Taraxasterane- and Ursane-Type Triterpenes from *Nerium oleander* and Their Biological Activities

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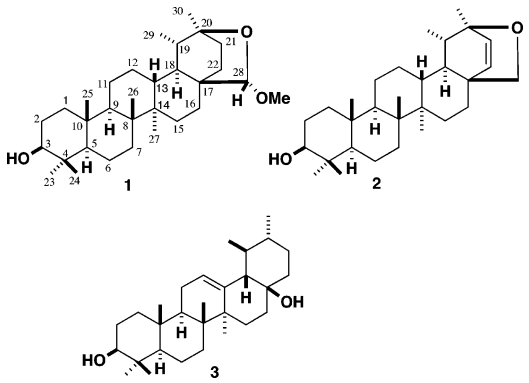
Two new taraxasterane-type triterpenes, 20β,28-epoxy-28α-methoxytaraxasteran-3β-ol (**1**) and 20β,28-epoxytaraxaster-21-en-3β-ol (**2**), were isolated from an ethyl acetate extract of the leaves of *Nerium oleander*, together with ursane-type triterpenes, 28-nor-urs-12-ene-3β,17β-diol (**3**) and 3β-hydroxyurs-12-en-28-aldehyde (**4**). The structures of **1** and **2** were established on the basis of their spectroscopic data. Anti-inflammatory activity of **1**−**4** was examined on the basis of inhibitory activity against the induction of intercellular adhesion molecule-1 (ICAM-1). Cytotoxic activity of **1**−**4** was evaluated against four human cell lines, A-549, WI-38, VA-13, and HepG2 cells.

Nerium oleander L. (*Nerium indicum, Nerium odorum*)[1] (Apocynaceae) is a medium-sized evergreen flowering tree of 2−5 m in height and is planted throughout Japan as a garden and roadside tree. *N. oleander* was originally distributed in the Mediterranean region, subtropical Asia, and the Indo-Pakistan subcontinent. Ishidate and co-workers reported the isolation of triterpene carboxylic acids, ursolic acid, and oleanolic acid in the early stages of the study of this plant.[2] Since then, several ursane-type pentacyclic terpenoids such as kanetic acid and oleanderolic acid were isolated.[3,4] Triterpenoids have many biological activities such as inhibition of tumor-promoting action,[5,6] anti-inflammatory activities,[5,7] hepatoprotective effects,[7] antitumor activity,[7] antihyperlipidemic effects,[7] antiulcer effects,[7] and NO inhibitory activity.[8] Against this background, we became interested in the relationship between the biological activities of the plant and the triterpenes contained in the plant. Recently, we reported the results of the examination of the triterpenoid portion of this plant.[9] In this paper we report the results of further screening of the less polar part of the triterpene fraction of the leaves of this plant.

Results and Discussion

A methanol extract of air-dried leaves of this plant was partitioned with hexane, ethyl acetate, and butanol. The ethyl acetate portion gave a large amount of insoluble material in ethyl acetate after concentration. The crude fraction of triterpenes was obtained mainly from the less polar portion of the filtrate of the solid (45.83% of the ethyl acetate extract) and separated by silica gel column chromatography and HPLC. Two new taraxasterane-type triterpenes, 20β,28-epoxy-28α-methoxytaraxasteran-3β-ol (**1**) and 20β,28-epoxytaraxaster-21-en-3β-ol (**2**), were isolated together with the ursane-type triterpenes 28-nor-urs-12-ene-3β,17β-diol (**3**)[10] and 3β-hydroxyurs-12-en-28-aldehyde (**4**).[11]



