.4	1.30 m 1.41 m	19.4	1.09 m 1.80 m	26.1	1.37 m 1.48 m	19.1	1.25 m 1.50 m	20.0
9	1.42 m 1.54 m	34.5	1.42 m 1.62 m	41.4	1.97 m 2.35 dd (12.8, 3.6)	36.4	1.37 m 1.68 m	40.3	1.48 m 1.70 dd (12.8, 4.0)	34.7
10		72.2		71.2		152.9		70.9		72.1
11	1.91 m	26.7	2.09 m	26.9	2.02 m	25.8	2.00 m	25.7	2.07 sep d (6.8, 3.2)	26.9
12	0.83 d (6.8)	15.4	0.75 d (7.2)	15.2	0.73 d (6.8)	15.4	0.79 d (6.9)	15.5	0.78 d (6.8)	14.9
13	0.87 d (6.8)	21.5	0.90 d (7.2)	21.5	0.93 d (6.8)	21.6	0.92 d (6.9)	21.5	0.88 d (6.8)	21.5
14	1.19 s	29.4	1.46 s	32.1	4.67 d (1.6) 4.57 d (1.6)	104.7	1.18 s	28.8	1.18 s	29.5
15	4.43 s	68.9	1.67 br s	23.1	1.27 s	28.0	1.25 s	27.7	1.24 s	29.1
$OCO\underline{CH}_3$	2.05 s	21.0								
$O\underline{C}OCH_3$		171.0								

entation because its spilling pattern is singlet. The structure of **3** is cadin-10(14)-ene-4β,5α-diol.

Compound **4** was isolated as a colorless needle (mp 186—188 °C) and had a molecular ion at *m/z* 256, as analyzed for $C_{15}H_{28}O_3$ from high-resolution mass spectroscopy. Analysis of its IR spectrum suggested that **4** contains a hydroxyl (3441 cm^{-1}) and isopropyl groups (1385, 1376 cm^{-1}). Only *sp*^{3} carbon signals, including three oxygenated carbon signals [two C (δ 71.3, 70.9) and one CH (δ 73.0)] were observed in the ^{13}C-NMR spectrum (Table 1) of **4**. Two IHD were determined from the ^{13}C-NMR spectrum and DEPT experiments. Three oxygen atoms all had the hydroxyl form. Two methyl groups [δ 1.18 and 1.25 (s each)] and an isopropyl group [δ 0.79, 0.92 (d, J=6.9 Hz), 2.00 (1H, m, COSY correlation with δ 0.79 and 0.92)] were revealed from its ^1H-NMR spectrum. In addition to four CH_2 and two C signals (^{13}C-NMR), compound **4** was assumed to be a cadinane-type sesquiterpene with two hydroxyl groups located at C-4 and C-10, respectively. The HMBC correlation [H-5 (δ_H 3.58)/δ_C 27.7 (C-15), 71.3 (C-4); H-15 (δ_H 1.25)/δ_C 71.3 (C-4), 73.0 (C-5)] assigned the glycol to be 1,2-diol, and δ_H 3.58 (d, J=2.3 Hz) has NOESY (see structure **17**) correlation with H-11, H-12, and H-13 showing this carbinyl proton locating at C-5. H-5 (δ 3.58) had COSY correlation with δ 1.80 (H-6; this location was confirmed by the HMBC spectrum). The signal at δ 1.80 expressed a triple-doublet (J=11.2, 11.2, 2.3 Hz) coupling pattern. This evidence gave the *trans*-fusion conformation for compound **4**. A small coupling constant (J=2.3 Hz) between H-5 and H-6 clarified H-5 to be in the β-equatorial orientation. H_3-15 (δ 1.25) exhibited NOESY correlation with H_2-3 and H-5 and no NOESY correlation with H-6, and the evidence established H_3-15 to be in the α-equatorial orientation. Finally, H_3-14 is in the α-equatorial orientation since there is no NOESY correlation with H-6. Therefore the structure of **4** is assigned to be cadinane-4β,5α,10β-triol.

Fifteen ^{13}C-NMR signals and exact mass spectrum data confirmed the molecular formula of **5** to be $C_{15}H_{28}O_3$. Compound **5** exhibited all *sp*^{3} carbon signals in ^{13}C-NMR and an hydroxyl absorption band (3418 cm^{-1}) in its IR spectrum. Two IHD (from the DEPT experiment), ^{13}C-NMR data, and the molecular formula indicated that **5** is a sesquiterpene with three hydroxyl groups. The signals at δ 1.18 (3H, s), 1.24 (3H, s), 0.78 and 0.88 (3H each, d, J=6.8 Hz), and 2.07 (1H, H-11, with COSY correlation with δ 0.78 and 0.88) suggested that **5** has an isopropyl group and two singlet methyl groups attached to an oxygenated quartenary carbon. In addition to four CH_2, three CH, and a carbinyl carbon (δ 72.3), the structure of **5** was suggested to be a trihydroxycadinane, with two hydroxyl groups located at C-4 and C-10, due to the above evidence. The 1,2-glycol was revealed from the HMBC correlation [H-5 (δ_H 3.78)/δ_C 29.1 (C-15), 72.2 (C-4); H-15 (δ_H 1.24)/δ_C 72.3 (C-5), 72.2 (C-4)] and NOESY correlation (δ_H 3.78/δ_H 1.24). The structure of **5** was assumed to be a cis-fused muurolane type due to the NOESY correlation between H-15 and H-7, -11, -12, and -13. The evidence also confirmed that H_3-15 is in the α-axial orientation. Two H-9 signals also showed NOESY correlation with H_3-14, and α-equatorial H_3-14 was established. The presence of a carbinyl proton was suggested at C-5 with the α-equatorial orientation based on the following evidence. The carbinyl proton (δ 3.78, br s) showed NOESY (see structure **18**) correlation with H-11 (δ 2.07, sep d, J=6.8, 3.2 Hz) and H-12 (-13) (δ 0.78, 0.88). Based on the above evidence, muurolane-4β,5β,10β-triol is the structure of compound **5**.

