# Three New Triterpenes from *Nerium oleander* and Biological Activity of the Isolated Compounds

Liwei Fu,[†] Shujun Zhang,[†,¶] Na Li,[‡] Jinlan Wang,[†,¶] Ming Zhao,[†] Junichi Sakai,[‡] Toshiaki Hasegawa,[§] Tomokazu Mitsui,[⊥] Takao Kataoka,[⊥] Seiko Oka,[#] Miwa Kiuchi,[#] Katutoshi Hirose,[||] and Masayoshi Ando*,[‡]

*Graduate School of Science and Technology and Department of Chemistry and Chemical Engineering, Niigata University, Ikarashi, 2-8050, Niigata 950-2181, Japan, Mitsubishi Gas Chemical Company Inc., Niigata Research Laboratory, 182, Shinwari, Tayuhama, Niigata 950-3112, Japan, Division of Bioinformatics Center for Biological Resources and Informatics, Tokyo Institute of Technology, 4259 Nagatuta-cho, Midori-ku, Yokohama 226-8501, Japan, Center for Instrumental Analysis, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan, Kobe Natural Products and Chemicals Co., Ltd., 527 Nisikawanobe, Ichikawa-Cho, Kanzaki-Gun Hyogo 679-2315, Japan, and Department of Pharmacy Engineering, College of Chemistry and Chemical Engineering, Qiqihar University, 30 Wenhuadajie, Qiqihar, Heilongjiang Sheng, 161006, People's Republic of China*

*Received March 18, 2004*

New ursane-type triterpene **1**, oleanane-type triterpene **2**, and dammarane-type triterpene **15** were isolated from the leaves of *Nerium oleander* together with 12 known triterpenes, 3$\beta$-hydroxy-12-ursen-28-oic acid (ursolic acid, **3**), 3$\beta$,27-dihydroxy-12-ursen-28-oic acid (**4**), 3$\beta$,13$\beta$-dihydroxyurs-11-en-28-oic acid (**5**), 3$\beta$-hydroxyurs-12-en-28-aldehyde (**6**), 28-norurs-12-en-3$\beta$-ol (**7**), urs-12-en-3$\beta$-ol (**8**), urs-12-ene-3$\beta$,28-diol (**9**), 3$\beta$-hydroxy-12-oleanen-28-oic acid (oleanolic acid, **10**), 3$\beta$,27-dihydroxy-12-oleanen-28-oic acid (**11**), 3$\beta$-hydroxy-20(29)-lupen-28-oic acid (betulinic acid, **12**), 20(29)-lupene-3$\beta$,28-diol (betulin, **13**), and (20*S*,24*R*)-epoxydammarane-3$\beta$,25-diol (**14**). On the basis of their spectroscopic data, the structures of the new compounds **1**, **2**, and **15** were established as 3$\beta$,20α-dihydroxyurs-21-en-28-oic acid, 3$\beta$,12α-dihydroxy-oleanan-28,13$\beta$-olide, and (20*S*,24*S*)-epoxydammarane-3$\beta$,25-diol, respectively. The anti-inflammatory activity of the seven isolated compounds and methyl esters of ursolic acid and oleanoic acid in vitro was examined on the basis of inhibitory activity against the induction of the intercellular adhesion molecule-1 (ICAM-1). The anticancer activity of the 14 isolated compounds, including **1**, **2**, **15**, and methyl esters of ursolic acid and oleanolic acid in vitro was examined on the basis of the cell growth inhibitory activities toward three kinds of human cell lines.

*Nerium oleander* (*Nerium indicum, Nerium odorum*)[1] is a medium-sized flowering evergreen tree of 2−5 m in height and is planted throughout Japan as garden and roadside trees. *N. oleander* was originally distributed in the Mediterranean region, subtropical Asia, and Indo-Pakistan subcontinent. This plant possesses cardiotonic, antibacterial, anticancer, and antiplatelet aggregation activity and depresses the central nervous system.[2−6] In connection with these biological activities, many kinds of steroidal cardenolides and steroidal glycosidic cardenolides were reported.[7−13a] Ishidate and co-workers reported the isolation of triterpene carboxylic acids, ursolic acid, and oleanolic acid in the early stage of the study of this plant.[14] Since then, several ursane- and lupane-type pentacyclic terpenoids such as kanetic acid, oleanderolic acid, and oleanderol were isolated.[6,13a−c] Triterpenoids have many biological activities such as inhibition of tumor-promoting action,[15,16] anti-inflammatory activities,[15,17] hepatoprotective effects,[17] antitumor activity,[17] antihyperlipidemic effects,[17] antiulcer effects,[17] and NO inhibitory activity.[18] Against this background, we are interested in the relationship between the biological activities of the plant and the triterpenes contained in the plant. In this paper we report the results of phytochemical screening of the triterpene fraction of the leaves of *N. oleander*, possessing lower polarity than steroidal cardenolides and steroidal glycosidic cardenolides.

## Results and Discussion

A methanol extract of air-dried leaves of this plant was partitioned with hexane, ethyl acetate, and *n*-butanol. The ethyl acetate portion contained a large amount of low-soluble material in ethyl acetate. This was separated as a solid (2.57% of dried leaves) in the course of concentration of the solution and deduced to be a ca. 1:4.5 mixture of oleanolic acid and ursolic acid by analyses of the $^{1}$H NMR spectrum. The ratio was finally determined by the separation with HPLC after methylation of this mixture with $CH_2N_2$. The crude fraction of triterpenes was obtained mainly from the less polar portion of the filtrate of the solid and separated by silica gel column chromatography and HPLC. A new triterpene carboxylic acid named oleanderic acid (**1**) and a new triterpene $\gamma$-lactone named oleanderolide (**2**) (Figure 1) along with 13 triterpenes including the third new triterpene 24-*epi*-ocotillol (**15**) (Figure 2) were isolated.

Oleanderic acid (**1**) had the composition $C_{30}H_{48}O_4$, which was determined by a combination of EIMS, FABMS, and $^{1}$H and $^{13}$C NMR spectra. The IR spectrum of **1** indicated the existence of hydroxyl and carboxyl groups (3724, 3624, 3024, 1740 cm$^{-1}$). The $^{13}$C NMR displayed 30 carbon signals. The carbonyl carbon of a carboxyl group was located at $\delta$ 175.8. Signals for two carbons bearing oxygen were observed at $\delta$ 79.2 (d) and 84.0 (s). Olefin carbons were observed at $\delta$ 134.0 (d) and 138.7 (d). Judging from the DEPT and HMQC spectra, the remaining carbon reso-

* To whom correspondence should be addressed. Tel and Fax: +81-25-262-7326. E-mail: mando@eng.niigata-u.ac.jp.
[†] Graduate School of Science and Technology, Niigata University.
[‡] Department of Chemistry and Chemical Engineering, Niigata University.
[§] Mitsubishi Gas Chemical Company, Inc.
[⊥] Tokyo Institute of Technology.
[#] Hokkaido University.
[||] Kobe Natural Products and Chemicals Co. Ltd.
[¶] Qiqihar University.

10.1021/np040072u CCC: $30.25 © 2005 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/28/2005

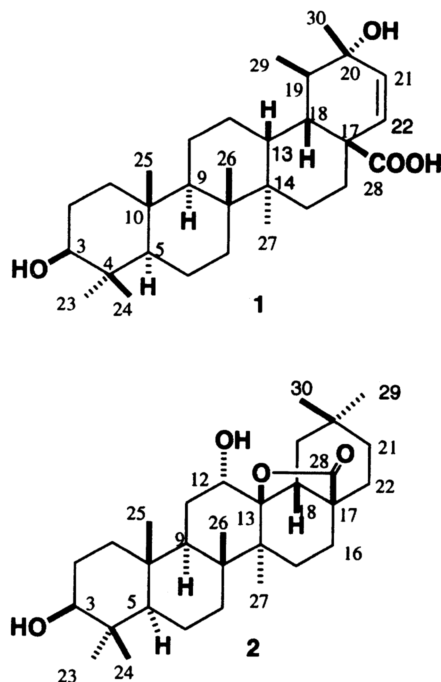

**Figure 1.**

nances were five quaternary carbons, five methine carbons, eight methylene carbons, and seven methyl carbons. The $^{1}$H NMR spectra showed six methyl singlets, one methyl doublet, and a pair of olefinic doublets ($\delta$ 6.07 and 6.10, $J$ = 7.6 Hz). The connections 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) were determined by analysis of the $^{1}$H−$^{1}$H COSY spectrum. An HMBC experiment was used to determine the carbon−carbon connections through the nonprotonated carbon atoms. The results are summarized in Table 1 and showed that compound **1** was a triterpene possessing an ursane skeleton. The correlation of the signal due to a carbon doublet bearing a hydroxyl group at $\delta$ 79.2 with those of H-23 and H-24 of the *gem*-dimethyl moiety placed the hydroxyl group at C-3. The carbonyl signal at $\delta$ 175.8 correlated with those of H-16α and H-18, and the signal due to the carbon singlet bearing a hydroxyl group at $\delta$ 84.0 correlated with those of H-19, H-21, H-22, H-29, and H-30. The results suggested that the carboxyl and tertiary hydroxyl groups were connected to C-17 and C-20, respectively, and the double bond was placed between C-21 and C-22.

