Labdane-Type Diterpenes and a Nordrimane-Type Sesquiterpene from the Stem Bark of *Thuja standishii*

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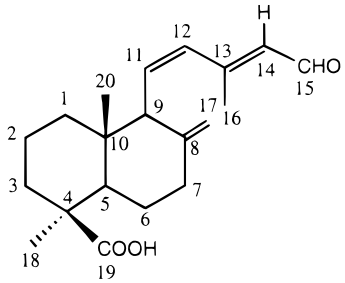
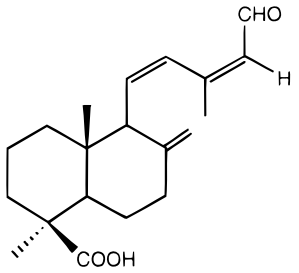
Two new labdane-type diterpene aldehydes, 15-oxolabda-8(17),11(*Z*),13(*E*)-trien-19-oic acid (**1**) and 15-oxolabda-8(17),11(*Z*),13(*Z*)-trien-19-oic acid (**2**), and a new nordrimane-type sesquiterpene, 12-oxo-11-nordrim-8-en-14-oic acid (**3**), along with a known diterpene, 15-nor-14-oxolabda-8(17),12(*E*)-dien-19-oic acid (**4**), were isolated from the stem bark of *Thuja standishii*. The structures of **1**−**3** were established by spectroscopic methods.

Recently, we reported the structure of standishinal [6α,12-dihydroxy-6(7→11)*abeo*-abieta-8,11,13-trien-7-al], a diterpene based on a novel carbon skeleton, and two known abietane-type diterpenoids, 12-hydroxy-6,7-*seco*-abieta-8,11,13-triene-6,7-dial and 6α-hydroxysugiol, from the stem bark of *Thuja standishii* (Gord.) Carr (Cupressaceae). It was verified that standishinal is biosynthesized from 12-hydroxy-6,7-*seco*-abieta-8,11,13-triene-6,7-dial.[1] Further careful examination of this plant part has now led to the isolation of two new labdane-type diterpene aldehydes, 15-oxolabda-8(17),11(*Z*),13(*E*)-trien-19-oic acid (**1**) and 15-oxolabda-8(17),11(*Z*),13(*Z*)-trien-19-oic acid (**2**), and a new nordrimane-type sesquiterpene aldehyde, 12-oxo-11-nordrim-8-en-14-oic acid (**3**), besides a known labdane-type diterpenoid, 15-nor-14-oxolabda-8(17),12(*E*)-dien-19-oic acid. This paper deals with the structure determination of **1**−**3**.



1



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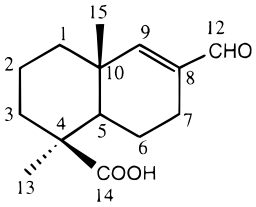

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Results and Discussion

The $CHCl_3$ extract of the stem bark of *T. standishii* was carefully chromatographed over Si gel and Sephadex LH-20, and then fractionated by medium-pressure liquid chromatography to afford three new (**1**−**3**) and a known (**4**) compound. The known compound was confirmed as 15-nor-14-oxolabda-8(17),12(*E*)-dien-19-oic acid (**4**).[2]