Compound **1** has the composition $C_{31}H_{52}O_3$, which was determined by a combination of HRFABMS and ^{1}H and ^{13}C NMR spectra. The IR spectrum of **1** indicated the presence of a hydroxyl group (3624 cm^{-1}). The ^{13}C NMR spectrum displayed 31 carbon signals. The acetal carbon appeared at δ 100.2, and signals for three carbons bearing oxygen were observed at δ 78.9 (d), 73.9 (s), and 55.2 (q, −OMe). As determined from the DEPT and HMQC spectra, the remaining carbon resonances were five quaternary carbons, five methine carbons, 10 methylene carbons, and seven methyl carbons. The ^{1}H NMR spectra showed six singlet methyls (δ 1.10, 0.98, 0.97, 0.93, 0.84, 0.77), one doublet methyl (δ 0.87, d, J = 7.1 Hz), and one methoxy group (δ 3.43). The connectivity of the protonated carbons (C-1 to C-2, C-2 to C-3; C-5 to C-6, C-6 to C-7; C-9 to C-11, C-11 to C-12, C-12 to C-13, C-13 to C-18, C-18 to C-19, C-19 to C-29; C-21 to C-22) was determined from the ^{1}H−^{1}H COSY spectrum. A HMBC experiment was used to determine the carbon−carbon connection through the nonprotonated carbon atoms. The results showed that compound **1** is a triterpene possessing a taraxasterane or ursane skeleton. The correlation of the signal due to a methine carbon bearing a hydroxyl group at δ 78.9 with those of H-23 and H-24 of a *gem*-dimethyl was used to

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place the hydroxyl group at C-3. The signal of an acetal carbon at δ 100.2 was correlated with those of H-16α, H-18, and OMe-28, and the signal due to an oxygenated tertiary carbon atom at δ 73.9 was correlated with those of H-19, H-21β, H-22β, H-28, H-29, and H-30. The results suggested that the acetal carbon at C-28 was connected with C-20 through an oxygen atom. Thus, the connection of the E ring and the acetal ring (F ring) became clear, as shown in structure **1**.

A NOESY experiment was used for assignment of the stereochemistry of **1**. The NOESY correlations observed [H-3 with H-23 (4α-Me) and H-5; H-7α with H-5 and H-27; H-9 with H-12α; H-25 with H-24 and H-26; H-27 with H-7α, H-12α, and H-16α; H-13 with H-26 and H-28; H-29 with H-21α and H-30; H-30 with H-21β and H-29; H-21α with H-18] indicated the full stereostructure of **1**. The correlation of H-28 with H-13 indicated *trans*-fusion in rings C and D and *anti-trans* stereochemistry in rings D and E and the existence of a β-epoxy bond between C-20 and C-28 with an α (*S*)-methoxy group at C-28. The NOE correlations of H-29 with H-30; H-29 with H-21α; and H-21α with H-18 indicated an α-configuration of the methyl groups at 19 and 20. Accordingly, compound **1** belongs to the taraxasterane series and was assigned as 20β,28-epoxy-28α-methoxytaraxasteran-3β-ol.

A second new triterpene, **2**, gave the elemental formula $C_{30}H_{48}O_2$, which was determined by HRFABMS and from the ^{1}H and ^{13}C NMR spectra. The IR spectrum of **2** indicated the presence of a hydroxyl moiety (3624 cm^{-1}). The ^{13}C NMR spectrum displayed 30 carbon signals, with signals for three carbons bearing oxygen observed at δ 79.0 (d), 74.2 (s), and 65.9 (t) and signals for two olefin carbons observed at δ 133.0 (d) and 140.6 (d). From the DEPT and HMQC spectra, the remaining carbon resonances were five quaternary carbons, five methine carbons, eight methylene carbons, and seven methyl carbons. The ^{1}H NMR spectra showed six singlet methyls (δ 1.30, 1.00, 0.97, 0.95, 0.85, 0.77), one doublet methyl [0.69 (J = 6.8 Hz)], and two *cis*-olefinic protons [5.98 (1 H, d, J = 8.2 Hz) and 6.10 (1 H, d J = 8.2 Hz)]. The connectivity of the protonated carbons (C-1 to C-2, C-2 to C-3; C-5 to C-6, C-6 to C-7; C-9 to C-11, C-11 to C-12; C-12 to C-13; C-15 to C-16; C-18 to C-19; C-19 to C-29; C-21 to C-22) and assignments of proton signals were determined by analysis of the ^{1}H−^{1}H COSY spectrum. A HMBC experiment was used to determine the carbon−carbon connection through the nonprotonated carbon atoms. The ^{13}C NMR spectrum of **2** was quite similar to that of **1** except for C-21, C-22, and C-28 and suggested that the structure of the A, B, C, and D rings of **2** is the same as that of **1**. The tertiary carbon signal bearing an oxygen atom at δ 74.2 correlated with H-19, H-21, H-22, H-28β, H-29, and H-30, and the signal due to a quaternary carbon signal at δ 37.6 correlated with that of H-15α, H-16α, H-18, H-21, H-22, and H-28α,β. These HMBC correlations suggested that C-20 and C-28 are connected by an oxygen atom, and a carbon−carbon double bond was located at the C-21 and C-22 positions.