The NOESY experiment was used for assignment of the configuration of **1**. The NOESY correlation [H-3 with H-23 (4α-Me) and H-5; H-9 with H-5, H-12α, and H-27; H-25 with H-26; H-13 with H-15$\beta$ and H-26; H-29 with H-18, H-19, and H-30; H-30 with H-21 and H-29; H-21 with H-30; H-22 with H-16$\beta$] indicated the relative configuration of oleanderic acid as shown in structure **1**. The correlation of H-22 with H-16$\beta$ indicated *cis*-fusion in the D−E ring and $\beta$ (*eq* in D ring)-orientation of the carboxyl group at C-17. Evidence regarding the configuration of the C-20 hydroxyl group could not be obtained from the NOESY experiment. The coupling constant between H-18 and H-19 was 7.8 Hz and the dihedral angle of H-18 and H-19 was 140° from the Karplus equation. This observation and an NOE between H-18 and H-29 suggested that the conformation of the E ring of **1** was a boat conformation (Figure 3). A chair conformation is excluded because no NOE is observed between H-19 and H-27 (14-Me) and H-16α. The observed NOE effect between H-29 and H-30 indicated that the orientation of 20-Me and 20-OH was $\beta$ (*eq*) and α (*ax*), respetively. This was also supported by a NOESY correlation between H-30 and H-21. In this structure, H-16α and 20α-OH are in close proximity. H-16α resonated at 1.61 ppm and 0.46 ppm lower than the H-16$\beta$ proton. H-30 (20-Me) appeared at 1.54 ppm and ca. 0.24 ppm lower than the usual methyl proton (ca. 1.30 ppm for *eq*-methyl in *cis*-ring fusion) connected to an oxygenated carbon probably because 20-Me is located in the plane of the 21,22-double bond. Accordingly, compound **1** was assigned as 3$\beta$,20α-dihydroxyurs-21-en-28-oic acid.

Oleanderolide (**2**) had the composition $C_{30}H_{48}O_4$, which was determined by a combination of HREIMS [*m/z* 472.3555 (calcd for $C_{30}H_{48}O_4$ 472.3553)] and $^{1}$H and $^{13}$C NMR spectra (Table 2). The IR spectrum of **2** indicated the existence of a hydroxyl and a $\gamma$-lactone moiety (3700, 3632, 1764 cm$^{-1}$).

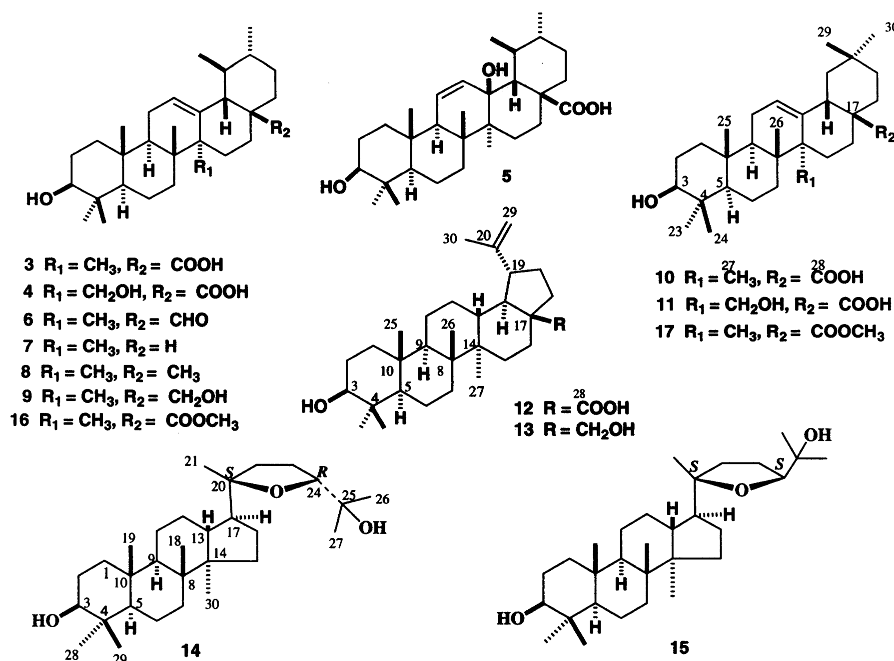


**Figure 2.**

**Table 1.** NMR Data of Compound **1** in $CDCl_3$

| position | $^{13}C$ | connected $^1H$ | $^1H-^1H$ COSY | HMBC | NOE |
|---|---|---|---|---|---|
| 1 | 39.1 (t) | α 1.69 (1H, ddd, 12.5, 3.0, 3.0) | H-1β, H-2α,β | H-5, H-25, H-9 | |
| | | β 0.92 (1H, m) | H-1α, H-2α,β | | |
| 2 | 27.6 (t) | α 1.62 (1H, m) | H-2β, H-1α,β, H-3 | H-1β | H-3 |
| | | β 1.57 (1H, m) | H-2α, H-1α,β, H-3 | | H-24 |
| 3 | 79.2 (d) | 3.21 (1H, dd, $J$ = 11.5, 5.0) | H-2α,β | H-2α, H-23, H-24 | H-2α, H-5, H-23 |
| 4 | 39.1 (s) | | | H-5, H-23, H-24 | |
| 5 | 55.6 (d) | 0.68 (1H, br d, 11.5) | H-6α,β | H-7α, H-23, H-24, H-25 | H-3, H-7α, H-9, H-23 |
| 6 | 18.5 (t) | β 1.52 (1H, m) | H-5, H-6α, H-7α,β | H-5, H-7α | H-24 |
| | | α 1.30 (1H, m) | H-5, H-6β, H-7α,β | | |
| 7 | 34.2 (t) | β 1.44 (1H, m) | H-7α, H-6α,β | H-5, H-26, H-6α | |
| | | α 1.38 (1H, m) | H-7β, H-6α,β | | H-5 |
| 8 | 40.8 (s) | | | H-6β, H-7β, H-9, H-26, H-27 | |
| 9 | 50.7 (d) | 1.28 (1H, m) | H-11α,β | H-7β, H-11α,β, H-25, H-26 | H-12α, H-5, H-27 |
| 10 | 37.4 (s) | | | H-5, H-6β, H-9, H-25 | |
| 11 | 21.3 (t) | α,β 1.27 (2H, m) | H-9, H12α,β | H-9 | |
| 12 | 27.6 (t) | α 1.60 (1H, m) | H-11α,β, H-12β, H-13 | H-11α,β | H-9, H-27 |
| | | β 1.10 (1H, m) | H-11α,β, H-12α, H-13 | | |
| 13 | 42.5 (d) | 1.34 (1H, m) | H-12α,β, H-18 | H-15α, H-18, H19, H-27 | H-15β, H-26 |
| 14 | 41.5 (s) | | | H-15α,β, H-16α, H-26, H-27 | |
| 15 | 27.1 (t) | β 2.12 (1H, ddd,14.0, 13.5, 4.5) | H-15α, H-16α,β | H-16α, H-27 | H-13, H-16β, H-26 |
| | | α 1.15 (1H, βr d, 14.0) | H-15β, H-16α,β | | H-27 |
| 16 | 25.7 (t) | β 2.25 (1H, ddd, 13.5, 4.5, 3.0) | H-16α, H-15α,β | H-15α,β | H-15β, H-22 |
| | | α 1.61 (1H, m) | H-16β, H-15α,β | | H-27 |
| 17 | 48.4 (s) | | | H-16α,β, H-18, H-21, H-22 | |
| 18 | 47.5 (d) | 0.98 (1H, m) | H-3, H-18, H-19 | H-13, H-29 | H-29 |
| 19 | 44.8 (d) | 1.72 (1H, dq, 7.0, 7.0) | H-18, H-29 | H-18, H-29, H-30 | H-29 |
| 20 | 84.0 (s) | | | H-19, H-21, H-22, H-29, H-30 | |
| 21 | 134.0 (d) | 6.07 (1H, d, $J$ = 7.6) | H-22 | H-19, H-30 | H-30 |
| 22 | 138.7 (d) | 6.10 (1H, d, $J$ = 7.6) | H-21 | H-16α, H-18 | H-16β |
| 23 | 28.2 (q) | 0.97 (3H, s) | | H-3, H-5, H-24 | H-3, H-5, H-24 |
| 24 | 15.6 (q) | 0.76 (3H, s) | | H-3, H-5, H-23 | H-2β, H-6β, H-23 |
| 25 | 15.9 (q) | 0.83 (3H, s) | | H-1α, H-5, H-9 | H-26 |
| 26 | 16.5 (q) | 0.95 (3H, s) | | H-7β, H-9 | H-13, H-15β, H-25 |
| 27 | 14.3 (q) | 0.95 (3H, s) | | H-15β | H-9, H-12α, H15α, H-16α |
| 28 | 175.8 (s) | | | H-16α, H-18 | |
| 29 | 19.9 (q) | 0.85 (3H, d, 7.1) | H-19 | H-18, H-19 | H-18, H-19, H-30 |
| 30 | 21.3 (q) | 1.54, (3H, s) | | H-19 | H-21, H-29 |

