Three New Diterpenoids from the Leaves of *Thuja orientalis*

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Abstract

Three new diterpenoids, 18-formyloxy-8*β*-hydroxysandaracopimar-15-ene (**1**), 15(*R*)-*n*-butoxypinusolidic acid (**2**), and 15,16-dihydro-15,16-dimethoxylambertianic acid (**3**), along with twelve known compounds (**4**–**15**) were isolated from MeOH extracts of leaves of *Thuja orientalis* L. The structures of the three new compounds were elucidated on the basis of spectroscopic analyses, including extensive 2D-NMR data. The absolute stereochemistry of compound **2** was clarified by a CD spectroscopic study. The isolated compounds were evaluated for cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines *in vitro* using the sulforhodamin B (SRB) bioassay.

Key words

Thuja orientalis · Cupressaceae · diterpene · cytotoxicity

Supporting information available online at
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Thuja orientalis L. (Cupressaceae) is an evergreen arbor that is widely distributed throughout Korea. This indigenous plant has been used as a Korean traditional medicine for the treatment of hypertension, hematemesis, epistaxis, and hemorrhoids [1]. Diterpenoids [2], essential oils [3], lignans [4], and flavonoids [5] were isolated from this plant and some of them showed neuroprotective [4], antiaggregative [6], antifibrotic [2], and fungitoxic [3] activities. In the course of our continuing search for biologically active compounds from natural Korean medicinal sources, we investigated MeOH extracts of the leaves of *T. orientalis.* The purification of the *n*-hexane-soluble fraction led to the isolation of three new diterpenoids (**1**–**3**) (**Fig. 1**) and twelve known compounds (**4**–**15**), which were identified on the basis of spectroscopic analyses. The isolated compounds were tested for cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cancer cell lines using the sulforhodamin B (SRB) bioassay.

Compound **1** showed a molecular ion at *m/z* 334.2511 $[M]^+$ in its HR-EI-MS (calcd. for 334.2508), suggesting a molecular formula of $C_{21}H_{34}O_3$. The IR spectrum indicated the presence of hydroxyl (3541 cm^{-1}), olefin (3083, 1635 cm^{-1}), and aldehydic carbonyl (1695 cm^{-1}) groups. The ^1H and ^{13}C-NMR data (**Tables 1** and **2**) were very similar to those of 8*β*-hydroxysandaracopimarene (**4**), which was isolated from *Dysoxylum lenticellare* [7]. The major differences were the appearance of the oxygenated carbon and proton signals (δ_C 72.4; δ_H 3.73 and 3.98, respectively) and the aldehydic carbon and proton signals (δ_C 161.4; δ_H 8.10, respectively), as well as the disappearance of the methyl carbon and proton signals (δ_C 33.8; δ_H 0.85, respectively). The correlation (H-CHO/C-18) in the HMBC spectrum confirmed the position of the formyloxy group at C-18 (**Fig. 1S**). The relative stereochemistry was assumed to be the same as that of **4** based on the *J* values. The ROESY correlations of H-5/H-9 and H-18, H-20/H-19, and H-11 (δ_H 1.65)/H-17 and H-20 confirmed the relative stereochemistry (**Fig. 1S**). The relative configuration of the formyloxy methylene group at C-4 was determined to be *α* by the ROESY correlation of H-5/H-18. The *β*-form of the OH group at C-8 was reconfirmed by observing the pyridine-induced solvent shift; the chemical shifts of H-17 and H-20 measured in pyridine-d_5 showed a downfield shift compared with those measured in $CDCl_3$ ($\Delta\delta = \delta CDCl_3 - \delta$pyridine-$d_5$; H-17, $\Delta\delta$ – 0.22; H-20, $\Delta\delta$ – 0.17) [8]. Therefore, the structure of **1** was established as 18-formyloxy-8*β*-hydroxysandaracopimar-15-ene.

