Triterpenoid Constituents Isolated from the Bark of *Abies sachalinensis*

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Three new lanostane-type triterpenoids (**1**−**3**) were isolated from the bark of *Abies sachalinensis* along with a known compound (**4**). The structures of **1**−**4** were characterized by spectroscopic methods including NMR and MS. Compound **4** and some derivatives were tested for inhibitory effects on *in vitro* DNA topoisomerases I and II and found to be selective catalytic inhibitors of topoisomerase II activity with IC_{50} values in the range 43−76 μM.

Abies sachalinensis (Japanese name: Todomatsu; Pinaceae) is a tall evergreen tree growing in Hokkaido, Japan, and Sakhalin, Russia. As a part of our work on utilization of chemical constituents in the leaves and the bark of coniferous trees, which are considered to be waste products in the forest industry, we investigated the bark of *A. sachalinensis*. The constituents obtained were screened for inhibitory effects on in vitro DNA topoisomerase (Topo) I and II activities as candidates for anticancer drugs.[1]

Results and Discussion

The air-dried bark of *A. sachalinensis* was extracted with $CHCl_3$. The extract obtained was repeatedly fractionated to give three new lanostane-type triterpenoids (**1**−**3**) along with known compound **4**. The known compound was identified as (23*R*,25*R*)-3,4-*seco*-9β*H*-lanosta-4(28),7-dien-26,23-olid-3-oic acid, which was previously isolated as abiesolidic acid from the oleoresin of *Abies sibirica*.[2] The ^{13}C NMR data of **4** are listed in Table 1.

The molecular formula of **1** was determined to be $C_{31}H_{48}O_4$ (*m*/*z* 484.3543, M^+) by high-resolution EIMS (HREIMS) analysis. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of **4** except for the signals due to a methoxycarbonyl group. These data suggested that **1** could be the methyl ester derivative of **4**. This assumption was substantiated by the preparation of **1** from **4**. Therefore, **1** was confirmed to be methyl (23*R*,25*R*)-3,4-*seco*-9β*H*-lanosta-4(28),7-dien-26,23-olid-3-oate. This is the first report of the isolation of **1**.

HREIMS analysis of **2** indicated a molecular formula of $C_{30}H_{44}O_4$. The UV spectrum of **2** showed an absorption band at 251 nm (log ϵ 4.12) indicative of a conjugated diene structure. The ^{13}C NMR spectrum of **2** showed signals for 30 carbons (Table 1). The IR and ^{1}H and ^{13}C NMR spectral data indicated three tertiary and two secondary methyl groups, a vinylic methyl group, a disubstituted double bond, a tetrasubstituted double bond, a carboxyl group, an exomethylene group, and a γ-lactone ring. These data suggested that **2** was similar in structure to **1** and **4**. The NMR data of the A−D ring part of **2** showed signal patterns similar to those of 3,4-*seco*-4(28)6,8(14)22*Z*,24-mariesapentaen-26,23-olid-3-oic acid.[3,4] Comparison of the ^{1}H and ^{13}C NMR spectral data of **2** with those of **1** and **4** suggested that **2** had the same side chain. Therefore, **2** was suspected to be a 3,4-*seco*-17,14-*friedo*-9β*H*-lanostane-type triterpenoid with 2-methylbutanolide in the side chain. Analysis of the ^{1}H−^{1}H COSY spectrum of **2** showed five ^{1}H−^{1}H spin systems leading to five partial fragments assigned to C1−C2, C5−C7, C9−C12, C15−C16, and C21−C27. The HMBC correlations (C to H) from C-5 to exomethylene protons, the exomethylene carbon to vinylmethyl protons, and the vinylmethyl carbon to H-5 helped to establish the presence of the isopropenyl unit. The carboxyl group C-3 displayed HMBC correlations to H_2-2 and H_2-1; the tetrasubstituted olefinic carbons, C-8 and C-14, showed correlations to H-7 and H-9, and to H-7, H-15, and Me-30, respectively; C-17 showed correlations to H-20 and Me-18; the γ-lactone carbon, C-26, showed correlations to H-25 and Me-27. These data confirmed the 3,4-*seco*-17,14-*friedo*-9β*H*-lanosta-4(28),6,8(14)-triene structure and the positions of the functional groups. Furthermore, NOE correlation data between H-9β and Me-18, 19, and 29; Me-21 and Me-30; and Me-30 and H-11α supported the skeletal structure. The

