Four New Trisnorlanostene-Type Triterpenoids from the Stem Bark of *Pinus luchuensis*

Shun-ich Wada* and Reiko Tanaka

Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan

Received March 29, 2000

Four new 25,26,27-tris*nor*lanostene-type triterpenoids were isolated from the stem bark of *Pinus luchuensis*, together with five known compounds. These new compounds were characterized as 25,26,-27-tris*nor*-3α-hydroxy-lanost-9(11)-en-24-oic acid (**1**), 25,26,27-tris*nor*-3α-methoxy-lanost-9(11)-en-24-oic acid (**2**), 25,26,27-tris*nor*-3*β*-methoxy-lanost-9(11)-en-24-oic acid (**3**), and 25,26,27-tris*nor*-3-oxo-lanost-9(11)-en-24-oic acid (**4**) on the basis of MS and NMR evidence.

We have investigated bioactive triterpenoids from plant sources and reported that some triterpenoids exhibit antitumor promotion[1] and topoisomerase-II inhibitory activities.[2] In continuation of our studies on the constituents of the genus *Pinus*,[3] we investigated the constituents of *P. luchuensis* Mayr. (Pinaceae), a tall evergreen tree. In 1975, Cheng, et al.[4] reported the isolation of some serratane-type triterpenoids, 3*β*-methoxyserrat-14-en-21-one, serrat-14-en-3,21-dione, 3*β*-hydroxyserrat-14-en-21-one, 3*β*,-21α-dimethoxyserrat-14-ene, and 3*β*-methoxyserrat-14-en-21α-ol, from the bark of *P. luchuensis*. A search for additional constituents in the $CHCl_3$ extract has led to the isolation of four new 25,26,27-trisnorlanostene-type triterpenoids, together with five known compounds. This paper deals with the characterization of the above new nortriterpenoids.

Results and Discussion

The air-dried stem bark of *P. luchuensis* was extracted with $CHCl_3$. The $CHCl_3$ extract was separated by a combination of normal-phase Si gel, LH-20 column chromatography and medium-pressure liquid chromatography (MPLC) followed by preparative TLC to afford five known compounds and four new compounds (**1**−**4**). The known compounds were identified as friedelin, *β*-sistosterol, 13-epimanoyloxide, dehydroabietic acid, and methyl dehydroabietate by direct comparison of the NMR, MS, and physicochemical data with the authentic samples.

Compound **1** was assigned the molecular formula $C_{27}H_{44}O_3$ by HREIMS. The IR spectrum indicated absorption bands for a hydroxyl (ν_{max} 3451 cm^{-1}) and carboxyl (ν_{max} 1713 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed five tertiary methyl groups, a secondary methyl group, an axial-oriented secondary hydroxyl group [δ_H 3.43 (t, J = 3.0 Hz), δ_C 76.3 (d)], a trisubstituted double bond [δ_H 5.26 (dt), δ_C 148.5 (s) and 114.6 (d)], and a carboxyl group [δ_C 178.6 (s)]. The gross structure of compound **1** was established by detailed analyses of the $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectral data. Figure 1(a) shows the $^1H-^1H$ connectivities observed in the $^1H-^1H$ COSY spectrum, and the two- and three-bond $^{13}C-^1H$ correlations observed in the HMBC spectrum. These data established compound **1** as a trisnorlanostene-type triterpenoid having a hydroxyl group, a carboxyl group, and a double bond at positions 3, 20, and 9(11), respectively. The HMBC spectrum indicated cross-peaks between C-3 (δ_C 76.3, d) and H-2α (δ_H 1.68, m), H-2*β* (δ_H 2.00, dddd), H_3-28 (δ_H 0.96, s), and H_3-29 (δ_H 0.88, s); C-24 (δ_C 178.6, s) and H_2-23 (δ_H 2.28 and 2.42, each ddd); C-9 (δ_C 148.5, s) and H-8 (δ_H 2.18, br d) and H_3-19 (δ_H 1.06; s), and C-11 (δ_C 114.6, d) and H-12α (δ_H 2.08, br d) and H-12*β* (δ_H 1.89, m). Furthermore, the NOESY spectrum showed NOE correlations between H-5 (δ_H 1.31) and H_3-28α (δ_H 0.96) and H-1α (δ_H 1.79) and between H-8 (δ_H 2.18) and H_3-18*β* and H_3-19*β* (δ_H 0.65 and 1.06) [Figure 1(b)]. The data indicated that the configuration at both H-5 and H-8 should be α. Therefore, compound **1** was determined to be a new compound, 25,26,27-tris*nor*-3α-hydroxy-lanost-9(11)-en-24-oic acid.