A NOESY experiment was used for assignment of the stereochemistry of **2**. The NOESY correlations [H-3 with H-23 (4α-Me) and H-5; H-9 with H-12α; H-12β with H-19; H-18 with H-27; H-25 with H-24 and H-26; H-27 with H-9 and H-18; H-29 with H-19 and H-30] indicated the full stereostructure of the ring A−D portion of **2**. The *trans* configuration of rings C and D, the *anti-trans* configuration of rings D and E, and the β-configuration of the methylenoxy bridge between C-17 and C-20 were also confirmed by NOESY correlations [H-28β with H-13, H-15β, and H-28α; H-28α with H-16β and H-28β]. Compound **2** was established as 20β,28-epoxytaraxaster-21-en-3β-ol.

Compound **3** gave the molecular formula $C_{29}H_{48}O_2$, which was determined by HRFABMS. The IR spectrum of **3** indicated the presence of a hydroxyl group (3624 cm^{-1}), and the ^{13}C NMR spectrum displayed 29 carbon signals. Signals for two carbons bearing oxygen were observed at δ 79.0 (d) and 72.1 (s). Olefinic carbons were observed at δ 127.8 (d) and 137.9 (s). From the DEPT and HMQC spectra, the remaining ^{13}C NMR resonances were four quaternary carbons, five methine carbons, nine methylene carbons, and seven methyl carbons. The ^{1}H NMR spectra showed five singlet methyls, two doublet methyls, and an olefinic proton (δ 5.30, t, J = 3.6 Hz). The connectivity of the protonated carbons (C-1 to C-2, C-2 to C-3; C-5 to C-6; C-9 to C-11, C-11 to C-12; C-15 to C-16; C-18 to C-19, C-19 to C-29; C-20 to C-21, C-21 to C-22) was determined by analysis of the ^{1}H−^{1}H COSY spectrum. An HMBC experiment was used to determine the carbon−carbon connection through the nonprotonated carbon atoms. Thus, the HMBC correlations (C-10 with H-1β, H-5, H-6α,β, H-9, H-11, H-25; C-8 with H-6β, H-7α, H-9, H-11, H-15β, H-26, H-27; C-14 with H-12, H-15β, H-16α,β, H-26, H-27) indicated clearly the connection of rings A−D. The correlation of the signal due to the methine carbon bearing a hydroxyl group at δ 79.0 with those of H-5, H-1β, H-2α, and H-23, -24 of a *gem*-dimethyl placed the hydroxyl group at C-3. The signal due to a tertiary carbon bearing a hydroxyl group at δ 72.1 correlated with those of H-15α, H-16β, H-21α, and H-22α. The signal due to a quaternary carbon at δ 137.9 correlated with those of H-11, H-18, and H-27. The results suggested that the tertiary hydroxyl group was connected to C-17 and a double bond was placed between C-12 and C-13. The above-mentioned correlation also indicated clearly the connection of the C, D, and E rings. The HMBC correlations (C-29 with H-18 and 19; C-19 with H-18, H-21α, H-29, and H-30; C-30 with H-19 and H-21α; C-20 with H-19, H-21α, H-22α, H-29, and H-30) indicated the vicinal methyl groups at C-19 and C-20.