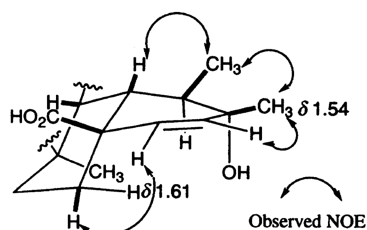


**Figure 3.** Stereochemistry of D−E rings of **1**.

The $^{13}C$ NMR displayed 30 carbon signals. The carbonyl carbon of a γ-lactone moiety was located at δ 179.9. Signals for three oxygenated carbons were observed at δ 78.8 (d), 76.4 (d), and 90.5 (s). Judging from the DEPT and HMQC spectra, the remaining carbon resonances were six quaternary carbons, three methine carbons, 10 methylene carbons, and seven methyl carbons. The $^1H$ NMR spectra showed seven methyl singlets (δ 0.78, 0.88, 0.91, 0.99, 1.00, 1.15, 1.30). The connections 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-15 to C-16; C-18 to C-19; C-21 to C-22) were determined by analysis of the $^1H-^1H$ COSY spectrum. An HMBC experiment was used to determine the carbon−carbon connection through the nonprotonated carbon atom. The results showed that compound **2** was a triterpene possessing an oleanane skeleton. The correlation of the signal due to a carbon doublet bearing a hydroxyl group at δ 78.8 with those of H-23 and H-24 of the *gem*-dimethyl moiety placed the hydroxyl group at C-3. The coupling constant of H-3 ($J$ = 11.5, 4.6 Hz) indicated that the orientation of the hydroxyl group at C-3 was β (*eq*). The carbonyl carbon signal at δ 179.9 correlated with those of H-16 and H-22, and the signal due to a doublet carbon bearing a hydroxyl group at δ 76.4 correlated with that of H-11. The signal due to a singlet carbon bearing a lactone

**Table 2.** NMR Data of Compound **2** in $CDCl_3$

| position | $^{13}C$ | connected $^1H$ |
|---|---|---|
| 1 | 38.8 (t) | 1.73 (1H, ddd, $J$ = 11.1, 3.4, 3.4), 0.95 (1H, m) |
| 2 | 27.5 (t) | 1.68 (1H, m), 1.65 (1H, m) |
| 3 | 78.8 (d) | 3.23 (1H, dd, $J$ = 11.5, 4.6) |
| 4 | 38.9 (s) | |
| 5 | 55.2 (d) | 0.76 (1H, m) |
| 6 | 17.7 (t) | 1.50 (1H, m) |
| 7 | 34.0 (t) | 1.56 (1H, m), 1.26 (1H, m) |
| 8 | 42.1 (s) | |
| 9 | 44.6 (d) | 1.54 (1H, m) |
| 10 | 36.4 (s) | |
| 11 | 28.8 (t) | 2.00 (1H, m), 1.48 (1H, m) |
| 12 | 76.4 (d) | 3.90 (1H, br s, $W_{h/2}$ = 9.0) |
| 13 | 90.5 (d) | |
| 14 | 42.3 (s) | |
| 15 | 28.0 (t) | 1.89 (1H, m) |
| | | 1.18 (1H, m) |
| 16 | 21.2 (t) | 2.13 (1H, ddd, $J$ = 13.3, 13.4, 5.6) |
| | | 1.25 (1H, m) |
| 17 | 44.7 (s) | |
| 18 | 51.1 (d) | 2.04 (1H, m) |
| 19 | 39.4 (t) | 2.00 (1H, m), 1.85 (1H, m) |
| 20 | 31.6 (s) | |
| 21 | 34.1 (t) | 1.39 (1H, m), 1.36 (1H, m) |
| 22 | 27.2 (t) | 1.62 (1H, m), 1.59 (1H, m) |
| 23 | 28.0 (q) | 1.00 (3H, s) |
| 24 | 15.4 (q) | 0.78 (3H, s) |
| 25 | 15.9 (q) | 0.88 (3H, s) |
| 26 | 18.5 (q) | 1.15 (3H, s) |
| 27 | 18.6 (q) | 1.30 (3H, s) |
| 28 | 179.9 (s) | |
| 29 | 33.3 (q) | 0.99 (3H, s) |
| 30 | 23.9 (q) | 0.91(3H, s) |

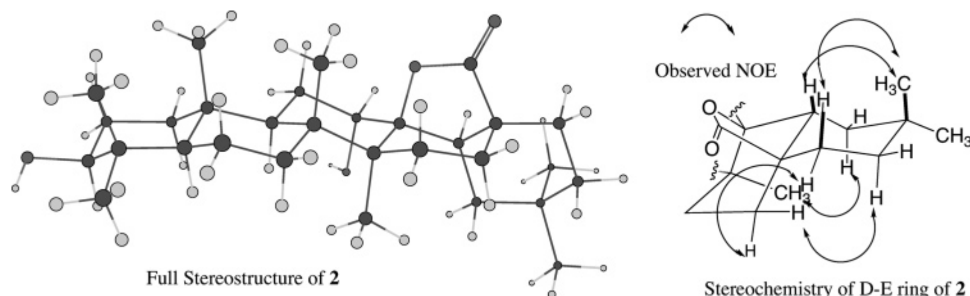


**Figure 4.** Stereostructure of **2**.

oxygen at δ 90.5 correlated with those of H-15, H-18, and H-27. Two carbon signals assigned to the geminal dimethyl moiety at δ 23.9 and 33.3 were correlated with those of H-19, H-21 and H-19, H-30, respectively. The results suggested that the carbonyl group and tertiary oxygen bond of a γ-lactone moiety were connected to C-17 and C-13, and a *sec*-hydroxyl group was connected to C-12. The small coupling constant of H-12 (δ 3.90, br s, $W_{h/2}$ = 9.0 Hz) indicated that the orientation of the hydroxyl group at C-12 is α (*ax*).

The NOESY experiment was used for assignment of the configuration of **2**. The NOESY correlation [H-3 with H-23 (4α-Me) and H-5; H-5 with H-9; H-12 with H-18; H-16 (α) with H-21 (α); H-16 (β) with H-22 (α); H-18 with H-30; H-25 with H-26; H-27 with H-19 (α); H-30 with H-22 (β)] indicated the relative configuration as shown in structure **2**. The correlation of H-21 (α) with H-16 (α), H-22 (α) with H-16 (β), and H-27 with H-19 (α) indicated *cis*-fusion of the D−E ring and a β-orientation of the γ-lactone ring (Figure 4). The lower chemical shift of H-27 (δ 1.30) than that of oleanolic acid (**10**) (δ 1.06) was explained by the anisotropic effect of the α(*ax*)-hydroxyl group at C-12. The observed NOE effect between H-12 and H-11β and between H-12 and H-18 also indicated that the orientation of 12-OH was α (*ax*). Accordingly, compound **2** was assigned as 3β,12α-dihydroxyoleanan-28,13β-olide (Table 2).

Kitagawa et al. reported structure **2** as the major product of photooxidation of oleanolic acid (**10**).[19a,b] Their compound indicated seven methyl singlets at δ 0.76, 0.88, 0.88, 0.96, 0.96, 0.96, and 1.12. These are different from those of 3β,12α-dihydroxyoleanan-28,13β-olide (**2**) that was isolated from *N. oleander* by us, as shown in Table 2. More recently, Yoshikawa et al. reported the glycoside of **2** as kochianolide III (3β,12α-dihydroxyolean-28,13β-olide 3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid),[19c] whose $^{13}$C NMR shifts were identical with those of **2** except that of C-3. This means that kochianolide III was the C-3 glycoside of **2**.