Compounds **1** and **2** had the same molecular formula, $C_{20}H_{28}O_3$, based on HREIMS. The UV spectra of **1** and **2** showed the presence of a HC═HC−CMe═CH−CHO chromophore. The IR spectra of **1** and **2** exhibited bands indicative of transoid diene, terminal methylene, carboxyl, and aldehyde groups. The ^1H and ^{13}C NMR spectra (Table 1) exhibited two tertiary methyl groups, five methylene groups, two methine groups, a cis-disubstituted double bond [**1**: δ_H 6.20 (d), 6.34 (dd), δ_C 136.1 (d), 137.1 (d); **2**: δ_H 7.06 (d), 6.24 (dd), δ_C 128.1 (d), 138.0 (d)], a trisubstituted double bond [**1**: δ_H 5.91 (d), δ_C 128.9 (d), 154.2 (s); **2**: δ_H 5.85 (d), δ_C 127.8 (d), 154.4 (s)], an exocyclic methylene group [**1**: δ_H 4.44 (d), 4.78 (d), δ_C 108.4 (t), 148.8 (s); **2**: δ_H 4.44 (d), 4.81 (d), δ_C 108.5 (t), 148.8 (s)], an α,β-unsaturated aldehyde group [**1**: δ_H 10.12 (d), δ_C 191.5 (d); **2**: δ_H 10.17 (d), δ_C 190.3 (d)], and a carboxyl group [**1**: δ_C 182.9 (s); **2**: δ_C 182.6 (s)]. Accordingly, **1** and **2** had the same planar structure represented by 15-oxolabda-8(17),11(*Z*),13-trien-19-oic acid, and a comparative study of the ^1H and ^{13}C, and 2D NMR spectra of those compounds readily determined that they are geometric isomers differing in the positioning of a formyl group about a double bond at C-13 and C-14. The extensive 2D NMR experiments involving HMQC, HMBC, $^1H-^1H$ COSY, and NOESY spectra supported the structures of **1** and **2**. In the HMBC spectra of these compounds (Table 1), C-12 correlated with H-9α, H-11, H-14, and Me-16, and C-15 correlated with H-14 and Me-16. The relative structures of **1** and **2** were determined from their NOESY spectra. In the NOESY spectrum of **1** (Figure 1), significant NOEs were observed for H-12 with H-14, and for Me-16 with H-15. On the other hand, NOEs were shown for H-12 with H-15 and for Me-16 with H-14 in compound

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Table 1. ^{1}H, ^{13}C, HMBC, and NOESY NMR Data for Compounds **1** and **2** ($CDCl_3$)[a]

	1			**2**		
position	δ_C	δ_H	HMBC (C→H)	δ_C	δ_H	HMBC (C→H)
1α	41.0 t	1.11 m	20	40.0 t	1.12 m	20
1*β*		1.47 m			1.49 m	
2α	19.6 t	1.47 m	1α, 3α, 18	19.6 t	1.49 m	1α, 3α, 18
2*β*		1.82 m			1.80 m	
3α	38.0 t	1.11 m	18	38.0 t	1.11 m	18
3*β*		2.19 m			2.19 m	
4	44.1 s		3α, 5α, 18	44.1 s		18
5α	55.5 d	1.36 dd (3, 12.3)	6*β*, 18, 20	55.5 d	1.36 dd (3, 12.5)	6*β*, 18, 20
6α	24.9 t	2.00 m	5α	24.9 t	1.98 m	7*β*
6*β*		1.92 m			1.91 m	
7α	37.1 t	2.48 m	6*β*, 17A, 17B	37.1 t	2.47 m	17A, 17B
7*β*		2.06 m			2.04 m	14
8	148.8 s		6α, 7α, 9α, 11, 17B	148.8 s		7*β*, 9α
9α	60.7 d	2.49 m	1α, 11, 12, 17A, 17B, 20	61.0 d	2.50 m	11, 12, 17A, 17B, 20
10	40.1 s		1α, 5α, 7α, 9α, 11, 20	40.0 s		1α, 5α, 9α, 11, 20
11	137.1 d	6.34 dd (10, 15.5)	9α, 12	138.0 d	6.24 dd (10, 15.5)	9α, 12
12	136.1 d	6.20 d (15.5)	9α, 11, 14, 16	128.1 d	7.06 d (10.0)	9α, 14, 16
13	154.2 s		11, 12, 16	154.4 s		11, 12, 16
14	128.9 d	5.91 d (8.0)	12, 15, 16	127.8 d	5.85 d (8.0)	12, 15, 16
15	191.5 d	10.12 d (8.0)	14, 16	190.3 d	10.17 d (8.0)	
16	13.3 q	2.29 d (1.0)	12, 14	21.6 q	2.11 s	12, 14
17A	108.4 t	4.44 d (1.5)	7α, 9α	108.5 t	4.44 d (1.5)	9α
17B		4.78 d (1.5)			4.81 d (1.5)	
18	28.9 q	1.28 s	3α, 5α	28.9 q	1.28 s	
19	182.9 s		3α, 5α, 18	182.6 s		5α, 18, 19
20	13.7 q	0.79 s	1α, 9α	13.7 q	0.79 s	1α

[a] Operated at 500 and 125 MHz for ^{1}H and ^{13}C NMR experiments, respectively; δ in ppm, J (in parentheses) in Hz; assignments made from ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY data.