The NOESY correlations [H-3 with H-23 (4α-Me) and H-5; H-9 with H-5 and H-27; H-24 with H-25; H-29 with H-12 and H-19; H-30 with H-19; H-22α with H-16β; H-21α with H-16α and H-19] indicated the full stereostructure of **3**. The correlation of H-19 with H-30 and H-27 supported the 19β-Me and 20α-Me configurations. The correlation of H-22α with H-16β and H-21α with H-16α and H-19 indicated *cis*-fusion in the D and E rings and β (eq in the E ring and ax in the D ring)-configuration of the hydroxyl group at C-17. Unfortunately, direct evidence of the stereochemistry of the hydroxyl group at the C-17 position of **3** was not obtained from the NOESY experiment. The chemical shift of H-15β (ax, δ 2.04) is ca. 0.5 ppm lower than the usual ax-H in **3** probably because of the anisotropy effect of β(ax)-OH at C-17. Accordingly, compound **3** was assigned 28-nor-urs-12-ene-3β,17β-diol, which is identical with the hydrolysis product of cladocalol reported recently by Benyahia et al.[10]

The anti-inflammatory activity of the compounds in vitro is estimated by an assay on inhibitory activity of induction of intercellular adhesion molecule-1 (ICAM-1).[12,13] We previously reported synthetic and natural compounds that strongly inhibit induction of ICAM-1.[14,15] The four above-mentioned compounds, **1**−**4**, were screened for inhibition of induction of ICAM-1 using human cultured A549 cells (lung carcinoma), an in vitro model of human endothelial cells, and the results are expressed by IC_{50} values (Table 2). Cell viability was measured by MTT assay and reported as IC_{50} values. The results of the MTT assay may also be evaluated as anticancer activity of the compounds toward lung carcinoma in vitro. Compound **1** showed weak inhibitory activity on induction of ICAM-1. The results of the MTT assay indicated that compound **1** showed weak cell growth inhibitory activity on A549 cells.

It has been reported that tumor initiation and promotion are inhibited by ursolic acid and oleanolic acid.[7] Cytotoxic and antileukemic activities of lupane triterpenes were also reported quite recently.[16] With this in mind, we have examined the cell growth inhibitory activity of compounds **1**−**4**, which were isolated in this work. The cell growth inhibitory activity was evaluated on WI-38 cells (normal human fibroblast cells induced from lung cells), VA-13 cells (malignant lung tumor cells induced from WI-38), and HepG2 cells (human liver cancer cells) (Table 3). Compounds **1**

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1**−**3** ($CDCl_3$, 125 MHz for ^{13}C NMR and 500 MHz for ^{1}H NMR, δ in ppm and J in Hz)[a]