We isolated a further 13 triterpenes:[20] ursolic acid (1.12%, **3**),[14,21,22a−c] 3β,27-dihydroxy-12-ursen-28-oic acid (0.00007%, **4**),[23] 3β,13β-dihydroxyurs-11-en-28-oic acid (0.0055%, **5**),[24] 3β-hydroxyurs-12-en-28-aldehyde (0.0033%, **6**),[25] 28-norurs-12-en-3β-ol (0.0021%, **7**),[25] urs-12-en-3β-ol (0.0024%, **8**),[26] urs-12-ene-3β,28-diol (0.0026%, **9**),[27] 3β-hydroxy-12-oleanen-28-oic acid (0.25%, oleanolic acid, **10**),[14,21,28] 3β,27-dihydroxy-12-oleanen-28-oic acid (0.00004%, **11**),[29] 3β-hydroxy-20(29)-lupen-28-oic acid (0.045%, betulinic acid, **12**),[30] 20(29)-lupene-3β,28-diol (0.0030%, betulin, **13**),[31] (20*S*,24*R*)-epoxydammarane-3β,25-diol (0.00061%, **14**),[32] and (20*S*,24*S*)-epoxydammarane-3β,25-diol (0.0014%, **15**).[33a]

In the course of the survey of compounds **14** and **15**, we found that confusion existed in the structures and the names of ocotillol and ocotillol II. $^1$H and $^{13}$C NMR data of **14** and **15** are summerized in Tables 3 and 4. The configuration of C-20 and C-24 was determined by the NOESY experiment (Figure 5). Halls and Warnhoff originally isolated a natural product, ocotillol, from *Fouquieria splendens*[32a,b] and deduced the structure to be **14** or **15** by their chemical conversion of dammarenediol II into ocotillol monoacetate. Since the structure of dammarenediol II was known,[34a−d] the stereostructure of ocotillol became clear by this chemical correlation including the C-20 (*S*) configuration except the newly formed stereochemistry at C-24. They also mentioned the possibility of the existence of the C-24 isomer of ocotillol in the same plant material and the existence of a C-24 isomer of ocotillol monoacetate in the reaction products, but unfortunately they failed in the isolation of the isomers. The reported $^1$H NMR data of ocotillol [δ 0.78, 0.85, 0.87, 0.97, 0.97, 1.12, 1,12, 1.21 (s, Me), 3.18 (H-3α), 3.73 (H-24)] are identical with those of **14** [δ 0.77, 0.83, 0.87, 0.95, 0.97, 1.11, 1.13, 1.21 (s, Me), 3.20 (H-3α), 3.73 (H-24)] but different from those of **15** [δ 0.77, 0.85, 0.87, 0.97, 0.97, 1.11, 1.15, 1.19 (s, Me), 3.20 (H-3α), 3.64 (H-24)]; especially a big difference was observed in the chemical shift of H-24 of ocotillol and **15**. It is concluded that ocotillol is the 20*S*, 24*R* isomer and identical with compound **14**, which was isolated by us from *N. oleander*, and that consequently **15** is the 24*S* isomer of ocotillol **14**. In 1983, Shuarez et al. reported the structure and absolute configuration of trevoagenins A, B, and C

**Table 3.** NMR Data of Compound **14** in $CDCl_3$

| position | $^{13}$C | connected $^1$H |
|---|---|---|
| 1 | 39.1 (t) | 1.66 (1H, m), 0.94 (1H, m) |
| 2 | 27.4 (t) | 1.63 (1H, m), 1.56 (1H, m) |
| 3 | 79.00 (d) | 3.20 (1H, dd, *J* = 11.2, 4.9) |
| 4 | 39.00 (s) | |
| 5 | 55.9 (d) | 0.73 (1H, dd, *J* = 11.0, 2.0) |
| 6 | 18.3 (t) | 1.53 (1H, m), 1.44 (1H, m) |
| 7 | 35.3 (t) | 1.50 (1H, m), 1.26 (1H, m) |
| 8 | 40.4 (s) | |
| 9 | 50.8 (d) | 1.30 (1H, dd, *J* = 14.0, 3.0) |
| 10 | 37.1 (s) | |
| 11 | 21.6 (t) | 1.50 (1H, m), 1.46 (1H, m) |
| 12 | 25.7 (t) | 1.77 (1H, m), 1.47 (1H, m) |
| 13 | 43.00 (d) | 1.55 (1H, m) |
| 14 | 50.0 (s) | |
| 15 | 26.1 (t) | 1.61 (1H, m), 1.44 (1H, m) |
| 16 | 31.5 (t) | 1.84 (1H, m), 1.07 (1H, m) |
| 17 | 49.5 (d) | 1.78 (1H, m) |
| 18 | 15.4 (q) | 0.95 (3H, s) |
| 19 | 16.2 (q) | 0.83 (3H, s) |
| 20 | 86.4 (s) | |
| 21 | 23.5 (q) | 1.13 (3H, s) |
| 22 | 35.7 (t) | 1.62 (1H, m), 1.55 (1H, m) |
| 23 | 27.4 (t) | 1.84 (1H, m), 1.78 (1H, m) |
| 24 | 83.3 (d) | 3.73 (1H, dd, *J* = 7.5, 7.5) |
| 25 | 71.4 (s) | |
| 26 | 27.5 (q) | 1.21 (3H, s) |
| 27 | 24.2 (q) | 1.11 (3H, s) |
| 28 | 28.0 (q) | 0.97 (3H, s) |
| 29 | 15.3 (q) | 0.77 (3H, s) |
| 30 | 16.5 (q) | 0.87 (3H, s) |

**Table 4.** NMR Data of Compound **15** in $CDCl_3$

| position | $^{13}C$ | connected $^1H$ |
|---|---|---|
| 1 | 39.1 (t) | 1.68 (1H, m), 0.95 (1H, m) |
| 2 | 27.4 (t) | 1.64 (1H, m), 1.56 (1H, m) |
| 3 | 79.0 (d) | 3.20 (1H, dd, $J$ = 11.2, 4.9) |
| 4 | 39.0 (s) | |
| 5 | 55.9 (d) | 0.73 (1H, dd, $J$ = 11.7, 2.0) |
| 6 | 18.3 (t) | 1.51 (1H, m) |
| 7 | 35.3 (t) | 1.53 (1H, m), 1.25 (1H, m) |
| 8 | 40.4 (s) | |
| 9 | 50.8 (d) | 1.31 (1H, m) |
| 10 | 37.2 (s) | |
| 11 | 21.8 (t) | 1.87 (1H, m), 1.48 (1H, m) |
| 12 | 25.9 (t) | 1.74 (1H, m), 1.34 (1H, m) |
| 13 | 42.8 (d) | 1.62 (1H, m) |
| 14 | 50.0 (s) | |
| 15 | 27.0 (t) | 1.45 (1H, m), 1.06 (1H, m) |
| 16 | 31.5 (t) | 1.78 (1H, m), 1.65 (1H, m) |
| 17 | 49.8 (d) | 1.84 (1H, m) |
| 18 | 15.5 (q) | 0.97 (3H, s) |
| 19 | 16.2 (q) | 0.85 (3H, s) |
| 20 | 86.5 (s) | |
| 21 | 27.2 (q) | 1.15 (3H, s) |
| 22 | 34.8 (t) | 1.86 (1H, m), 1.64 (1H, m) |
| 23 | 26.4 (t) | 1.80 (1H, m) |
| 24 | 86.3 (d) | 3.64 (1H, dd, $J$ = 10.0, 5.1) |
| 25 | 70.2 (s) | |
| 26 | 27.8 (q) | 1.19 (3H, s) |
| 27 | 24.1 (q) | 1.11 (3H, s) |
| 28 | 28.0 (q) | 0.97 (3H, s) |
| 29 | 15.4 (q) | 0.77 (3H, s) |
| 30 | 16.4 (q) | 0.87 (3H, s) |

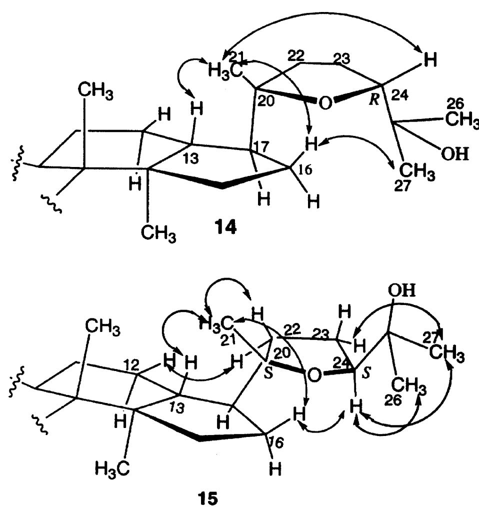


**Figure 5.** NOESY correlations of **14** and **15**.

based on their X-ray crystallographic analysis.[32c] Furthermore they performed the chemical transformation of trevoagenin B into ocotillol and concluded that the C-24 configuration of ocotillol was *R*.[32c] In their reported $^1H$ NMR, H-3 and H-24 chemical shifts are in good agreement with **14**. Strangely, many publications (Nagai et al.,[32d] Dev et al.,[32e] Ohmoto et al.,[32f] Nagai et al.,[32g] Connolly et al.,[32h] Tanaka et al.[32i]) use the name ocotillol-II for **14** and their reference was the publication by Halls and Warnhoff.[32a,b] Their $^1H$ NMR data in Tables 5 and 6 also suggested that ocotillol and ocotillol-II were indeed the same compound. We concluded that ocotillol and ocotillol-II are the same compound **14** possessing the (20*S*,24*R*)-configuration and should be called ocotillol as in the original nomenclature by Halls and Warnhoff.[32a,b]

The 3-epimer of **15** has been reported as cabraleadiol.[33b] The structure of the 3-OAc derivative of this compound was determined by X-ray crystallographic analysis.[33c] The reported $\delta$ values in $^1H$ and $^{13}C$ NMR of carbraleadiol are in good agreement with those of **15**, except H-29 (C-4$\beta$ Me), H-3, and C-3, and different from those of **14**. It is clear that the configuration at the C-3 and C-24 position of 20*S*,24$\xi$-epoxydammarane-3$\xi$,25-diol can be determined by $\delta$ values of $^1H$ and $^{13}C$ NMR. This NMR data also supported the structure of **15**, and we proposed calling **15** 24-*epi*-ocotillol-[(20*S*,24*S*)-epoxydammarane-3$\beta$,25-diol)].