Compound **2** showed a molecular ion at *m/z* 405.2642 $[M + H]^+$ in its HR-FAB-MS (calcd. for 405.2641), suggesting a molecular formula of $C_{24}H_{36}O_5$. The IR spectrum showed the presence of a carboxylic carbonyl (1692 cm^{-1}), an olefin (3078, 1645 cm^{-1}), and an *α,β*-unsaturated *γ*-lactone (1768 cm^{-1}) group. The ^1H and ^{13}C-NMR data (**Table 1** and **2**) were quite similar to those reported for 15-methoxypinusolidic acid, which was isolated from *Calocedrus microlepic* var. *formosana* [9]. The major differences were the absence of the methoxy carbon and proton signals (δ_C 56.9; δ_H 3.55, respectively) and the presence of *n*-butoxy carbon [δ_C 70.3 (C-21), 31.8 (C-22), 19.3 (C-23), and 14.0 (C-24)] and proton [δ_H 3.66 (m, H-21a), 3.86 (m, H-21b), 1.62 (m, H-22), 1.40 (m, H-23) and 0.93 (t, H-24)] signals. The ^1H-^1H COSY correlations (H-22/H-21 and H-23, and H-23/H-24) and the HMBC correlations (H-21/C-15, C-22 and C-23 and H-24/C-22 and C-23) supported the position of the *n*-butoxy group at C-15 (**Fig. 2S**). The relative stereochemistry was assumed to be the same as that of 15-methoxypinusolidic acid [9] based on the *J* values. The NOESY correlations confirmed the stereochemistry (**Fig. 2S**). The negative

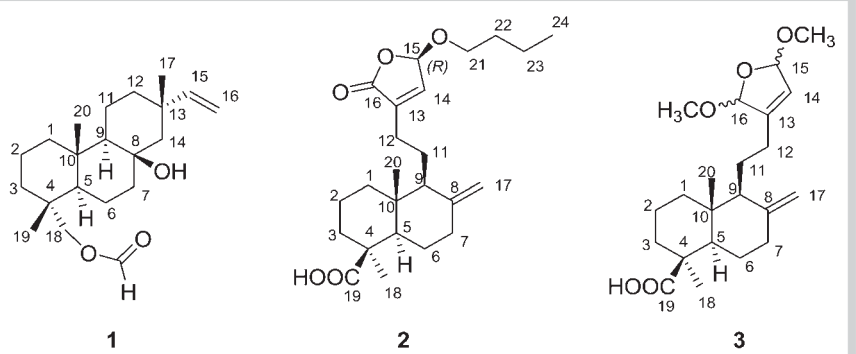

Fig. 1 Structures of new compounds **1**–**3** from the leaves of *T. orientalis*.

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

H	1	2	3
1	0.85 m, 1.75 m	1.06 m, 1.89 m	1.10 m, 1.90 m
2	1.50–1.70 m	1.51 m, 1.84 m	1.55 m, 1.84 m
3	1.30–1.42 m	1.04 m, 2.16 m	1.12 m, 2.18 m
5	1.19 m	1.33 m	1.36 m
6	1.45–1.70 m	1.89 m, 2.00 m	1.91 m, 2.00 m
7	1.40 m, 1.66 m	1.90 m, 2.44 m	1.90 m, 2.43 m
9	0.91 m	1.62 m	1.65 m
11	1.50 m, 1.65 m	1.58 m, 1.77 m	1.59 m, 1.78 m
12	1.33 m, 1.60 m	2.10 m, 2.50 m	1.88 m, 2.35 m
14	1.28 m, 1.35 m	6.77 brs	5.67 brs
15	5.71 dd (17.5, 10.5)	5.79 brs	5.40 brs
16	4.82 dd (10.5, 1) 4.87 dd (17.5, 1)		5.53 brs
17	1.22 s	4.57 brs, 4.89 brs	4.51 brs, 4.86 brs
18	3.73 d (11) 3.98 d (11)	1.24 s	1.24 s
19	0.89 s		
20	1.04 s	0.60 s	0.61 s
21		3.66 m, 3.86 m	
22		1.62 m	
23		1.40 m	
24		0.93 t (7.5)	
CHO	8.10 s		
15-OCH_3			3.38 s
16-OCH_3			3.42 s