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

position	1	2	3	4
1	28.9 t	28.4 t	34.1 t	28.7 t
2	29.2 t	29.6 t	34.3 t	29.1 t
3	175.1 s	179.6 s	219.2 s	180.6 s
4	149.7 s	145.8 s	46.9 s	149.6 s
5	45.3 d	50.5 d	52.3 d	45.3 d
6	29.6 t	126.6 d	22.9 t	29.6 t
7	117.9 d	125.2 d	121.3 d	117.9 d
8	146.3 s	125.0 s	148.7 s	146.3 s
9	38.7 d	39.5 d	45.4 d	38.6 d
10	36.3 s	37.1 s	35.7 s	36.3 s
11	18.6 t	23.8 t	20.8 t	18.5 t
12	34.0 t	32.4 t	34.2 t	33.9 t
13	43.8 s	47.5 s	43.9 s	43.7 s
14	51.6 s	146.7 s	51.8 s	51.5 s
15	34.0 t	19.7 t	33.0 t	33.9 t
16	28.3 t	36.2 t	28.2 t	28.2 t
17	53.5 d	49.0 s	53.0 d	53.5 d
18	21.7 q	15.6 q	22.4 q	21.7 q
19	24.1 q	21.8 q	23.1 q	24.0 q
20	33.1 d	34.4 d	36.5 d	33.1 d
21	18.2 q	15.2 q	18.5 q	18.1 q
22	42.6 t	38.7 t	33.3 t	42.5 t
23	76.0 d	76.6 d	28.6 t	76.0 d
24	36.4 t	36.5 t	79.5 d	36.4 t
25	34.2 d	34.1 d	73.1 s	34.2 d
26	180.2 s	179.9 s	23.1 q	180.2 s
27	15.9 q	15.8 q	26.5 q	15.9 q
28	112.0 t	115.4 t	28.0 q	112.0 t
29	25.9 q	24.8 q	21.2 q	25.9 q
30	27.4 q	21.7 q	27.3 q	27.4 q
OMe	51.6 q			

[a] Assignments were confirmed by DEPT, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectra.

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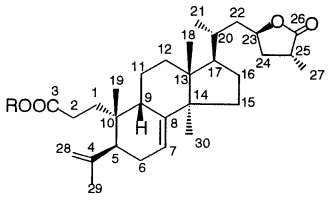
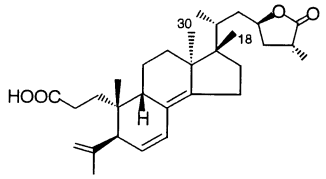


1 R = Me, **4** R = H



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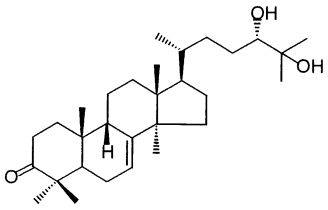

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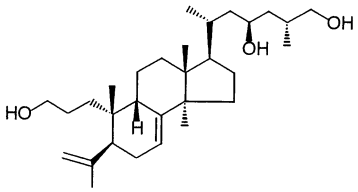

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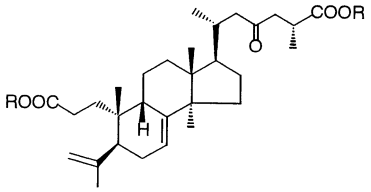

6 R = H **7**, R = Me

stereochemistry of C-23 and C-25 was determined as *R* by comparison with the ^{1}H and ^{13}C NMR spectra of those of **1** and **4**. Therefore, **2** is (23*R*,25*R*)-3,4-*seco*-17,14-*friedo*-9β*H*-lanosta-4(28),6,8(14)-trien-26,23-olid-3-oic acid. Compound **2** may be biosynthesized from **4** by enzymatic dehydrogenation of H-17 or dehydroxylation of 17-OH followed by successive 1,2-shifts of methyl groups to give **2**.[4,5]