The anti-inflammatory activity of the compounds in vitro was examined by an assay on the inhibitory activity of induction of intercellular adhesion molecule-1 (ICAM-1). Expression of an excess amount of ICAM-1 on the surface of endothelial cells of a blood vessel plays an important role in the progress of the inflammatory reaction. These facts suggest that one reason for the anti-inflammatory activities of traditional medicinal plants may exist in the inhibitory activities on induction of ICAM-1 by compounds in plants. Recently, anti-inflammatory effects of **3** and **10** have been reported.[17] With this in mind, we began to examine four natural ursane triterpenes, **3**, **4**, **5**, and **9**, one natural oleanane triterpene, **10**, and two natural lupane triterpenes, betulinic acid (**12**) and betulin (**13**), which were isolated from *N. oleander* with **1**, **2**, and **15**. The seven compounds and methyl ursolate (**16**) and methyl oleanolate (**17**) were screened for inhibition of induction of ICAM-1 using human cultured A549 cells (lung carcinoma), an in vitro model of the human endothelial cell, and the results were expressed by $IC_{50}$ values. Cell viability was measured by an MTT assay in $IC_{50}$ (Table 7). The results of the MTT assay may also be evaluated as anticancer activity of the compounds toward lung carcinoma in vitro.

We previously reported synthetic and natural compounds that strongly inhibit ICAM-1.[35−38] In contrast to these compounds, ursolic acid (**3**) showed moderate inhibitory activity on induction of ICAM-1 ($IC_{50}$ = 21.6 $\mu$M) under concentrations that did not decrease cell viability. Toxic activity of compound **3** ($IC_{50}$ = 55.8 $\mu$M) was about 2.6-fold higher than those required for the inhibition of ICAM-1 expression. The result of the MTT assay also indicated that compound **3** inhibited the cell growth of the lung carcinoma cell line A 549 in moderate concentration. Biological activities of both the methyl ester of **3** (compound **16**) and natural products possessing one more hydroxyl group at C-27 or C-13 of **3** (compounds **4** and **5**) decreased. Oleanolic acid (**10**) showed moderate inhibitory activity on induction of ICAM-1 under concentrations that did not decrease cell viability. Betulinic acid (**12**) and methyl oleanolate (**17**) showed moderate inhibitory activity on induction of ICAM-1 and cell growth of lung carcinoma A 549 cells. They have comparable activities in inhibitory activities on induction of ICAM-1 and cell viability measured by MTT assays, respectively. No inhibitory activity on ICAM-1 expression and cytotoxic activities toward A549 cells were observed in compounds **9** and **13**, which possess a hydroxymethyl group instead of a carboxyl group at C-17.

In conclusion, the order of the inhibitory activity of ICAM-1 in the test samples was **3** $>$ **17** $\geq$ **12** $\geq$ **10** $>$ **4** $\geq$ **5** $>$ **16** $\gg$ **9**, **13**. All compounds possessing a carboxyl moiety, such as **3**, **4**, **5** **10**, and **12**, and methyl esters **16** and **17** showed moderate inhibitory activity on induction of ICAM-1. Hence, it is inferred that a carboxyl or methyl carboxylate moiety in the molecule is essential for the activity in the case of these compounds. This might be consistent with the previous paper showing that most of the oleanane-type triterpenes with a C-17 methyl group except for abrisapogenol E did not inhibit ICAM-1 expression more than 50% at nontoxic compounds.[39] In fact, no

**Table 5.** $^{1}$H NMR Data of **14**, **15**, and Ocotillol and Related Data Reported in References

| | | 14 | 15 | ocotillol[32c] | ocotillol[32b] | cabraleadiol[33b] | ocotillol-II[32i] | 3-*epi*-ocotillol-II[32f] | ocotillol-II[32f] |
|---|---|---|---|---|---|---|---|---|---|
| Me-group | H-29 | 0.77 | 0.77 | 0.78 | 0.78 | 0.84 | 0.77 | | |
| | H-19 | 0.83 | 0.85 | 0.85 | 0.85 | 0.86 | 0.84 | | |
| | H-30 | 0.87 | 0.87 | 0.87 | 0.87 | 0.89 | 0.87 | | |
| | H-18 | 0.95 | 0.97 | 0.97 | 0.97 | 0.97 | 0.95 | | |
| | H-28 | 0.97 | 0.97 | 0.97 | 0.97 | 0.94 | 0.97 | | |
| | H-27 | 1.11 | 1.11 | 1.13 | 1.12 | 1.11 | 1.12 | | |
| | H-21 | 1.13 | 1.15 | 1.13 | 1.12 | 1.15 | 1.13 | | |
| | H-26 | 1.21 | 1.19 | | 1.21 | 1.19 | 1.21 | | |
| | H-3 | 3.20 (3β-OH) | 3.20 (3β-OH) | 3.18 (3β-OH) | 3.18 (3β-OH) | 3.40 (3α-OH) | 3.20 (3β-OH) | 3.38 (3α-OH) | 3.22 (3β-OH) |
| | H-24 | 3.73 (20*S*,24*R*) | 3.64 (20*S*,24*S*) | 3.73 (20*S*,24*R*) | 3.73 (20*S*,24*R*) | 3.65 (20*S*,24*S*) | 3.73 (20*S*,24*R*) | 3.71 (20*S*,24*R*) | 3.71 (20*S*,24*R*) |

**Table 6.** $^{13}$C NMR Data of **14**, **15**, Carbreadiol, and Ocotillol-II

| position | 14 | ocotillol-II[32i] | cabraleadiol[33b] | 15 |
|---|---|---|---|---|
| 3 | 78.95 (d) | 78.95 | 78.3 | 78.96 (d) |
| 20 | 86.41 (s) | 86.44 | 86.5 | 86.54 (s) |
| 21 | 23.54 (q) | 23.55 | 27.1 | 27.17 (q) |
| 22 | 35.67 (t) | 35.67 | 34.7 | 34.75 (t) |
| 23 | 27.37 (t) | 27.43 | 26.3 | 26.36 (t) |
| 24 | 83.30 (d) | 83.31 | 86.2 | 86.30 (d) |
| 25 | 71.42(s) | 71.43 | 70.2 | 70.23 (s) |
| 26 | 27.49 (q) | 27.43 | 27.8 | 27.84 (q) |
| 27 | 24.26 (q) | 24.28 | 24.0 | 24.05 (q) |
| stereochemistry | 3β-OH, (20*S*,24*R*) | 3β-OH, (20*S*,24*R*) | 3α-OH, (20*S*,24*S*) | 3β-OH, (20*S*,24*S*) |

**Table 7.** Effect of Triterpenoids on Induction of ICAM-1 and Cell Viability[a]

| | ursane | | | | | oleanane | | lupane | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 | 16 | 4 | 5 | 9 | 10 | 17 | 12 | 13 |
| ICAM-1 $IC_{50}$ (μM)[b] | 21.6 | 191 | 144 | 159 | >316 | 96.2 | 55.7 | 72.8 | >316 |
| MTT $IC_{50}$ (μM)[c] | 55.8 | 191 | 236 | >316 | >316 | 209 | 84 | 85.3 | >316 |

[a] A549 cells (3 × 10$^{4}$ cells/well) were pretreated with various concentrations of the compounds for 1 h and then incubated in the presence of IL-1β 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 as described in the Experimental Section. The experiments were carried out in triplicate cultures. $IC_{50}$ was calculated by using the formula in the Experimental Section. Cell viability was measured by the MTT assay. [b] A549 cells were pretreated with serial dilutions of the compounds for 1 h and then incubated in the presence of IL-1α for 6 h. Expression of ICAM-1 (% of control) was calculated by using the formula in the Experimental Section and used for determination of $IC_{50}$. The experiments were carried out in triplicate. The SD values of ICAM-1 expression ranged within 11.5%. [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. The SD values of cell viability ranged within 9.9%.

inhibitory activity on ICAM-1 expression was observed in compounds **9** and **13**, which possess a hydroxymethyl group instead of a carboxyl group at C-17. Compounds **3**, **17**, and **12** showed moderate cytotoxic activity to the lung carcinoma A 549 cell line.

It has been already reported that tumor initiation and promotion are inhibited by ursolic acid (**3**) and oleanolic acid (**10**).[17] Quite recently, cytotoxic and antileukemic activities of lupane triterpenes were reported.[40] Cell growth inhibitory activities of seven kinds of ursane-type triterpenes, **1**, **3**–**5**, and **7**–**9**, three kinds of oleanane-type triterpenes, **2**, **10**, and **11**, two kinds of lupane-type triterpenes, **12** and **13**, two kinds of damarane-type triterpenes, **14** and **15**, methyl ursolate (**16**), and methyl oleanolate (**17**) were examined (Table 8). Compounds **1**, **3**, **12**, **13**, **14**, and **15** showed significant cell growth inhibitory activity, and compounds **2**, **11**, **16**, and **17** showed no cell growth inhibitory activity to WI-38 cells.