[a] ^{1}H-NMR run at 500 MHz in $CDCl_3$. Chemical shifts are given in δ values. Proton coupling constants (*J*) in Hz are given in parentheses

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

C	1	2	3
1	39.1 t	39.4 t	39.4 t
2	18.0 t	20.1 t	20.1 t
3	36.0 t	38.2 t	38.3 t
4	36.8 s	44.4 s	44.4 s
5	50.3 d	56.5 d	56.5 d
6	17.8 t	26.2 t	26.3 t
7	43.4 t	38.8 t	38.9 t
8	72.7 s	147.5 s	147.8 s
9	57.2 d	55.9 d	56.2 d
10	37.4 s	40.7 s	40.8 s
11	17.3 t	21.9 t	21.9 t
12	38.3 t	24.8 t	25.9 t
13	36.7 s	139.2 s	146.3 s
14	51.8 t	142.0 d	123.5 d
15	151.7 d	102.0 d	108.4 d
16	108.8 t	171.7 s	107.1 d
17	24.5 q	107.0 t	106.9 t
18	72.4 t	29.2 q	29.2 q
19	17.7 q	183.4 s	182.0 s
20	16.2 q	13.0 q	13.0 q
21		70.3 t	
22		31.8 t	
23		19.3 t	
24		14.0 q	
CHO	161.4 d		
15-OCH_3			53.8 q
16-OCH_3			54.6 q

[a] ^{13}C-NMR run at 125 MHz in $CDCl_3$. Chemical shifts are given in δ values

Cotton effect at 252 nm in the CD spectrum determined the absolute configuration at C-15 as the *R* form [10]. Thus, the structure of **2** was elucidated as 15(*R*)-*n*-butoxypinusolidic acid.

Compound **3** showed a molecular ion at *m/z* 401.2304 $[M + Na]^+$ in its HR-FAB-MS (calcd. for 401.2305), suggesting a molecular formula of $C_{22}H_{34}O_5$. The IR spectrum indicated the presence of a carboxylic carbonyl (1691 cm^{-1}) and an olefin (3078, 1641 cm^{-1}) group. The ^{1}H and ^{13}C-NMR data (**Tables 1** and **2**) were very similar to those reported for 15-methoxypinusolidic acid [9] and **2**. By comparison with the NMR data of 15-methoxypinusolidic acid, the major differences were the disappearance of the carbonyl carbon signal (δ_C 171.3) and the presence of the methine carbon (δ_C 107.1), methoxy carbon (δ_C 54.6), and methine proton (δ_H 5.53) signals. The HMBC correlations (H-14/C-12 and C-13, H-15/C-14, C-16 and C-15-OCH_3, and H-16/C-13 and C-16-OCH_3) confirmed the position of the 2,5-dihydro-2,5-dimethoxyfuran ring (**Fig. 3S**). The relative stereochemistry was assumed to be the same as that of 15-methoxypinusolidic acid [9] based on the *J* values. The ROESY correlations of H-5/H-9 and H-11/H-20 confirmed the stereochemistry (**Fig. 3S**), but the stereochemistries at C-15 and C-16 could not be determined. Thus, the structure of **3** was established as 15,16-dihydro-15,16-dimethoxylambertianic acid.

Compounds **4**–**15** were identified by comparing the ^{1}H and ^{13}C-NMR, and MS spectra with the literature data. They were determined to be 8β-hydroxysandaracopimarene (**4**) [7], 8(14),15-isopimaradien-3β,19-diol (**5**) [11], pimara-8(14),15-dien-3β-ol (**6**) [12], isopimara-7,15-dien-3β-ol (**7**) [13], isopimara-8(9),15-dien-3β-ol (**8**), 15-isopimaren-3β,8β-diol (**9**) [11], abietatriene-3β-ol (**10**) [14], 7α-hydroxysandaracopimaric acid (**11**) [15], pinusolide (**12**) [16], *trans*-communic acid (**13**) [17], *cis*-communic acid (**14**) [18], and 14,15-dinor-13-oxo-8(17)-labden-19-oic acid (**15**) [19].