Compound **3** was assigned the molecular formula $C_{30}H_{50}O_3$ by HREIMS. The IR and ^{1}H and ^{13}C NMR (Table 1) spectral data indicated five tertiary and one secondary methyl group, a hydroxyisopropyl group, a hydroxymethine group, and a six-membered ring ketone. These data were similar to those of 3-oxolanost-9(11)-en-24*S*,25-diol isolated from the bark of *Pinus luchuensis*.[6] However, the chemical shifts of **3** due to a trisubstituted double bond were at low-field in the ^{1}H NMR spectrum and high-field in the ^{13}C NMR spectrum as compared with those of 3-oxolanost-9(11)-en-24*S*,25-diol. The gross structure of **3** was also proved by detailed analyses of extensive 2D NMR data. The cross-peaks between olefinic C-8 and H-9, Me-30, and H-7 were observed in the HMBC spectrum. Analysis of ^{1}H−^{1}H COSY spectrum of **3** indicated a ^{1}H−^{1}H spin system assigned to C5−C7. Thus, the double bond was located at position 7, and the planar structure of **3** was assigned as 3-oxolanost-7-en-24,25-diol. The NOESY spectrum showed NOE correlations between H-5 and Me-28α and H-1α; and H-9 and Me-18β and Me-19β, respectively. The configuration of the hydroxyl group at position 24 was established by CD using 2% Eu(dpm)$_3$ in CCl_4 as the solvent.[7,8] The CD spectrum of **3** exhibited a positive Cotton effect curve at 285 nm ($\Delta\epsilon$ −1.34) and 310 nm ($\Delta\epsilon$ +1.21), indicating *S*-configuration of the hydroxyl group at C-24. Accordingly, **3** was elucidated as 3-oxolanost-9β*H*-7-en-24*S*,25-diol.

The main component, **4**, its methyl ester derivative, **1**, and the open lactone derivatives, **5**−**7**, were tested for inhibitory effects on *in vitro* human DNA Topos I and II activities. The preparation of the triol derivative (**5**) was carried out by reduction of **4** with lithium aluminum hydride. The diacid derivative (**6**) was obtained by oxidation of **5** with CrO_3 and pyridine. Compound **6** was converted to the dimethyl ester derivative (**7**) using diazomethane. The conversion of supercoiled plasmid DNA to relaxed DNA by Topos I and II was examined in the presence of these compounds. No Topo I inhibitory effects were observed up to 200 μM. However, **1**, **4**, **5**, and **7** showed complete inhibition of the catalytic activity of Topo II at 200 μM. Compound **6** showed 4% inhibition of Topo II relaxation activity at 200 μM. The inhibitory effects of **1**, **4**, **5**, and **7** were dose-dependent. The IC_{50} values were 75.7 for **1**, 42.6 for **4**, 50.4 for **5**, and 67.3 μM for **7**, which were comparable to a Topo II inhibitor, etoposide (IC_{50}: 50 μM). These data therefore provide evidence of the selective inhibitory activities of **4** and its derivatives against Topo II activity.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. ^{1}H 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 silica gel (70−230 mesh), and MPLC was carried out with silica gel (230−400 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 HF_{254}) and ^{1}H NMR. Preparative TLC was carried out on Merck silica gel PF_{254} plates (20 × 20 cm, 0.5 mm thick).

Plant Material. The stem bark of *Abies sachalinensis* was collected in mountainous terrain under the control of National Eniwa Forestry Office, Hokkaido Prefecture, Japan, in September 1997. A voucher specimen (AS-9709-1) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences. The extraction was carried out in October 1997.

Extraction and Isolation. The chopped stem bark (17.0 kg) of *A. sachalinensis* was extracted with $CHCl_3$ in an automatic percolator at 60 °C for 1 week. The $CHCl_3$ extract (657 g) was chromatographed on silica 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 nine fractions (I−XI) were collected. Fraction IV (99.7 g) was rechromatographed on silica gel using $CHCl_3$, then LH-20 ($CHCl_3$−MeOH, 1:1) followed by preparative TLC ($CHCl_3$) to give compound **1** (5.0 mg). Fraction V (123.9 g) was fractionated on silica gel using $CHCl_3$ to afford crystalline mixtures (5.9 g). Further fractionation of the mixtures on LH-20 ($CHCl_3$−MeOH, 1:1) and PTLC ($CHCl_3$−MeOH, 98:2) yielded compounds **2** (5.4 mg), **3** (19.8 mg), and **4** (218 mg).