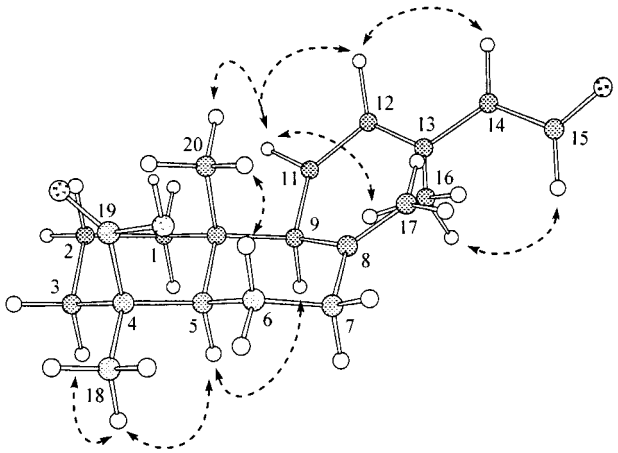

Figure 1. NOESY correlations for **1**.

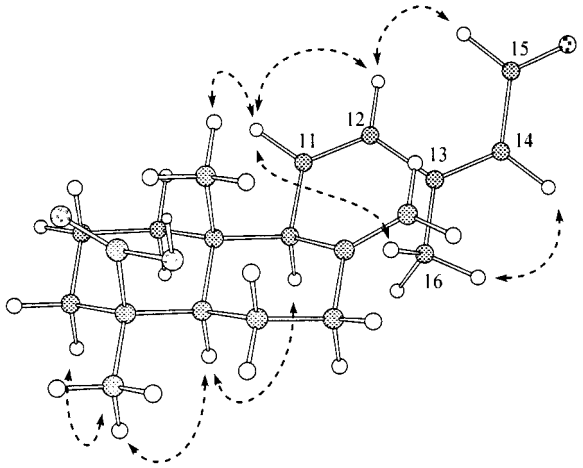

Figure 2. NOESY correlations for **2**.

2 (Figure 2). Thus, the relative stereostructures of the new compounds, **1** and **2** were determined as 15-oxolabda-8(17),11(*Z*),13(*E*)-trien-19-oic acid and 15-oxolabda-8(17),11(*Z*),13(*Z*)-trien-19-oic acid, respectively. The exact absolute stereostructures were not determined.

Compound **3** was assigned the molecular formula of $C_{14}H_{20}O_3$ (HREIMS). The UV and IR spectra showed absorption bands for α,*β*-unsaturated aldehyde and carboxyl groups. The ^{1}H and ^{13}C NMR spectra (Table 2) showed two tertiary methyl groups, five methylene groups, and a methine group, a trisubstituted double bond [δ_H 6.34 (d), δ_C 138.2 (s), 160.9 (d)], an α,*β*-unsaturated aldehyde group [δ_H 9.41 (s), δ_C 194.8 (d)], and a carboxyl group [δ_C 181.2 (s)]. The gross structure of **3** was determined from its HMQC, HMBC, ^{1}H−^{1}H COSY, and NOESY spectra. In the HMBC spectrum (Table 2), C-8 correlated with H-6α, H-7α, H-7*β*, and H-12 and C-14 correlated with H-3α, H-5α, and Me-13. In the ^{1}H−^{1}H COSY spectrum, correlations were observed between H-2 and H-1α, H-1*β*, H-3α, and H-3*β*, and between H-6 and H-5α, H-7α, and H-7*β*; and between H-9; and H-7α and H-7*β* (allylic coupling). The relative structure of **3** was determined from its NOESY spectrum (Table 2), with significant NOEs observed for Me-15 with H-1*β*, H-2*β*, H-6*β*, and H-9; and for H-5α with H-3α, H-6α, and H-7α; and for Me-13 with H-3α, H-5α, and H-6α. Hence, the structure of the new compound **3** was established as 12-oxo-11-nordrim-8-en-14-oic acid. Previously, poligonal,[3] isopoligonal,[4] and polygonone[4] have been isolated from *Polygonum hydropiper* (Polygonaceae) as other nordrimane-type sesquiterpene constituents.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. UV spectra were recorded using a Hitachi 150−20 spectrophotometer. IR spectra were recorded using a Perkin-Elmer 1720X FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse

Table 2. ^{1}H, ^{13}C, HMBC, and NOESY NMR Data for Compound **3** ($CDCl_3$)[a]

position	δ_C	δ_H	HMBC (C→H)	NOESY
1α	38.4 t	1.35 m	9, 15	3α
1β		1.63 m		15
2α	19.1 t	1.58 m	3α, 13	
2β		1.98 m		1β, 15
3α	37.7 t	1.10 ddd (4.0, 13.8, 13.5)	13	1α, 13
3β		2.26 m		
4	43.6 s		3α, 5α, 13	
5α	52.1 d	1.37 m	6α, 6β, 7β, 9, 13, 15	3α, 6α, 7α
6α	19.5 t	2.12 m	5α	5α, 13
6β		1.82 m		7β, 15
7α	23.7 t	2.04 m	5α, 6α, 9, 12	5α, 6α
7β		2.47 ddd (17.5, 6.0, 1.5)		6β
8	138.2 s		6α, 7α, 7β, 12	
9	160.9 d	6.34 d (1.5)	7α, 7β, 12, 15	1β, 12, 15
10	37.4 s		1α, 15	
12	194.8 d	9.41 s	7β, 9	9
13	28.1 q	1.30 s	3α	3α, 5α, 6α
14	181.2 s		3α, 5α, 13	
15	18.5 q	0.99 s	5α	1β, 2β, 6β, 9

[a] Operated at 500 and 125 MHz for ^{1}H and ^{13}C NMR experiments, respectively; δ in ppm, *J* (in parentheses) in Hz; assignments made from ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY data.

sequences, operating at 500 and 125 MHz, respectively. $CDCl_3$ was used as the solvent and TMS as the internal standard. EIMS were recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out over Si gel (70−230 mesh, Merck) and MPLC was conducted with Si gel (230−400 mesh, Merck) and Cosmocil 40C_{18}−PREP (ODS, Nacarai Tesque). Fractions obtained from column chromatography were monitored by TLC (Si gel 60 F_{254}, Merck). Preparative TLC was carried out on Merck Si gel F_{254} plates (20 × 20 cm, 0.5 mm thick).