**Table 8.** Cell Growth Inhibitory Activities of Compounds against WI-38, VA-13, and HepG2 Cells

| compd | WI-38 $IC_{50}$ (μM) | VA-13 $IC_{50}$ (μM) | HepG2 $IC_{50}$ (μM) |
|---|---|---|---|
| **1** | 3.4 | >212 | 159 |
| **3** | 1.8 | 93.6 | 145 |
| **4** | 7.2 | 67.1 | 99.7 |
| **5** | 14 | 19.3 | 92.7 |
| **7** | 8 | 121 | 65 |
| **8** | 12.7 | >235 | 220 |
| **9** | 11.8 | 58.3 | 214 |
| **10** | 14.5 | 123 | 165 |
| **11** | >212 | 36.2 | 117 |
| **2** | >212 | >212 | >212 |
| **12** | 1.3 | 11.6 | 21 |
| **13** | 1.4 | 20.3 | 17 |
| **14** | 1.3 | 15 | 136 |
| **15** | 2.4 | 199 | 142 |
| **16** | >213 | 96.1 | 105 |
| **17** | >213 | 121 | 90.6 |
| Taxol | 0.04 | 0.005 | 8.1 |
| ADM | 0.66 | 0.38 | 1.2 |

Compounds **5**, **11**, **12**, **13**, and **14** showed moderate cell growth inhibitory activity to malignant lung tumor model, VA-13 cells. The $IC_{50}$ values are 19.3, 36.2, 11.6, 20.3, and 15.0 μM, respectively. The other compounds, **3**, **4**, **7**, **9**, **10**, **15**, **16**, and **17**, showed weak cell growth inhibitory activities to VA-13 cells. The assay result of **11** to VA-13 is interesting because its $IC_{50}$ values to WI-38 cells, a normal human lung cell model, is >212 μM, and **11** is not toxic to WI-38.

Compounds **12** and **13** showed moderate cell growth inhibitory activities to human liver cancer model, HepG2. The $IC_{50}$ values are 21.0 and 17.0 μM. The other compounds, **1**, **3**–**5**, **7**–**11**, and **14**–**17**, showed weak cell growth inhibitory activities.

It is interesting that compounds **11**, **16**, and **17** showed no cytotoxicity to a normal human lung cell model, WI-38 cells ($IC_{50}$ >212), but showed moderate to weak cell growth inhibitory activities to malignant lung tumor and human liver cancer models, VA-13 cells and HepG2 cells.

Finally, the compounds with a lupane skeleton such as **12** and **13** showed stronger activity than the corresponding ursanes (**3** and **9**) and oleanane (**10**), which possess the same functional groups at the corresponding positions, respectively.

## Experimental Section

**General Experimental Procedures.** Melting points are uncorrected. Optical rotation $[\alpha]_D$ values were measured using a Horiba Sepa-200 polarimeter. IR spectra were recorded on a Hitachi 270-30. $^1H$ and $^{13}C$ NMR spectra were measured with a Varian Unity-plus instrument at 500 and 125 MHz. $^1H$ NMR assignments were determined by $^1H-^1H$ COSY experiments. $^{13}C$ NMR assignments were determined using DEPT, HMBC, and HMQC experiments. EIMS was recorded on a JEOL LMS-FABmate instrument, and FABMS was 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 Kyotikuto (*Nerium oleander*) were collected in Niigata City, Niigata Province, Japan, in November 10, 2000. 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 10.0 L and extracted with hexane (5 × 5.0 L). The hexane extracts were dried ($Na_2SO_4$) and concentrated to give an oily residue (120.24 g). 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 ($Na_2SO_4$) and concentrated to give an oily material (518.98 g). A part of the EtOAc extract (219.79 g) was separated into five fractions ($F_A-F_E$) [8 cm i.d. column packed with silica gel (1130 g); $F_A$: $CHCl_3$ (1800 mL, 69.06 g), $F_B$: EtOAc (6000 mL, 54.91 g), $F_C$: EtOAc−MeOH (1:1, 8000 mL, 52.65 g), $F_D$: MeOH (8000 mL, 29.09 g), $F_E$: acetone (2000 mL, 2.42 g)]. Fraction $F_A$ was further separated into six fractions [8 cm i.d. column packed with silica gel (1005 g); $F_{A-1}$ and $F_{A-2}$: hexane−EtOAc (8:2, 8000 mL, 0.32 g and 14.77 g), fractions $F_{A-3}$ and $F_{A-4}$: hexane−EtOAc (1:1, 5750 mL, 6.12 and 0.93 g), $F_{A-5}$: EtOAc (6000 mL, 6.15 g), $F_{A-6}$: MeOH (4000 mL, 2.61 g)]. A part of fraction $F_{A-4}$ (659 mg) was further separated by column chromatography [silica gel (53 g)] into five fractions [$F_{A-4-1}-F_{A-4-4}$; hexane−EtOAc−THF (8:1:1, 1700 mL, 513 mg), $F_{A-4-5}$: hexane−EtOAc−THF (7:2:1, 1000 mL, 70 mg)]. The fraction $F_{A-4-5}$ was separated with HPLC [silica gel, hexane−EtOAc (1:1)]. The fourth peak ($F_{A-4-5-4}$) gave crystalline **2** (1.4 mg). The third peak ($F_{A-4-5-3}$) was further separated by HPLC [ODS, MeOH−5% ammonium acetate aqueous solution] to give **4** (3.1 mg) and **11** (1.8 mg).

A part of the EtOAc extract (105 mg) was separated into 12 fractions ($F_1-F_{12}$) [silica gel (20 g), $F_1-F_8$: hexane−EtOAc (9:1, 4.7 L), $F_9-F_{12}$: hexane−EtOAc (6:4, 500 mL)]. The combined fraction ($F_1-F_4$, 26.1 mg) was separated by HPLC [silica gel, hexane−EtOAc (75:25)] to give **12** (0.9 mg, 0.045%).

In the course of the separation procedures, we noticed that the crude EtOAc plant extract contained insoluble compounds in the solvent used for column chromatography. Since the efficient chromatographic separation was hampered, we tried another separation procedure. Into the crude EtOAc plant extract (9.491 g), 100 mL of EtOAc was added at room temperature, stirred for 1 h, and filtered to give a pale green powder, S-1 (4.656 g, 49.05% based on EtOAc extract), and the filtrate was concentrated to give a viscous oil, VO-1 (4.835 g, 50.94% based on EtOAc extract). A part of S-1 (1.00 g) was recrystallized from MeOH, repeatedly, to give pure **3** (300 mg, 14.7% based on EtOAC extract and 1.66% based on air-dried leaves). The viscous oil, VO-1 (4.835 g), was separated by column chromatography [silica gel (326 g)] and divided to 22 fractions, f1−f8 [hexane−EtOAc (9:1), 665 mg], f9 [hexane−EtOAc (7:3), 284 mg], f10−f13 [hexane−EtOAc (7:3), 834 mg], f14−f18 (EtOAc, 1106 mg), f19−f22 [EtOAc−MeOH (1:1), 1786 mg]. Fractions f1−f9 were combined to give 949 mg of a semisolid material. An insoluble solid (139 mg) in EtOAc was obtained by filtration. Recrystallization of this solid from MeOH gave pure **3** (80 mg). The residue (811 mg) was named LSF (low-polarity soluble fraction) and separated by flash chromatography [silica gel 230−400 mesh, 67 g, hexane−EtOAc (9:1)] and divided into 13 fractions ($LSF_1-LSF_{13}$). The combined $LSF_3$, $LSF_4$, $LSF_5$ (A, 93.2 mg) fraction was separated by HPLC [silica gel, hexane−EtOAc (75:25)] to $A_1$ (30 mg) and $A_2$ (30.8 mg). $A_1$ was separated by reversed-phase HPLC [ODS, MeOH−$H_2O$ (95:5)] to give **7** (3.8 mg) and **8** (4.3 mg). A2 (30.8 mg) was purified by normal-phase HPLC [silica gel, hexane−EtOAc (9:1)] and reversed-phase HPLC [ODS, MeOH−$H_2O$ (93:7)] to give **6** (6 mg). $LSF_{11}$ (B, 45 mg) was separated by normal HPLC [silica gel, hexane−EtOAc (75:25)] to give $B_1$ (7.1 mg), $B_2$ (2.9 mg), and $B_3$ (13.4 mg). $B_3$ was further purified by reversed-phase HPLC [ODS, MeOH−$H_2O$ (9:1)] to give **1** (2.7 mg) and **13** (2.9 mg). EtOAc was added to $LSF_{12}$ (127 mg), and an insoluble solid (61 mg) was separated by filtration. The insoluble solid was recrystallized from MeOH to give pure **10** (41 mg). The filtrate was concentrated to give a viscous oil (C, 121 mg), which was separated by normal-phase HPLC [silica gel, hexane−EtOAc (9:1 and 75:25)] followed by reversed-phase HPLC [ODS, MeOH−$H_2O$ (9:1)] to give **5** (10.0 mg), **9** (4.7 mg), **15** (2.5 mg), and **14** (1.1 mg), and additional **1** (1.2 mg) and **13** (2.6 mg).