Methyl (23*R*,25*R*)-3,4-*seco*-9β*H*-lanosta-4(28),7-dien-26,23-olid-3-oate (1): colorless needles (MeOH−$CHCl_3$); mp 151−153 °C; $[\alpha]^{22}_D$ +9.3° (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3074 (OH), 1767 (γ-lactone ring), 1743 (−COOMe), 1637 and 903

(CH_2=C<) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.31 (1H, dt, J = 3.5, 3.0 Hz, H-7), 4.88, 4.81 (each 1H, d, J = 1.5 Hz, H-28), 4.66 (1H, m, H-23), 3.67 (3H, s, OMe), 2.70 (1H, ddq, J = 15.0, 7.5, 7.0 Hz, H-25), 2.56 (1H, m, H-9β), 2.29 (2H, m, H-2), 2.28 (1H, m, H-6β), 2.08 (1H, m, H-5α), 2.04 (2H, m, H-24), 1.98 (1H, m, H-6α), 1.91 (1H, m, H-16α), 1.83 (1H, m, H-12α), 1.79 (3H, s, Me-29), 1.79 (1H, m, H-22a), 1.70 (1H, m, H-1a), 1.68 (1H, m, H-12β), 1.65 (1H, m, H-11β), 1.58 (1H, m, H-1b), 1.54 (1H, m, H-11α), 1.51 (1H, m, H-15β), 1.47 (1H, m, H-20), 1.46 (1H, m, H-17α), 1.46 (1H, m, H-15α), 1.26 (1H, m, H-16β), 1.29 (3H, d, J = 7.5 Hz, Me-27), 1.17 (1H, ddd, J = 14.5, 10.5, 3.0 Hz, H-22b), 1.02 (3H, s, Me-30), 0.96 (3H, d, J = 6.5 Hz, Me-21), 0.84 (3H, s, Me-19), 0.76 (3H, *s*, Me-18); ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* 484 (100) $[M]^+$, 469 (10) $[M - Me]^+$, 437 (3), 397 (47) $[C_{27}H_{41}O_2]^+$, 355 (4), 316 (6), 301 (7), 235 (13), 175 (13), 148 (18), 99 (20). HREIMS *m/z* 484.3543 (calcd for $C_{31}H_{48}O_4$, 484.3550).

(23*R*,25*R*)-3,4-*seco*-17,14-*friedo*-9β*H*-Lanosta-4(28),6,8(14)-trien-26,23-olid-3-oic acid (2): amorphous solid; $[\alpha]^{22}_D$ −136.8° (*c* 0.46, $CHCl_3$); UV (EtOH) λ_{max} 251.2 (log ϵ 4.12) nm; IR (film) ν_{max} 2965 (=CH−), 1770 (γ-lactone ring), 1707 (−COOH), 1638 and 921 (CH_2=C<) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.21 (1H, d, J = 10.0 Hz, H-7), 5.36 (1H, dd, J = 10.0, 5.5 Hz, H-6), 4.96, 4.75 (each 1H, d, J = 2.0 Hz, H-28), 4.62 (1H, m, H-23), 2.70 (1H, ddq, J = 15.0, 7.5, 7.0 Hz, H-25), 2.62 (1H, d, J = 5.5 Hz, H-5α), 2.40 (1H, m, H-9β), 2.38 (1H, m, H-11β), 2.30 (1H, m, H-11α), 2.28 (2H, t, J = 8.0 Hz, H-2), 2.11 (1H, m, H-20), 2.08 (2H, m, H-24), 1.91 (1H, m, H-22a), 1.78 (3H, s, Me-29), 1.72 (1H, m, H-16α), 1.63 (2H, m, H-12), 1.62 (2H, m, H-1), 1.60 (1H, m, H-15), 1.52 (1H, m, H-16β), 1.29 (3H, d, J = 7.5 Hz, Me-27), 1.23 (1H, m, H-22b), 1.02 (3H, s, Me-30), 0.94 (3H, d, J = 6.0 Hz, Me-21), 0.85 (3H, s, Me-19), 0.66 (3H, s, Me-18); ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* 468 (100) $[M]^+$, 395 (66) $[C_{27}H_{39}O_2]^+$, 327 (53), 299 (22), 287 (36), 213 (57), 185 (54), 171 (63), 157 (46), 99(57); HREIMS *m/z* 468.3230 (calcd for $C_{30}H_{44}O_4$, 468.3238).