carbon	1	2	3	proton	1	2	3
1	38.8 (t)	38.8 (t)	38.7 (t)	H-β	1.69 (1H, m)	1.70 (1H, m)	1.66 (1H, m)
				H-α	0.95 (1H, m)	0.92 (1H, m)	1.02 (1H, m)
2	27.4 (t)	27.4 (t)	27.2 (t)	H-α	1.62 (1H, m)	1.62 (1H, m)	1.63 (1H, m)
				H-β	1.59 (1H, m)	1.58 (1H, m)	1.59 (1H, m)
3	78.9 (d)	79.0 (d)	79.0 (d)	H-α	3.19 (1H, dd, 11.5, 4.4)	3.19 (1H, dd, 11.2, 4.4)	3.22 (1H, dd, 11.0, 5.1)
4	38.9 (s)	38.9 (s)	38.8 (s)				
5	55.4(d)	55.4 (d)	55.2 (d)	H-α	0.69 (1H, br d, 11.2)	0.69 (1H,m)	0.76 (1H, br d, 11.7)
6	18.3 (t)	18.2 (t)	18.3 (t)	H-α	1.52 (1H, m)	1.54 (1H, m)	1.54 (1H, m)
				H-β	1.37 (1H, m)	1.40 (1H, m)	1.39 (1H, m)
7	33.8 (t)	33.9 (t)	33.0 (t)	H-β	1.40 (1H, m)	1.38 (2H, m)	1.52 (1H, m)
				H-α	1.34 (1H, m)		1.43 (1H, m)
8	40.8 (s)	40.7 (s)	39.8 (s)				
9	50.8 (d)	50.6 (d)	47.6 (d)	H-α	1.37 (1H, m)	1.33 (1H, m)	1.54 (1H, m)
10	37.2 (s)	37.2 (s)	37.0 (s)				
11	21.1 (t)	21.3 (t)	23.6 (t)	H_2	1.25 (2H, m)	1.27 (2H, m)	1.94 (2H, dd, 9.0, 3.6)
12	25.1 (t)	27.3 (t)	127.8 (d)	H-α	1.69 (1H, m)	1.10 (1H, m)	5.30 (1H, t, 3.6)
				H-β	1.64 (1H, m)	1.64 (1H, m)	(H-12 olefin proton)
13	39.3 (d)	38.7 (d)	137.9 (s)	H-β	1.56 (1H, m)	1.73 (1H, m)	
14	41.3 (s)	41.6 (s)	41.9 (s)				
15	26.3 (t)	26.6 (t)	26.0 (t)	H-β	1.56 (1H, m)	1.52 (1H, m)	2.04 (1H, m)
				H-α	1.02 (1H, m)	1.04 (1H, m)	1.05 (1H, m)
16	27.7 (t)	27.5 (t)	28.4 (t)	H-β	1.56 (1H, m)	1.50 (1H, m)	1.25 (1H, m)
				H-α	1.15 (1H, m)	1.76 (1H, m)	2.02 (1H, m)
17	35.2 (s)	37.6 (s)	72.1 (s)				
18	48.6 (d)	45.4 (d)	60.6 (d)	H-α	0.88 (1H, m)	0.74 (1H, m)	1.59 (1H, m)
19	42.6 (d)	44.2 (d)	41.6 (d)	H-β	1.38 (1H, m)	1.47 (1H, m)	1.27 (1H, m)
20	73.9 (s)	74.2 (s)	39.3 (d)				0.92 (1H, m)
21	27.4 (t)	133.0 (d)	32.3 (t)	H-β	1.64 (1H, m)	5.98 (1H, d, 8.2)	1.57 (1H, m)
				H-α	1.62 (1H, m)	(H-21 olefn)	1.18 (1H, m)
22	28.8 (t)	140.6 (d)	40.4 (t)	H-β	1.73 (1H, m)	6.10 (1H, d, 8.2)	1.57 (1H, m)
				H-α	0.91 (1H, m)	(H-22 olefin)	1.73 (1H, m)
23	28.0 (q)	28.0 (q)	28.2 (q)	H_3	0.97 (3H, s)	0.97 (3H, s)	1.01 (3H, s)
24	15.4 (q)	15.4 (q)	15.6 (q)	H_3	0.77 (3H, s)	0.77 (3H, s)	0.80 (3H, s)
25	16.3 (q)	16.3 (q)	15.5 (q)	H_3	0.84 (3H, s)	0.85 (3H, s)	0.95 (3H, s)
26	15.7 (q)	15.7 (q)	17.1 (q)	H_3	0.98 (3H, s)	1.00 (3H, s)	0.99 (3H, s)
27	14.6 (q)	14.3 (q)	23.0 (q)	H_3	0.93 (3H, s)	0.95 (3H, s)	1.08 (3H, s)
28	100.2 (d)	65.9 (t)		H-β	4.87 (1H, s)	β 4.17 (1H, d, 7.8)	
						α 2.81 (1H, dd, 7.8, 1.5)	
29	20.1 (q)	20.9 (q)	17.3 (q)	H_3	0.87 (3H, d, 7.1)	0.69 (3H, d, 6.8)	0.82 (3H, d, 6.6)
30	25.0 (q)	22.2 (q)	20.7 (q)	H_3	1.10 (3H, s)	1.30 (3H, s)	1.10 (3H, d, 6.1)
OMe	55.2 (q)			H_3	3.43 (3H,s)		

[a] Assignments are based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

Table 2. Effect of Triterpenoids **1**−**4** on Induction of ICAM-1 and Cell Viability[a]