The cytotoxicity of the compounds (**1**–**15**) against A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT-15 (colon cancer cells) human tumor cell lines were evaluated using the SRB assays *in vitro* [20]. Compounds **8, 10**, and **12** showed moderate cytotoxicity, with IC_{50} values ranging from 5.72 µM to 34.84 µM. New compounds **1** and **2** exhibited selective cytotoxicity against A549 cell lines (IC_{50}: 26.05 µM and 27.51 µM, respectively) and **3** against A549 (IC_{50}: 25.61 µM) and SK-MEL-2 (IC_{50}: 23.17 µM) cell lines (**Table 1S**).

Materials and Methods

The leaves of *T. orientalis* were collected in Yeongcheon City, Korea during May 2009, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU NPL 0819) of the plant was deposited at the herbarium of the School of Pharmacy at the Sungkyunkwan University, Suwon, Korea. The plant material (4 kg) was extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (405 g), which was dissolved in water (800 mL × 2) and solvent-partitioned to give *n*-hexane (73 g), $CHCl_3$ (41 g), EtOAc (42 g), and *n*-BuOH (104 g) fractions. The *n*-hexane fraction (36 g) was applied to repeated column chromatography to purify the compounds **1**-**15** (Supporting Information).

Isolates

18-formyloxy-8β-hydroxysandaracopimar-15-ene (**1**): White powder, mp 124 °C; $[\alpha]_D^{25}$ +7.3 (*c* 0.35, $CHCl_3$); IR (KBr) ν_{max} 3541, 3083, 2936, 2908, 2868, 2847, 1695, 1635, 1463, 1387, 1254, 1192, 1043, 987, 968, 929, 905, 850, 771 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz), see **Table 1**; ^{13}C-NMR ($CDCl_3$, 125 MHz), see **Table 2**; HR-EI-MS *m/z* 334.2511 $[M]^+$ (calcd. for 334.2508).

15(R)-n-butoxypinusolidic acid (**2**): Colorless gum, UV (CH_3CN) λ_{max} (log ε) 221.0 (6.10), 248.0 (1.02) nm; $[\alpha]_D^{25}$ +29.5 (*c* 0.35, $CHCl_3$); CD (CH_3CN) λ_{max} ($\Delta\varepsilon$) 252.0 (−1.04), 216.0 (+0.20), 207.0 (+0.16) nm; IR (KBr) ν_{max} 3078, 2957, 2933, 2873, 2848, 1768, 1692, 1645, 1466, 1343, 1263, 1144, 1086, 1029, 931, 889, 771 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz), see **Table 1**; ^{13}C-NMR ($CDCl_3$, 125 MHz), see **Table 2**; HR-FAB-MS *m/z* 405.2642 $[M + H]^+$ (calcd. for 405.2641).

15,16-dihydro-15,16-dimethoxylambertianic acid (**3**): Colorless gum, $[\alpha]_D^{25}$ +36.6 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3078, 2932, 2845, 1691, 1641, 1263, 1164, 1099, 1029, 970, 942, 891, 772 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz), see **Table 1**; ^{13}C-NMR ($CDCl_3$, 125 MHz), see **Table 2**; HR-FAB-MS *m/z* 401.2304 $[M + Na]^+$ (calcd. for 401.2305).

The positive controls, doxorubicin and gemcitabine (purity ≥ 98 %), were purchased from Sigma. Tested compounds were demonstrated to be pure as evidenced by NMR and HPLC analyses (purity ≥ 95 %).

Supporting information

The general experimental procedures, isolation details, ^{1}H and ^{13}C-NMR, DEPT, HMQC, HMBC, ^{1}H-^{1}H COSY, NOESY, and ROESY spectra for new compounds **1**–**3**, structures of the twelve known compounds **4**–**15**, and CD spectrum of **3** are available as Supporting Information.

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Conflict of Interest

All authors declare that there are no conflicts of interest.

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