Compounds **2**−**4** were assigned the molecular formulas, $C_{28}H_{46}O_3$, $C_{28}H_{46}O_3$, and $C_{27}H_{42}O_3$, respectively by HREIMS. The signal patterns in the IR and the ^1H and ^{13}C NMR spectra of compounds **2**−**4** are similar to those of compound **1**. However, the signals, originating from an α-methoxy group [δ_H 3.31, s (OCH_3) and 2.81, t ($CHOCH_3$)] in compound **2** or from a *β*-methoxy group [δ_H 3.37, s (OCH_3) and 2.65, dd ($CHOCH_3$)] in compound **3**, were observed and thus indicated the methylation of the free hydroxyl group in compounds **2** and **3**. A downfield carbonyl group (δ_C 217.2, s; ν_{max} 1698 cm^{-1}) indicated the oxidation of the hydroxyl group in compound **4**. The structures of compounds **2**−**4** were confirmed by extensive 2D NMR experiments. The correlation patterns in the 2D NMR spectra of compounds **2**−**4** resembled those of compound **1**. In the HMBC spectra, cross-peaks were observed between C-3 and α-OMe, H_3-28, H_3-29, and H_2-2 in compound **2**, between C-3 and *β*-OMe, H_3-28, H_3-29, and H_2-2 in compound **3**, and between the carbonyl group and H_3-28, H_3-29, and H_2-2 in compound **4**. From the above data, compounds **2**−**4** were identified as new compounds, 25,-26,27-tris*nor*-3α-methoxy-lanost-9(11)-en-24-oic acid (**2**), 25,26,27-tris*nor*-3*β*-methoxy-lanost-9(11)-en-24-oic acid (**3**), and 25,26,27-tris*nor*-3-oxo-lanost-9(11)-en-24-oic acid (**4**). This is the first report of the isolation of compounds **1**−**4** in the literature; however, another trisnortriterpenoid, 25,-26,27-tris*nor*-3*β*-hydroxy-24-dimethoxycycloartane, has been

* To whom correspondence should be addressed. Fax: (+81)726-90-1005. E-mail: wada@oysun01.oups.ac.jp.