Plant Material. The stem bark of *T. standishii* (Gord.) Carr. was collected at Hashimoto City, Wakayama Prefecture, Japan, in September 1995. A voucher specimen (TS-95−01) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Extraction and Isolation. The chopped stem bark (5.3 kg) of *T. standishii* was extracted for 7 days at 50 °C with $CHCl_3$ (20 L) employing an automatic percolator. The $CHCl_3$ was evaporated under reduced pressure, and the resulting dark green residue (558.8 g) was subjected to Si gel column chromatography (10 kg). Elution of the column with $CHCl_3$ afforded residues A (fraction nos. 1−4, 6.3 g), B (fraction nos. 5−8, 28.7 g), C (fraction nos. 9−11, 28.7 g), D (fraction nos. 12−20, 27.6 g), E (fraction nos. 21−31, 15.4 g), and F (fraction nos. 32−46, 22.2 g). Elution was continued to give residues G (53.2 g), H (15.5 g), I (12.5 g) and J (17.8 g), respectively, from the fraction numbers 47−53, 54−59, 60−71, and 72−81 eluted with $CHCl_3$−EtOAc (10:1), and residues K (47.5 g) and L (52.2 g), respectively, were obtained from fraction numbers 82−96, and 97−121, eluted from $CHCl_3$-EtOAc (2:1). Subsequent elution of the column with EtOAc afforded residue M (fraction nos. 122−142; 71.2 g). Repeated column chromatography of residue G on Si gel (2.0 kg) furnished a yellow gum (48.4 g) eluted with $CHCl_3$ from fraction numbers 14−22, which was subjected to Sephadex LH-20 with $CHCl_3$−MeOH (1:1) to give a crude mass (44.2 g, fraction nos. 8−23). This material was subjected to Si gel column chromatography (1.5 kg) to give a diterpene mixture (3.3 g, fraction nos. 17−35), followed by MPLC with ODS (100 g) gave residues a (120 mg, fraction nos. 67−72) and b (559 mg, fraction nos. 73−99). Residue a was separated by preparative TLC (*n*-hexane−EtOAc, 2:1) to give compounds **1** (13.9 mg) and **2** (3.7 mg). Repeated MPLC of residue b on Si gel with *n*-hexane−EtOAc (1:1) afforded compound **4** (2.9 mg). Residue H was rechromatographed over Si gel (1.0 kg) to afford a gummy product (1.3 g, fraction nos. 30−38) eluted with *n*-hexane−EtOAc (5:1), which was subjected to MPLC on Si gel (200 g), eluted with *n*-hexane−EtOAc (5:1), to give a crude product (246 mg, fraction nos. 79−81), and this was purified by HPLC with ODS, eluting with CH_3-CN−H_2O (4:1), to give compound **3** (2.0 mg).

15-Oxolabda-8(17),11(*Z*),13(*E*)-trien-19-oic acid (1): colorless oil; $[\alpha]^{25}_D$ −45° (*c* 0.88, $CHCl_3$); UV (EtOH) λ_{max} 284 (log ε 4.3) (C=C−C=C−C=O) nm; IR (film) ν_{max} 3200−2800 and 1694 (COOH), 2937, 2853, 1664 (−CH=C−CHO), 1629 and 893 (>C=CH_2), 1450, 1395, 979 (*trans*-diene) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 316 $[M]^+$ (100), 298 (9), 287 (8), 257 (7), 255 (7), 203 (7), 161 (31), 147 (38), 135 (24), 121 (34), 107 (14), 105 (10), 95 (19), 91 (5); HREIMS *m*/*z* 316.2037 $[M]^+$ ($C_{20}H_{28}O_3$ requires 316.2037).

15-Oxolabda-8(17),11(*Z*),13(*Z*)-trien-19-oic acid (2): colorless oil; $[\alpha]^{25}_D$ −28° (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} 280 (log ε 3.9) (C=C−C=C−C=O) nm; IR (film) ν_{max} 3200−2800 and 1694 (COOH), 2928, 2852, 1668 (−CH=C−CHO), 1631 and 893 (>C=CH_2), 1452, 1408, 981 (*trans*-diene) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 316 $[M]^+$ (83), 298 (14), 287 (7), 257 (19), 255 (7), 203 (11), 161 (64), 147 (35), 135 (56), 121 (100), 107 (55), 105 (76), 95 (59), 91 (74); HREIMS *m*/*z* 316.2040 $[M]^+$ ($C_{20}H_{28}O_3$ requires 316.2037).

12-Oxo-11-nordrim-8-en-14-oic Acid (3): colorless oil; $[\alpha]^{25}_D$ +140° (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} 226.5 (log ε 3.9) (C=C−C=O) nm; IR (film) ν_{max} 3200−2700 and 1690 (COOH), 2931, 1643 (C=C−CHO), 1470, 1413, 1375, 1262, 1189, 975, 757 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 236 $[M]^+$ (80), 221 $[M - Me]^+$ (20), 207 $[M - CHO]^+$ (47), 190 (32), 175 (27), 161 (100); HREIMS *m*/*z* 236.1580 $[M]^+$ ($C_{14}H_{20}O_3$ requires 236.1580).

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