A part (1.01 g) of S-1 was dissolved in THF (200 mL), and 0.5 M ether solution of $CH_2N_2$ (140 mL) was added. The solution was stored in a refrigerator for 3 days and concentrated to give an oily crude residue (1.323 g), which was separated by flash column chromatography [silica gel (230−400 mesh, 150 g), hexane−EtOAc (7:3), 250 mL each fraction] into 10 fractions. Fractions 2−5 (656.9 mg) were further separated by reversed-phase HPLC [ODS, MeOH−$H_2O$ (95:5)] to give **16** (457.4 mg) and **17** (101.1 mg).

**3*β*,20α-Dihydroxyurs-21-en-28-oic acid (oleanderic acid, 1):** colorless microcrystals; mp 160−162 °C; $[\alpha]^{20}_D$ +37.5° (*c* 0.208, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3724, 3624, 3024, 1740, 1620, 1600 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m/z* 454 [$(M - H_2O)^+$, 3.6], 410 (53), 247 (53), 189 (37), 120 (100), 105 (42); FABMS (+NaI) *m/z* 477 [$(M - H_2O + Na)^+$]; FABMS (Na free) *m/z* 455 [$(M - H_2O + 1)^+$].

**3*β*,12α-Dihydroxyoleanan-28,13*β*-olide (oleanderolide, 2):** colorless microcrystals; mp 229−231 °C; $[\alpha]_D^{20}$ +74.7° (*c* 0.062, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3700, 3632, 1764, 1715, 1604 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz), see Table 2; HREIMS *m/z* 472.3555 (calcd for $C_{30}H_{48}O_4$, 472.3553).

**(20*S*,24*S*)-Epoxydammarane-3*β*,25-diol (15):** colorless microcrystals; mp 185−187 °C; $[\alpha]_D^{20}$ +25.52° (*c* 0.192, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3700, 3624, 3571 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz), see Table 3; EIMS *m/z* 445 $[M - Me]^+$; HRFABMS *m/z* 461.3993 [calcd for $C_{30}H_{53}O_3$ ($M^+$ +1), 461.3994].

**Inhibitory Activity on Induction of ICAM-1.** Human lung carcinoma A549 cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal calf serum (JRH Bioscience, Lenexa, KS) and a penicillin−streptomycin−neomycin antibiotic mixture (Invitrogen).

Mouse anti-human ICAM-1 antibody C167 was purchased from Leinco Technologies, Inc. (Ballwin, MO), and peroxidase-conjugated goat anti-mouse IgG antibody was obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Recombinant human IL-1α was kindly provided by Dainippon Pharmaceutical Co Ltd. (Osaka Japan).

A549 cells (lung carcinoma, ATCC, CCL 185), an in vitro model of human endothelial cells, were seeded in a microtiter plate at $2 \times 10^4$ cells/well the day before the assay. After 1 h, A549 cells with or without test compounds in 75 $\mu$L, 25 $\mu$L of IL-1α (1 ng/mL) were added to the culture, and the cells were further incubated for 6 h. The cells were washed once with phosphate-buffered saline (PBS) and fixed by 15 min of incubation with 1% paraformaldehyde−PBS for 15 min and then washed once with PBS. After blocking with 1% bovine serum albumin−PBS overnight, the fixed cells were treated with mouse anti-human ICAM-1 antibody for 60 min. After being washed three times with 0.02% Tween 20-PBS, the cells were treated with peroxidase-linked anti-mouse IgG antibody for 60 min. The cells were washed three times with 0.02% Tween 20−PBS. The cells were incubated with the substrate (0.1% *o*-phenylenediamine dihydrochloride and 0.02% $H_2O_2$ in 0.2 M sodium citrate buffer, pH 5.3) for 20 min at 37 °C in the dark and assayed for the absorbance at 415 nm by using a microplate reader. Expression of ICAM-1 was calculated as follows:

$$\text{Expression of ICAM-1 (\% of control)} = [(\text{absorbance with sample and IL-1}\alpha\text{ treatment} - \text{absorbance without IL-1}\alpha\text{ treatment}) / (\text{absorbance with IL-1}\alpha\text{ treatment} - \text{absorbance without IL-1}\alpha\text{ treatment})] \times 100$$

A549 cells ($2 \times 10^4$ cell/well) were seeded in a microtiter plate the day before the assay and incubated in the presence or absence of test compounds for 24 h. At the last 4 h of incubation, the cells were pulsed with 500 $\mu$g/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) for 4 h. MTT formazan was solubilized with 5% sodium dodecyl sulfate (SDS) overnight. Absorbance at 595 nm was measured. Cell viability (%) was calculated as [(experimental absorbance − background absorbance)/(control absorbance − background absorbance)] × 100.

**Cell Growth Inhibitory Activity of Compounds to WI-38 Fibroblast Cells, VA-13 Malignant Tumor Cells, and HepG2 Human Liver Tumor Cells in Vitro. Cells.** WI-38 is the normal human fibroblast derived from female human lung. VA-13 is malignant tumor cells induced from WI-38 by infection of SV-40 virus. HepG2 is human liver tumor cells. These cell lines are available from the Institute of Physical and Chemical Research (RIKEN), Tukuba, Ibaraki, Japan. WI-38 and VA-13 cells were maintained in Eagle's MEM medium (Nissui Pharmaceutical Co., Tokyo, Japan) and RITC 80-7 medium (Asahi Technoglass Co., Chiba, Japan), respectively, both supplemented with 10% (v/v) fetal bovine serum (FBS) (Filtron PTY Ltd., Australia) with 80 $\mu$g/mL of kanamycin. HepG2 cells were maintained in D-MEM medium (Invitrogen) supplemented with 10% (v/v) FBS (Filtron PTY Ltd., Australia) with 80 $\mu$g/mL of kanamycin.

**Procedures.** A 100 $\mu$L sample of medium containing ca. 5000 cells (WI-38, VA-13, HepG2) was incubated at 37 °C in humidified atmosphere of 5% $CO_2$ for 24 h in a 96-well microplate. Then test samples dissolved in DMSO were added to the medium, and incubation was continued further for 48 h under the same conditions. Coloration substrate, WST-8 [2-(2-methyl-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt], was added to the medium. The resulting formazan concentration was determined by the absorption at 450 nm. Cell viability (%) was calculated as [(experimental absorbance − background absorbance)/(control absorbance − background absorbance)] × 100. Cell viability at different concentrations of compounds was plotted, and 50% inhibition of growth was calculated as $IC_{50}$.

**Acknowledgment.** This work was performed as a part of the Japan-China Scientific Cooperation Program supported by Japan Society for Promotion of Science (JSPS) and National Natural Science Foundation of China (NNSFC). We thank Research Professor Y. Zhang, Professor W. Zhang, and Associate Professor L. Zhang at Dalian Institute of Chemical Physics, Chinese Academy of Science, for their useful discussions about analytical and separation methods.