10.1021/np000155k CCC: $19.00
Published on Web 07/11/2000

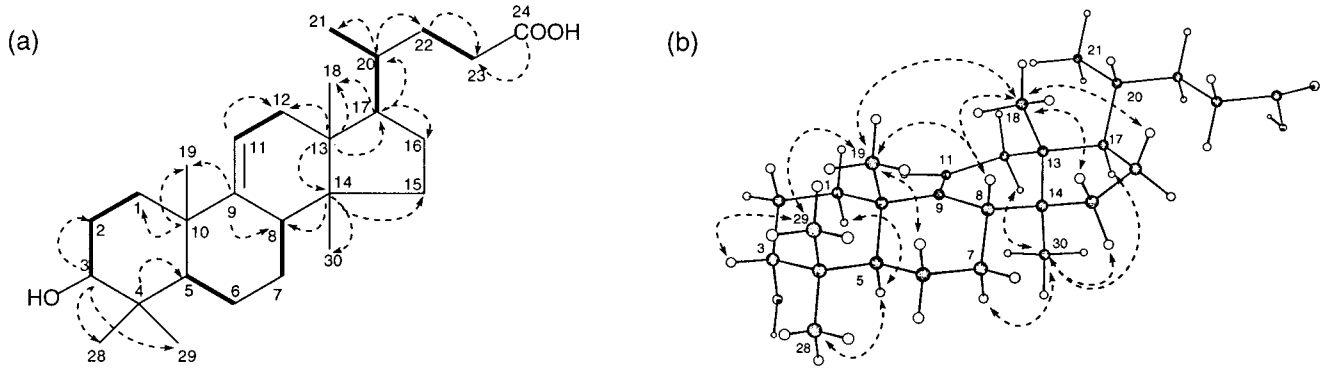


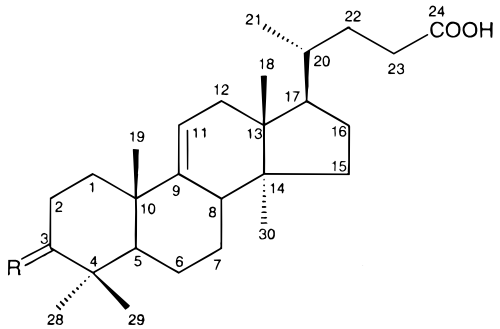
Figure 1. (a) Partial $^1H-^1H$ COSY (━) and HMBC (---->, C to H) and (b) NOESY correlations of compound **1**.

Table 1. ^1H NMR Data for Compounds **1**−**4** (500 MHz, $CDCl_3$)[a,b]

position	1	2	3	4
1α	1.79 m	1.71 m	1.38 m	1.80 m
1β	1.50 m	1.42 m	1.82 m	2.10 m
2α	1.68 m	1.85 m	1.71 m	2.41 ddd (15.5, 5.0, 3.0)
2β	2.00 dddd (14.5, 14.5, 4.0, 3.0)	1.75 m	1.48 m	2.72 ddd (15.5, 13.5, 6.5)
3α			2.65 dd (11.5, 4.0)	
3β	3.43 t (3.0)	2.81 t (3.0)		
5α	1.31 m	1.30 m	0.86 m	1.37 m
6α	1.92 m	1.90 m	1.92 m (2H)	1.64 m (2H)
6β	1.36 m	1.35 m		
7α	1.65 m	1.60 m	1.66 m	1.70 m (2H)
7β	1.37 m	1.40 m	1.33 m	
8β	2.18 br d (11.0)	2.16 br d (10.5)	2.17 br d (11.5)	2.23 br d (11.5)
11	5.26 dt (6.0, 2.0)	5.22 dt (6.5, 2.0)	5.22 dt (6.0, 1.5)	5.29 dt (6.0, 2.0)
12α	2.08 br d (17.5)	2.06 br d (18.0)	2.07 br d (19.5)	2.09 m
12β	1.89 m	1.88 m	1.89 m	1.92 m
15α	1.36 m (2H)	2.35 t (7.5)	1.36 m (2H)	1.38 m (2H)
15β		1.34 m		
16α	1.58 m	1.58 m	1.67 m	1.95 m
16β	1.46 m	1.40 m	1.46 m	1.36 m
17	1.61 m	1.60 m	1.60 m	1.62 m
18	0.65 s	0.65 s	0.65 s	0.68 s
19	1.06 s	1.06 s	1.04 s	1.23 s
20	1.45 m	1.44 m	1.44 m	1.46 m
21	0.90 d (6.0)	0.90 d (6.5)	0.90 d (6.5)	0.91 d (6.5)
22	1.34 m	1.34 m	1.34 m	1.35 m
	1.85 m	1.84 m	1.84 m	1.86 m
23	2.28 ddd (16.0, 9.5, 6.5)	2.27 ddd (15.8, 9.6, 6.5)	2.28 ddd (15.8, 10.0, 6.5)	2.29 ddd (15.8, 10.0, 6.5)
	2.42 ddd (16.0, 10.0, 5.0)	2.42 ddd (15.6, 10.0, 5.5)	2.42 ddd (15.8, 10.0, 5.5)	2.43 ddd (15.8, 10.0, 5.0)
28	0.96 s	0.93 s	0.97 s	1.08 s
29	0.88 s	0.87 s	0.80 s	1.07 s
30	0.75 s	0.74 s	0.74 s	0.75 s
OMe		3.31 s	3.37 s	

[a] Assignments were determined by $^1H-^1H$ COSY, NOESY, HMQC, and HMBC spectra. [b] J values are given in Hz.

reported from the aerial part of *Euphorbia broteri*.[5]



1 R = α-OH

2 R = α-OMe

3 R = β-OMe

4 R = O

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. ^1H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse 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 Sephadex LH-20 (Pharmacia Fine Chem.), and MPLC was carried out with Si gel (230−400 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (Si gel 60 HF_{254}) and ^1H NMR. Preparative TLC was carried out on Merck Si gel PF_{254} plates (20 × 20 cm, 0.5 mm thick).