3-Oxolanost-9β*H*-7-en-24*S*,25-diol (3): colorless needles (MeOH); mp 148−150 °C; $[\alpha]^{22}_D$ +38° (*c* 1.30, $CHCl_3$); IR (KBr) ν_{max} 3443 (OH), 2971 (=CH−), 2879, 1708 (>C=O), 1467, 1382, and 1338 (gem-dimethyl), 1164, 1075, 993, 818 cm^{-1} (−CH=C<); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.64 (1H, dt, J = 8.0, 3.0 Hz, H-7), 3.29 (1H, dd, J = 10.5, 2.0 Hz, H-24), 2.49 (2H, m, H-2), 2.22 (1H, m, H-9β), 1.99 (1H, m, H-16α), 1.89 (1H, m, H-6α), 1.83 (1H, m, H-12α), 1.80 (1H, m, H-6β), 1.80 (1H, m, H-22a), 1.72 (1H, m, H-1α), 1.64 (2H, m, H-11), 1.62 (1H, m, H-1β), 1.62 (1H, m, H-12β), 1.59 (1H, m, H-23a), 1.58 (1H, m, H-15β), 1.53 (1H, m, H-17α), 1.42 (1H, m, H-5α), 1.42 (1H, m, H-15α), 1.40 (1H, m, H-20), 1.35 (1H, m, H-16β), 1.22 (3H, s, Me-27), 1.16 (1H, m, H-23b), 1.16 (3H, s, Me-26), 1.10 (3H, s, Me-29), 1.09 (3H, s, Me-28), 1.02 (3H, s, Me-30), 1.01 (1H, m, H-22b), 0.99 (3H, s, Me-19), 0.90 (3H, d, J = 6.5 Hz, Me-21), 0.78 (3H, s, Me-18); ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* 458 (14) $[M]^+$, 440 (9) $[M - H_2O]^+$, 425 (100) $[M - Me - H_2O]^+$, 407 (26) $[M - Me - 2H_2O]^+$, 339 (8), 313 (11), 271 (12) $[C_{19}H_{27}O]^+$, 257 (19), 145 (11) $[C_8H_{17}O_2]^+$, 125 (8), 59 (25); HREIMS *m/z* 458.3761 (calcd for $C_{30}H_{50}O_3$, 458.3758); CD [*c* 0.145, 2% Eu(dpm)$_3$ in CCl_4] $\Delta\epsilon_{285}$ 1.34 and $\Delta\epsilon_{310}$ +1.21.

(23*R*,25*R*)-3,4-*seco*-9β*H*-lanosta-4(28),7-dien-26,23-olid-3-oic acid (4): colorless needles (MeOH−$CHCl_3$); mp 195−197 °C; $[\alpha]^{22}_D$ −0.6° (*c* 1.01, $CHCl_3$); IR (KBr) ν_{max} 3075 (OH), 1767 (γ-lactone ring), 1715 (−COOH), 1639 and 900 (CH_2=C<) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.32 (1H, dt, J = 3.5, 3.0 Hz, H-7), 4.88, 4.82 (each 1H, d, J = 1.5 Hz, H-28), 4.66 (1H, m, H-23), 2.71 (1H, ddq, J = 15.0, 7.5, 7.0 Hz, H-25), 2.58 (1H, m, H-9β), 2.30 (2H, m, H-2), 2.26 (1H, m, H-6β), 2.08 (1H, m, H-5α), 2.04 (2H, m, H-24), 1.99 (1H, m, H-6α), 1.90 (1H, m, H-16α), 1.83 (1H, m, H-12α), 1.80 (3H, s, Me-29), 1.78 (1H, m, H-22a), 1.72 (1H, m, H-1a), 1.70 (1H, m, H-20), 1.68 (1H, m, H-12β), 1.62 (1H, m, H-11β), 1.59 (1H, m, H-1b), 1.55 (1H, m, H-11α), 1.51 (1H, m, H-15β), 1.46 (1H, m, H-17α), 1.45 (1H, m, H-15α), 1.28 (1H, m, H-16β), 1.29 (3H, d, J = 7.0 Hz, Me-27), 1.18 (1H, ddd, J = 14.3, 10.5, 3.0 Hz, H-22), 1.03 (3H, s, Me-30), 0.96 (3H, d, J = 6.0 Hz, Me-21), 0.85 (3H, s, Me-19), 0.76 (3H, s, Me-18); ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* 470 (100) $[M]^+$, 455 (9) $[M - Me]^+$, 437 (5) $[M - Me - H_2O]^+$, 397 (48) $[C_{27}H_{41}O_2]^+$, 316 (8), 301 (11), 235 (24), 175 (26) 148 (36), 99 (23); HREIMS *m/z* 470.3391 (calcd for $C_{30}H_{46}O_4$, 470.3393).