	1	2	3	4
ICAM-1 IC50 (μM)[b]	80	>316	>316	158
MTT IC50 (μM)[c]	76	>316	>316	205

[a] A549 cells were pretreated with various concentrations of the compound for 1 h and then incubated in the presence of IL-1α for 6 h. Absorbency of 415 nm was assayed after treatment of the cells with primary and secondary antibodies and addition of the enzyme substrate. [b] The experiments were carried out in triplicate cultures. [c] A549 cells were incubated with serial dilutions of the compounds for 24 h. Cell viability (%) was measured by MTT assay and used for determination of IC_{50}. The experiments were carried out in triplicate cultures.

Table 3. Cell Growth Inhibitory Activities of Compounds on WI-38, VA-13, and HepG2 Cells[a]

compound	WI-38 IC_{50} (μM)	VA-13 IC_{50} (μM)	HepG2 IC_{50} (μM)
1	11.8	11.7	10.3
2	83.8	111	155
3	20.1	108	21.1
4	94.7	85.4	99
Taxol	0.04	0.005	8.1
ADM	0.66	0.38	1.2

[a] Cell growth inhibitory effects on three cells were determined with Taxol and ADM as positive control, and IC_{50} was defined as the compound concentration causing 50% growth inhibition.

and **3** showed significant and moderate cell growth inhibitory activity and compounds **2** and **4** showed weak cell growth inhibitory activity for WI-38 cells, respectively. Compound **1** showed significant cell growth inhibitory activity for VA-13 cells. Compounds **1** and **3** showed significant and moderate cell growth inhibitory activity and compound **4** showed weak cell growth inhibitory activities for HepG2 cells, respectively. It is interesting that new taraxasterane derivative **1** showed significant cell growth inhibitory activities for both VA-13 cells and HepG2 cells (IC_{50} = 11.7 and 10.3 μM, respectively) and comparable inhibitory activities for WI-38 cells (IC_{50} = 11.8 μM).

Experimental Section

General Experimental Procedures. Melting points are uncorrected. Optical rotation values were measured using a Horiba Sepa-200 polarimeter. IR spectra were recorded on a Hitachi 270-30 infrared spectrometer. ^{1}H and ^{13}C NMR spectra were measured with a Varian Unity-plus instrument at 500 and 125 MHz. ^{1}H NMR assignments were determined by ^{1}H−^{1}H COSY experiments. ^{13}C NMR assignments were determined using DEPT, HMBC, and HMQC experiments. FABMS were recorded on a JEOL JMS-HX110 instrument. Silica gel (70−230 mesh) was employed for column chromatography and silica gel (230−400 mesh) for flash column chromatography. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10 × 250 mm stainless steel column and an Inertsil Prep-ODS GL 10 × 250 mm stainless steel column and monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.

Plant Material. Leaves of *Nerium oleander* L. were collected in Niigata City, Niigata Province, Japan, in November 2001. The plant was identified by Dr. K. Yonekura, Department of Biology, Faculty of Science, Tohoku University, Sendai, Japan. A voucher specimen (2001-

11-10) was deposited at the Department of Chemistry and Chemical Engineering, Niigata University.