Plant Material. The stem bark of *P. luchuensis* Mayr. was collected at Okinawa Forest Experiment Station, Okinawa Prefecture, Japan, in September 1998. The extraction was carried out in October 1998.

Extraction and Isolation. The chopped stem bark (25.2 kg) of *P. luchuensis* was extracted with $CHCl_3$ in an automatic percolator at 60 °C for 1 week. The $CHCl_3$ extract (420 g) was chromatographed on Si gel using *n*-hexane−$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$−EtOAc (5:1→1:1), EtOAc, and EtOAc−MeOH (1:1) as eluents, and was collected in nine fractions (I−IX). Fractions IV and VI gave a positive color with Liebermann−Burchard reagent.

Fraction IV (56.7 g) was rechromatographed on a Si gel column using $CHCl_3$, to afford 5 fractions (IV-A−IV-E). Repeated column chromatography of fraction IV-A (3.83 g) using Si gel gave friedelin (20 mg), β-sistosterol (52 mg), 13-

Table 2. ^{13}C NMR Data for Compounds **1**−**4** (125 MHz, $CDCl_3$)[a]

position	1	2	3	4
1	30.5 t	30.8 t	36.0 t	36.7 t
2	25.7 t	20.4 t	22.5 t	34.9 t
3	76.3 d	85.9 d	88.6 d	217.2 s
4	37.9 s	38.1 s	39.0 s	47.7 s
5	46.7 d	47.3 d	53.0 d	53.4 d
6	27.9 t	27.9 t	27.9 t	22.6 t
7	28.0 t	27.9 t	28.1 t	27.7 t
8	41.9 d	41.9 d	41.8 d	41.9 d
9	148.5 s	148.6 s	148.7 s	147.1 s
10	39.4 s	39.4 s	39.4 s	39.1 s
11	114.6 d	114.2 d	114.7 d	116.2 d
12	37.1 t	37.1 t	37.1 t	37.2 t
13	44.3 s	44.3 s	44.4 s	44.3 s
14	47.2 s	47.2 s	47.1 s	47.0 s
15	33.9 t	33.9 t	33.9 t	33.9 t
16	21.3 t	21.2 t	21.2 t	27.9 t
17	50.7 d	50.7 d	50.8 d	50.8 d
18	14.4 q	14.4 q	14.4 q	14.5 q
19	22.1 q	22.2 q	22.3 q	21.8 q
20	35.7 d	35.7 d	35.7 d	35.7 d
21	18.0 q	18.0 q	18.0 q	18.0 q
22	31.1 t	31.1 t	31.1 t	31.1 t
23	30.9 t	30.9 t	30.9 t	30.9 t
24	178.6 s	178.6 s	178.4 s	178.6 s
28	28.4 q	28.4 q	28.3 q	25.6 q
29	22.5 q	22.9 q	16.4 q	22.0 q
30	18.5 q	18.5 q	18.5 q	18.4 q
OMe		57.0 q	57.5 q	

[a] Assignments were determined by DEPT, $^1H-^1H$ COSY, NOESY, HMQC, and HMBC spectra.

epimanoyloxide (49 mg), dehydroabietic acid (450 mg), and methyl dehydroabietate (120 mg), which were identified by direct comparison with authentic samples. The IV-D fraction (6.83 g) was subjected to LH-20 column chromatography ($CHCl_3$−MeOH, 1:1), preparative TLC ($CHCl_3$−MeOH, 98:2), and recrystallization to give compounds **2** (14.2 mg), **3** (13.5 mg), and **4** (8.5 mg) as colorless needles ($CHCl_3$−MeOH).

Fraction VI (27.3 g) was fractionated by Si gel column chromatography using $CHCl_3$ into five fractions (VI-A−VI-E). Further fractionation of VI-D (509.5 mg) by LH-20 column chromatography ($CHCl_3$-MeOH, 1:1), preparative TLC ($CHCl_3$−MeOH, 98:2), and recrystallization yielded compound **1** (15.3 mg) as colorless needles ($CHCl_3$−MeOH).