## References and Notes

(1) The binomials *Nerium indicum, Nerium odorum* are used in the older literature: see p 79 of ref 6 and ref 10.
(2) Abe, F.; Yamauchi, T.; Minato, K. *Phytochemistry* **1996**, *42,* 45−49, and references therein.
(3) Langford, S. D.; Boor, P. J. *Toxicology* **1996**, *109*, 1−13, and references therein.
(4) Begum, S.; Siddiqui, B. S.; Sultana, R.; Zia, A.; Suria, A. *Phytochemistry* **1999**, *50*, 435−438.
(5) Huq, M. M.; Jabbar, A.; Rashid, M. A.; Hasan, C. M. *Fitoterapia* **1999**, *70*, 5−9, and references therein.
(6) Burrows, G. E.; Tyrl, R. J. *Toxic Plants of North America;* Iowa State University Press: Ames, 2001; pp 78−82.
(7) Ishidate, M.; Tamura, Z. *Yakugaku Zasshi* **1950**, *70*, 239−241 (CA, ISSN 0031-6903, Accession Number, 45:4763).
(8) Yamauchi, T.; Abe, F.; Takahashi, M. *Tetrahedron Lett*. **1976**, 1115−1116.
(9) Abe, F.; Yamauchi, T. *Chem. Pharm. Bull*. **1978**, *26*, 3023−3027.
(10) Yamauchi, T.; Abe, F.; Tachibana, Y.; Atal, C. K.; Sharma, B. M.; Imre, Z. *Phytochemistry* **1983**, *22*, 2211−2214.
(11) Abe, F.; Yamauchi, T. *Phytochemistry* **1992**, *31,* 2459−2463.
(12) Hanada, R.; Abe, F.; Yamauchi, T. *Phytochemistry* **1992**, *31*, 3183−3187.
(13) (a) Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids*; Chapman and Hall: London, Vol. 2, Di- and Higher Terpenoids; 1991. (b) Siddiqui, S.; Begum, S.; Hafeez, F.; Siddiqui, B. S. *Phytochemistry* **1989**, *28*, 1187−1191. (c) Siddiqui, S.; Begum, S.; Hafeez, F.; Siddiqui, B. S. *J. Nat. Prod*. **1988**, *51,* 229−233.
(14) Ishidate, M.; Tamura, Z.; Okada, M. *Yakugaku Zasshi* **1947**, *67*, 206−208 (CA, ISSN 0031-6903, Accession Number, 45:52941).
(15) Shibata, S. *Food Phytochemical for Cancer Prevention II*; ACS Symposium Series 547; 1994; pp 308−321.
(16) Nishino, H.; Nishino, A.; Takayasu, J.; Hasegawa, T.; Iwashima, A.; Hirabayashi, K.; Iwata, S.; Shibata, S. *Cancer Res.* **1988**, *48*, 5210−5215.
(17) Liu, J. *J. Ethnopharm.* **1995**, *49*, 57−68, and references therein.
(18) Tapondjou, L. A.; Lontsi, D.; Sondengam, B. L.; Shaheen, F.; Choudhary, M. I.; Rahman, A.-U.; van Heerden, F. R.; Park, H. J.; Lee, K. T. *J. Nat. Prod*. **2003**, *66*, 1266−1269.
(19) (a) Kitagawa, I.; Kitazawa, K.; Yosioka, I. *Tetrahedron Lett.* **1968**, *4*, 509−512. (b) Kitagawa, I.; Kitazawa, K.; Yosioka, I. *Tetrahedron* **1972**, *28*, 907−921. (c) Yoshikawa, M.; Dai, Y.; Shimada, H.; Morikawa, T.; Matsumura, N.; Yoshizumi, S.; Matsuda, H.; Matsuda, H.; Kubo, M. *Chem. Pharm. Bull*. **1997**, *45*, 1052−1055.
(20) The structures including stereochemistry of the following 13 compounds were confirmed by full analyses of their NMR spectra at 500 MHz ($^1$H NMR, $^1$H−$^1$H COSY, NOESY, HMQC, HMBC) and at 125 MHz ($^{13}$C NMR, DEPT) as well as HREIMS or HRFABMS. The yield of each compound in parentheses is based on the weight of dry leaves of *Nerium oleander*.
(21) The yields of **3** and **10** are calculated on the basis of the isolated yields of their methyl esters that were obtained after methylation of the insoluble mixture of **3** and **10** by $CH_2N_2$.
(22) (a) Mills, J. S.; Werner, A. E. A. *J. Chem. Soc*. **1955**, 3132−3140. (b) *Merck Index*, 13 ed.; Merck & Co., Inc.: New Jersey; p 1761 (Compound Number 9957). (c) Mahato, S. M.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(23) Budzikiewicz, H.; Thomas, H. *Z. Naturforsch. B* **1980**, *35,* 226−232.
(24) Huang, H.; Sun, H. D.; Zhao, S. X. *Phytochemistry* **1996**, *42*, 1665−1666.
(25) Hota, R. K.; Bapuji, M. *Phytochemistry* **1994**, *35*, 1073−1074.
(26) (a) Seo, S.; Tomita, Y.; Tori, K. *Tetrahedron Lett* **1975**, 7−10. (b) Dekebo A.; Dagne, E.; Gautun, O. R.; Aasen, A. *J. Bull. Chem. Soc. Ethiop.* **2002**, *16*, 87−90.
(27) Siddiqui, S.; Hafeez, F.; Begum, S.; Siddiqui, B. S. *J. Nat. Prod*. **1986**, *49,* 1086−1090.
(28) (a) Adesina, S. K.; Reisch, J. *Phytochemistry* **1985**, *24*, 3003−3006. (b) *Merck Index*, 13th ed.; Merck & Co., Inc.: New Jersey; p 1761 (Compound Number 9957). (c) Mahato, S. M.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(29) (a) Maillard, M.; Adewunmi, C. O.; Hostettmann, K. *Phytochemistry* **1992**, *31*, 1321−1323. (b) Kashiwada Y.; Zhang, D. C. *J. Nat. Prod.* **1993**, *56*, 2077−2082.
(30) (a) Sholichin, M.; Yamasaki, K.; Kasai, R.; Tanaka, O. *Chem. Pharm. Bull*. **1980**, *28*, 1006−1008. (b) Otsuka, H.; Fujioka, S.; Komiya, T.; Goto, M.; Hiramatsu, Y.; Fujimura, H. *Chem. Pharm. Bull*. **1981**, *29*, 3099−3104.
(31) Tinto, W. F.; Blair, L. C.; Alli, A.; Reynolds, W. F.; McLean, S. *J. Nat. Prod*. **1992**, *55*, 395−398.
(32) (a) Halls, C. M. M.; Warnhoff, E. W. *Chem. Ind.* (*London*) **1963**, 1986. (b) Warnhoff, E. W.; Halls, C. M. M. *Can. J. Chem.* **1965**, *43*, 3311−3321. (c) Betancor, C.; Freire, R.; Hernandez, R.; Suarez, E. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1119−1126. (d) Nagai, M.; Tanaka, N.; Ichikawa, S.; Tanaka, O. *Tetrahedron Lett.* **1968**, *40*, 4239−4242. (e) Gupta, A. S.; Dev, S. *Tetrahedron* **1971**, *27*, 823−834. (f) Ohmoto, T.; Nikaido, Y.; Ikuse, M. *Chem. Pharm. Bull*. **1978**, *26*, 1437−1443. (g) Nagai, M.; Tanaka, N.; Tanaka, O.; Ichikawa, S. *Chem. Pharm. Bull*. **1973**, *21*, 2061−2065. (h) Anjaneyulu, V.; Harischandra Pasad, K.; Ravi, K.; Connoly, J. D. *Phytochemistry* **1985**, *24*, 2359−2367. (i)

Tanaka, R.; Masuda, K.; Matsunaga, S. *Phytochemistry* **1993**, *32*, 472−474. In this report the authors indicated the 20*R*, 24*S* structure for ocotillol-II. $^{1}$H and $^{13}$C NMR data in this report are in good accordance with those of **14**.

(33) (a) Klyne, W.; Buckingham, J. *Atlas of Stereochemistry*; Chapman and Hall: London, 1974; Vol. 1, p 128. In this book, the authors introduced the chemical transformation of dammarenediol II to (+)-ocotillol II and stereostructure **15** was shown there as (+)-ocotillol II, but the reference there is a publication by Halls and Warnhoff. So structure **15** should be replaced by **14**. (b) 3-epimer of **15** (carbraleadiol): Hisham, A.; Ajitha Bai, M. D.; Fujimoto, Y.; Hara, N.; Shimada, H. *Magn. Reson. Chem.* **1996**, *34*, 146−150. (c) Qiu, S. X.; Hung, N. V.; Xuan, L. T.; Gu, J. Q.; Lobkovsky, E.; Khanh, T. C.; Soejarto, D. D.; Clardy, J.; Pezzuto, J. M.; Dong, Y.; Tri, M. V.; Huong, L. M.; Fong, H. H. S. *Phytochemistry* **2001**, *56*, 775−780.

(34) (a) The C-20 configuration of dammarenediol II[(b)] was established by chemical correlation with betulafolienetriol[(c)], the structure of which was established by X-ray analysis of 20*R*-protopanaxadiol[(d)]. (b) Mills, J. S.; Werner, A. E. A. *J. Chem. Soc*. **1955**, 3132−3140. (c) Fisher, F. G.; Seiler, N. *Ann.* **1959**, *626,* 185−205; **1961**, *644*, 146−162. (d) Tanaka, O.; Nagai, M.; Ohsawa, T.; Tanaka, N.; Kawai, K.; Shibata, S. *Chem. Pharm. Bull*. **1972**, *20*, 1204−1211.

(35) Kawai, S.; Kataoka, T.; Sugimoto, H.; Nakamura, A.; Kobayashi, T.; Arao, K.; Higuchi, Y.; Ando, M.; Nagai, K. *Immunopharmacology* **2000**, *48*, 129−135.

(36) Sugimoto, H.; Kataoka, T.; Igarashi, M.; Hamada, M.; Takeuchi, T.; Nagai, K. *Biochem. Biophys. Rev. Commun*. **2000**, *277*, 330−333.

(37) Yuuya, S.; Hagiwara, H.; Suzuki, T.; Ando, M.; Yamada, A.; Suda, K.; Kataoka, T.; Nagai, K. *J. Nat. Prod*. **1999**, *62*, 22−30.

(38) Higuchi, Y.; Shimoma, F.; Koyanagi, R.; Suda, K.; Mitui, T.; Kataoka, T.; Nagai, K.; Ando, M. *J. Nat. Prod*. **2003**, *66*, 588−594.

(39) Ahn, K. S.; Kim, J. H.; Oh, S. R.; Min, B. S.; Kinjo, J.; Lee, H. K. *Biol. Pharm. Bull.* **2002**, *25*, 1105−1107.

(40) Recently antileukemic activity and cytotoxicity of lupane triterpenes were reported. (a) Hata, K.; Hori, K.; Ogasawara, H.; Takahashi, S. *Toxicol. Lett.* **2003**, *175*, 1−7, and references there. (b) Zuco, V.; Spino, R.; Righetti, S. C.; Cleris, L.; Marchesi, E. M.; Gambacorti-Passerini, C.; Formelli, F. *Cancer Lett.* **2002**, *175*, 17−25.

NP040072U