Preparation of the Methyl Ester of 4. An excessive amount of ethereal diazomethane was added to a solution of **4** (10 mg) in THF (3 mL). The mixture was kept at room temperature for 30 min. The mixture was evaporated in vacuo, and the solid obtained was recrystallized from $CHCl_3$−MeOH to afford the methyl ester **1** as colorless needles (9.8 mg, 96%): mp 152−154 °C ($CHCl_3$−MeOH); $[\alpha]^{22}_D$ +8.9 (*c* 0.83, $CHCl_3$); EIMS (70 eV) *m/z* 484 $[M]^+$ (100).

Reduction of 4 with Lithium Aluminum Hydride. Lithium aluminum hydride (100 mg) was added to a solution of **4** (67 mg) in dry ether (20 mL). The mixture was refluxed for 1.5 h. The ethereal solution was washed with 1% H_2SO_4, H_2O, and saturated NaCl solutions, then dried over anhydrous $MgSO_4$, and filtered. The filtrate was recrystallized from $CHCl_3$−MeOH to give **5** as colorless needles (59 mg, 91%): mp 176−178 °C ($CHCl_3$−MeOH); $[\alpha]^{27}_D$ −21° (*c* 0.67, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.32 (1H, dt, J = 3.5, 3.0 Hz,), 4.84 (1H, d, J = 2.0 Hz), 4.80 (1H, d, J = 2.0 Hz), 3.63 (2H, t, J = 5.6 Hz), 3.55 (2H, m), 1.80 (3H, s), 1.02 (3H, s), 0.95 (3H, d, J = 6.6 Hz), 0.93 (3H, d, J = 6.0 Hz), 0.86 (3H, s), 0.78 (3H, s); EIMS (70 eV) *m/z* 460 $[M]^+$ (100).

Oxidation of 5 with CrO_3 and Pyridine. To a stirred solution of CrO_3 (420 mg) in pyridine (4.2 mL) was added **5** (42 mg) in pyridine (14 mL) at room temperature. After 24 h, the reaction mixture was poured into ice water. It was extracted with ether. The extract was washed with 1 N HCl and saturated aqueous NaCl solutions, then dried over $MgSO_4$, and filtered. The filtrate was subjected to preparative TLC [$CHCl_3$−MeOH (85:15)] to give **4** (14 mg, 32%) and **6** (5.6 mg, 13%) as an amorphous solid: $[\alpha]^{27}_D$ −18° (*c* 0.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.32 (1H, dt, J = 3.5, 3.0 Hz,), 4.88 (1H, d, J = 2.0 Hz), 4.80 (1H, d, J = 2.0 Hz), 3.00 (1H, m), 2.82 (1H, t, J = 8.0 Hz), 1.80 (3H, s), 1.22 (3H, d, J = 7.2), 1.03 (3H, s), 0.87 (3H, d, J = 6.3 Hz), 0.85 (3H, s), 0.78 (3H, s); EIMS (70 eV) *m/z* 486 $[M]^+$ (35).

Methylation of 6 with Diazomethane. Compound **7** was prepared from **6** according to the same method as for the methyl ester of **4** to give an amorphous solid (98%): $[\alpha]^{27}_D$ −34° (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.32 (1H, dt, J = 3.5, 3.0 Hz,), 4.87 (1H, d, J = 2.0 Hz), 4.81 (1H, d, J = 2.0 Hz), 3.68 (3H, s), 3.66 (3H, s), 2.98 (1H, m), 2.81 (1H, *t*, J = 8.1 Hz), 1.79 (3H, s), 1.18 (3H, d, J = 6.9), 1.03 (3H, s), 0.87 (3H, d, J = 6.3 Hz), 0.84 (3H, s), 0.78 (3H, s); EIMS (70 eV) *m/z* 514 $[M]^+$ (62).

Topos I and II Assay. Human Topos I (2 U/μL) and IIα (p170 form, 2 U/μL) were purchased from TopoGen, Inc. (Columbus, OH). Supercoiled pBR 322 plasmid DNA was obtained from Toyobo (Osaka). Etoposide as a Topo II inhibitor was purchased from Sigma Chemicals. Test compounds were dissolved in DMSO at 20 mM as a stock solution. Assay conditions of Topos I and II were previously described.[9]

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References and Notes

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