Extraction and Isolation. Air-dried leaves (9.91 kg) were extracted two times with MeOH (66 and 39 L) for 3 and 4 days. The MeOH extract was concentrated to 10 L and extracted with hexane (5 × 5 L). Water (4 × 10 L) was added to the MeOH layer, and this was extracted with EtOAc (5 × 5.0 L). The EtOAc extracts were dried and concentrated to give an oil (519 g). To a part of the EtOAc extract (80.64 g), 3 × 200 mL of EtOAc were added, stirred for 1 h, and filtered. The filtrate was concentrated to give on drying a viscous oil, NE (36.69 g, 45.83% based on EtOAc extract), which was separated by column chromatography on silica gel (3.4 kg) into 17 fractions, NE-1−NE-17, using a gradient of hexane and EtOAc. Fraction NE-5 [hexane−EtOAc (7:3)] gave 4466 mg of a semisolid, which was washed with 20 mL of EtOAc and filtered. The filtrate was concentrated to give a semisolid, NE-5F (3013.8 mg). This was further separated by column chromatography [silica gel (274 g)] and divided into seven fractions, NE-5F-1−NE-5F-7, using a gradient of hexane and EtOAc. Fractions NE-3 [hexane−EtOAc (7:3)], NE-4 [hexane−EtOAc (7:3)], NE-5F-1 [hexane−EtOAc (9:1)], and NE-5F-2 [hexane−EtOAc (8:2)] were combined to give 2350 mg of a semisolid, which was named NE-A and separated by flash chromatography [silica gel 230−400 mesh, 235 g] to give nine fractions, (NE-A-1−NE-A-9), using a gradient of hexane and EtOAc. Fraction NE-A-4 [hexane−EtOAc (8:2)] gave on drying a viscous oil (450.8 mg), which was further separated by HPLC [silica gel, hexane−EtOAc (75:25)] into seven fractions (NE-A-4-1−NE-A-4-7). Fraction NE-A-4-3 gave on drying a viscous oil (280.1 mg), which separated by HPLC [silica gel, hexane−EtOAc (8:2)] into seven fractions (NE-A-4-3-1−NE-A-4-3-7). Fraction NE-A-4-3-2 gave on drying a viscous oil (195.1 mg), which was separated by HPLC [silica gel, hexane−EtOAc (9:1)] into eight fractions (NE-A-4-3-2-1−NE-A-4-3-2-8). Fraction NE-A-4-3-2-2 (9.7 mg) was purified by HPLC [ODS, MeOH−H_2O (95:5)] to give **1** (2.8 mg). Fraction NE-A-4-3-2-4 (9.6 mg) was purified by HPLC [ODS, MeOH−H_2O (9:1)] to give **2** (3.5 mg). Fraction NE-A-4-3-2-3 was separated by HPLC [ODS, MeOH−H_2O (95:5)] into seven fractions (NE-A-4-3-2-3-1−NE-A-4-3-2-3-7). Fraction NE-A-4-3-2-3-2 (36.4 mg) was purified by HPLC [ODS, MeCN−H_2O (75:25)] to give **3** (7.8 mg). Fraction NE-A-4-3-2-3-4 (4.3 mg) was purified by HPLC [ODS, MeCN−H_2O (85:15)] to give **4** (1.9 mg). The isolated yields of **1**, **2**, **3**, and **4** from the weight of dry leaves were 0.00018, 0.00023, 0.00051, and 0.00012%, respectively.

20β,28-Epoxy-28α-methoxytaraxasteran-3β-ol (1): colorless microcrystals; mp 172−174 °C; $[\alpha]^{20}_D$ +46.8 (*c* 0.231, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3624, 2997, 2947, 2872, 1468, 1377 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS (+NaI) *m*/*z* 495 [(M + Na)$^+$]; HRFABMS *m*/*z* 495.3803 [calcd for $C_{31}H_{52}O_3Na$ 495.3814].

20β,28-Epoxytaraxaster-21-en-3β-ol (2): colorless microcrystals; mp 226−229 °C; $[\alpha]^{20}_D$ +72.6 (*c* 0.215, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3624, 2948, 2872, 1612, 1452, 1380 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 440.3668 [calcd for $C_{30}H_{48}O_2$ 440.3654].

28-nor-Urs-12-ene-3β,17β-diol (3): colorless microcrystals; mp 152−155 °C; $[\alpha]^{20}_D$ +90.6 (*c* 0.362, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3624, 2949, 2928, 2862, 1454, 1379 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS (+NaI) *m*/*z* 451 [(M + Na)$^+$]; HRFABMS *m*/*z* 451.3554 [calcd for $C_{29}H_{48}O_2Na$ 451.3552].

Inhibitory Activity on Induction of ICAM-1. Experimental details were described in our previous paper.[9,14,15]

Cell Growth Inhibitory Activity to WI-38, VA-13, and HepG2 in Vitro. Experimental details were described in our previous paper.[9]

References and Notes

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