25,26,27-Tris*nor*-3α-hydroxy-lanost-9(11)-en-24-oic acid (1): mp 180−185 °C ($CHCl_3$−MeOH); $[\alpha]^{15}_D$ +59° ($CHCl_3$, *c* 0.24); IR (KBr) ν_{max} 3451 (OH), 3041 (═CH−), 2872, 1713 (COOH), 1451, 1373, 1183, 1066, 984, 816 (−CH═C<) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 416 $[M]^+$ (29), 401 $[M - Me]^+$ (54), 383 $[M - Me - H_2O]^+$ (100), 287 $[C_{20}H_{31}O]^+$ (9), 175 (12), 161 (9), 121 (10), 119 (12), 109 (10), 95 (13), 52 (10); HREIMS *m*/*z* 416.3295 (calcd for $C_{27}H_{44}O_3$, 416.3288).

25,26,27-Tris*nor*-3α-methoxy-lanost-9(11)-en-24-oic acid (2): mp 168−170 °C ($CHCl_3$−MeOH); $[\alpha]^{15}_D$ +23° ($CHCl_3$, *c* 0.16); IR (KBr) ν_{max} 3460 (OH), 3043 (═CH−), 2852, 1718 (COOH), 1466, 1373, 1103, 976, 815 (−CH═C<) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 430 $[M]^+$ (56), 415 $[M - Me]^+$ (57), 383 (100), 368 (9), 340 (9), 287 (10), 227 (13), 175 (16), 83 (43); HREIMS *m*/*z* 430.3445 (calcd for $C_{28}H_{46}O_3$, 430.3445).

25,26,27-Tris*nor*-3β-methoxy-lanost-9(11)-en-24-oic acid (3): mp 222−225 °C ($CHCl_3$−MeOH); $[\alpha]^{15}_D$ +35° ($CHCl_3$, *c* 0.11); IR (KBr) ν_{max} 3457 (OH), 3047 (═CH−), 2871, 1732 (COOH), 1459, 1371, 1090, 985, 814 (−CH═C<) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 430 $[M]^+$ (39), 415 $[M - Me]^+$ (68), 383 (100), 276 (12), 261 (11), 175 (15), 135 (14), 95 (16), 52 (14); HREIMS *m*/*z* 430.3445 (calcd for $C_{28}H_{46}O_3$, 430.3445).

25,26,27-Tris*nor*-3-oxo-lanost-9(11)-en-24-oic acid (4): mp 190−193 °C ($CHCl_3$−MeOH); $[\alpha]^{15}_D$ +34° ($CHCl_3$, *c* 0.13); IR (KBr) ν_{max} 3405 (OH), 3041 (═CH−), 2869, 1713 (COOH), 1698 (C═O), 1457, 1376, 1280, 1114, 985, 815 (−CH═C<) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 414 $[M]^+$ (29), 399 $[M - Me]^+$ (100), 381 $[M - Me - H_2O]^+$ (13), 313 $[C_{22}H_{33}O]^+$ (6), 272 $[C_{19}H_{28}O]^+$ (4), 173 (5), 159 (7), 125 (27), 119 (15), 107 (13), 93 (10), 52 (17); HREIMS *m*/*z* 414.3139 (calcd for $C_{27}H_{42}O_3$, 414.3132).

Acknowledgment. We thank Mr. G. Uechi, Okinawa Forest Experiment Station, Okinawa Prefecture, Japan, for the collection of plant material. The authors are also grateful to Mr. K. Minoura and Mrs. M. Fujitake, Osaka University of Pharmaceutical Sciences, for NMR and MS measurements, respectively.

References and Notes

(1) Takayasu, J.; Tanaka, R.; Matsunaga, S.; Ueyama, H.; Tokuda, H.; Hasegawa, T.; Nishino, A.; Nishino, H.; Iwashima, A. *Cancer Lett*. **1990**, *53*, 141−144.
(2) Wada, S.; Tanaka, R.; Iida, A.; Matsunaga, S. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2829−2832.
(3) Tanaka, R.; Matsunaga, S. *J. Pharm. Soc. Jpn*. **1999**, *119*, 319−339.
(4) Cheng, Y. S.; Chen, E. H. T.; Fang, G. J. M. *J. Chin. Chem. Soc. (Taipei)* **1975**, *22*, 341−347.
(5) Pascual, T. J.; Urones, J. G.; Marcos, I. S.; Basabe, P.; Sexmero, C. M. J.: Fernandez, M. R. *Phytochemistry* **1987**, *26*, 1767−1776.

NP000155K