Experimental

General Experimental Procedures Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity Plus 400 at 400 and 100 MHz in $CDC1_3$ solution with tetramethylsilane as an internal standard. EI-MS, FAB-MS, UV, and specific rotations were taken on a JEOL JMS-HX 300, a JEOL JMS-HX 300, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM).

Extraction and Isolation An air-dried of slice of *T. cryptomerioides* (15 kg) was extracted two times with acetone (125 l) at room temperature (7 d each time). The acetone extract was evaporated *in vacuo* to leave a black residue, which was suspended in H_2O (7 l), and then partitioned (3 times) with 1 l of ethyl acetate. The EtOAc fraction (365 g) was chromatographed on silica gel using a mixture of *n*-hexane and EtOAc of increasing polarity as eluent and further purified by HPLC. Ten components, T-cadinol (**6**) (1.5 g), T-muurolol (**7**) (1.5 g), α-cadinol (**8**) (20.5 g), δ-cadinol (**9**) (0.8 g), 15-acetoxy-T-muurolol (**1**) (5 mg), isokhusinodiol (**2**) (6 mg), cadin-10(14)-ene-4β,5α-diol (**3**) (7 mg), khusinodiol (**10**) (9 mg), cadinane-4β,5α,10β-triol (**4**) (10 mg), and muurolane-4β,5β,10β-triol (**5**) (7 mg) were eluted with 20%, 20%, 20%, 20%, 20%, 30%, 30%, 30%, 40%, and 40% EtOAc in a hexane solvent system, respectively.

15-Acetoxy-T-muurolol (**1**): Amorphous solid, $[\alpha]_D^{31}$ $-51.2°$ (c=0.08, $CHCl_3$). IR ν_{max} cm^{-1}: 3441, 1738, 1674, 1378, 1240, 1032. ^{1}H- and ^{13}C-NMR data see Table 1. EI-MS (70 eV) (rel. int. %) *m*/*z*: 280 (M$^+$, 2), 262 (5), 220 (10), 202 (63), 177 (23), 159 (100); HR-EI-MS *m*/*z*: 280.2043 (M$^+$, Calcd for $C_{17}H_{28}O_3$: 280.2039).

Isokhusinodiol (**2**): mp 105—107 °C, $[\alpha]_D^{27}$ $-5.9°$ (c=0.26, $CHCl_3$). IR ν_{max} cm^{-1}: 3407, 1646, 1386, 1373. ^{1}H- and ^{13}C-NMR data, see Table 1. EI-MS (70 eV) (rel. int. %) *m/z*: 238 (M$^+$, 1), 220 (76), 177 (100), 159 (87), 149 (74), 135 (42), 121 (38). HR-EI-MS *m/z*: 238.1935 (M$^+$, Calcd for $C_{15}H_{26}O_2$: 238.1926).

Cadin-10(14)-ene-4β,5α-diol (**3**): Amorphous solid, $[\alpha]_D^{27}$ $-5.9°$ (c=0.26, $CHCl_3$), IR ν_{max} cm^{-1}: 3435, 3050, 1646, 1389, 1375, 889. ^{1}H- and ^{13}C-NMR data see Table 1. EI-MS (70 eV) (rel. int. %) *m/z*: 238 (M$^+$, 1), 177 (22), 159 (100). HR-EI-MS *m/z*: 238.1937 (M$^+$, Calcd for $C_{15}H_{26}O_2$: 238.1926).

Cadinane-4β,5α,10β-triol (**4**): mp 186—188 °C, $[\alpha]_D^{27}$ $-6.0°$ (c=0.26, $CHCl_3$). IR ν_{max} cm^{-1}: 3441, 1385, 1376, 1163, 1021. ^{1}H- and ^{13}C-NMR data, see Table 1. EI-MS (70 eV) (rel. int. %) *m/z*: 256 (M$^+$, 4), 238 (39), 220(6), 195(36), 177 (69), 159 (79), 151 (81), 137 (100). HR-EI-MS *m/z*: 256.2039 (M$^+$, Calcd for $C_{15}H_{28}O_3$: 256.2039).

Muurolane-4β,5β,10β-triol (**5**): mp 168—170 °C, $[\alpha]_D^{18}$ $-6.4°$ (c=0.14, $CHCl_3$). IR ν_{max} cm^{-1}: 3418, 1382, 1371, 1119, 1049. ^{1}H- and ^{13}C-NMR data see Table 1. EI-MS (70 eV) (rel. int. %) *m/z*: 256 (M$^+$, 1), 238 (24), 220 (56), 177 (100), 159 (75). HR-EI-MS *m/z*: 256.2037 (M$^+$, Calcd for $C_{15}H_{28}O_3$: 256.2039).

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