Bioactive Pregnanes from *Nerium oleander*

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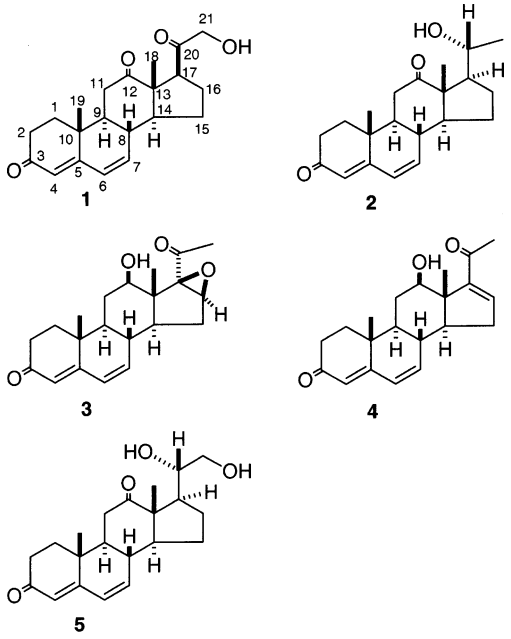
Three new pregnanes, 21-hydroxypregna-4,6-diene-3,12,20-trione (**1**), 20*R*-hydroxypregna-4,6-diene-3,12-dione (**2**), and 16β,17β-epoxy-12β-hydroxypregna-4,6-diene-3,20-dione (**3**), were isolated from *Nerium oleander*, together with two known compounds, 12β-hydroxypregna-4,6,16-triene-3,20-dione (neridienone A, **4**) and 20*S*,21-dihydroxypregna-4,6-diene-3,12-dione (neridienone B, **5**). The structures of compounds **1**−**3** were established on the basis of their spectroscopic data. The anti-inflammatory activity in vitro of compounds **2**−**4** was examined on the basis of inhibitory activity against the induction of intercellular adhesion molecule-1 (ICAM-1), and compound **4** was active. The cytotoxic activity of compounds **1**−**5** was evaluated against four human cell lines, normal human fibroblast cells (WI-38), malignant tumor cells induced from WI-38 (VA-13), human liver tumor cells (HepG2), and human lung carcinoma cells (A-549). Compound **4** showed significant cell growth inhibition of VA-13 and HepG2 cells. The MDR-reversal activity of compounds **1**−**5** was evaluated on the basis of the amount of calcein accumulated in MDR human ovarian cancer 2780AD cells in the presence of each compound. Compounds **1**, **2**, and **5** showed significant effects on calcein accumulation.

Nerium oleander L. (synonyms: *Nerium indicum*; *Nerium odorum*) (Apocyaceae) is a medium-sized evergreen flowering tree of 2−5 m in height and is planted throughout Japan as a garden and roadside tree. This species was distributed originally in the Mediterranean region, subtropical Asia, and the Indo-Pakistan subcontinent and has been used as a traditional medicine because of its antibacterial, anticancer, antidote, antileprotic, and cardiotonic properties.[1] Abe and Yamauchi reported the isolation of five pregnanes from the root bark of this plant.[2] Recently, we have investigated the triterpenoid constituents from the leaves of *N. oleander*.[3] In the present paper, we report the results of a phytochemical and biological investigation of pregnanes from the bark and twigs of this plant.



Results and Discussion

A methanol extract of air-dried bark and twigs of *N. oleander* was partitioned successively with hexane, ethyl acetate, and butanol. The ethyl acetate-soluble portion was separated by silica gel column chromatography and normal- and reversed-phase HPLC. Three new pregnanes, 21-hydroxypregna-4,6-diene-3,12,20-trione (**1**), 20*R*-hydroxypregna-4,6-diene-3,12-dione (**2**), and 16β,17β-epoxy-12β-hydroxypregna-4,6-diene-3,20-dione (**3**), were isolated together with two known derivatives, 12β-hydroxypregna-4,6,16-triene-3,20-dione (neridienone A, **4**)[2,4] and 20*S*,21-dihydroxypregna-4,6-diene-3,12-dione (neridienone B, **5**).[2]

Compound **1** gave the elemental composition $C_{21}H_{26}O_4$, which was determined by a combination of an analysis of the HREIMS and ^{1}H and ^{13}C NMR data. The IR spectrum of **1** indicated the presence of hydroxyl (3500 cm^{-1}), carbonyl (1709 cm^{-1}), and conjugated carbonyl (1663, 1630, and 1618 cm^{-1}) groups. The UV spectrum of **1** was consistent with the presence of a conjugated dienone chromophore [265 nm (log ϵ 4.23)]. The ^{13}C NMR spectrum displayed 21 carbon signals. Three carbonyl carbon signals appeared at δ 198.7, 210.8, and 211.6 and four olefin carbon resonances were located at δ 124.8 (d), 129.2 (d), 137.7 (d), and 161.1 (s). A signal for one carbon-bearing oxygen was observed at δ 69.5 (t). Judging from the DEPT and HMQC spectra, the remaining carbon resonances were two methyl carbons, five methylene carbons, four methine carbons, and two quaternary carbons. The ^{1}H NMR spectra showed two singlet methyls (δ 1.07 and 1.20). The connectivity of the protonated carbons (C-1 to C-2; C-6 to C-7, C-7 to C-8, C-8 to C-9, C-9 to C-11; C-8 to C-14, C-14 to C-15, C-15 to C-16) was determined from the ^{1}H−^{1}H COSY spectrum. A HMBC experiment was used to determine the carbon−carbon connections through the nonprotonated carbon atoms [HMBC correlations: C-10 (δ 36.2) with H_2-1, H_2-2, H-4, H-9, H_2-11, Me-19; C-13 (δ 59.1) with H-11α, H-14, H_2-15, H_2-

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Table 1. ^{13}C NMR Spectroscopic Data of Compounds **1**−**3** (125 MHz, δ in ppm, $CDCl_3$)

position	**1**	**2**	**3**
1	33.6 (t)	33.5 (t)	33.5 (t)
2	33.6 (t)	33.6 (t)	33.9 (t)
3	198.7 (s)	198.8 (s)	199.3 (s)
4	124.8 (d)	124.7 (d)	124.3 (d)
5	161.1 (s)	161.4 (s)	162.4 (s)
6	129.2 (d)	129.0 (d)	129.1 (d)
7	137.7 (d)	138.1 (d)	138.1 (d)
8	36.6 (d)	36.3 (d)	34.9 (d)
9	51.5 (d)	51.0 (d)	48.6 (d)
10	36.2 (s)	36.2 (s)	36.0 (s)
11	37.2 (t)	37.4 (t)	28.7 (t)
12	211.6 (s)	216.6 (s)	72.2 (d)
13	59.1 (s)	57.7 (s)	49.7 (s)
14	54.2 (d)	52.7 (d)	58.2 (d)
15	23.0 (t)	22.9 (t)	28.4 (t)
16	23.5 (t)	24.8 (t)	67.1 (d)
17	49.5 (d)	50.7 (d)	74.8 (s)
18	13.5 (q)	12.1 (q)	10.1 (q)
19	15.9 (q)	15.9 (q)	16.0 (q)
20	210.8 (s)	68.0 (d)	208.2 (s)
21	69.5 (t)	23.1 (q)	25.3 (q)

16, H-17, Me-18]. Interpretation of these results suggested that compound **1** has steroid A, B, C, and D rings.[5] HMBC correlations [the carbonyl carbon at δ 198.7 with H-1β and H_2-2; the disubstituted olefin carbon at δ 161.1 with H-1β, Me-19, and the olefin protons H-4, H-6, and H-7] were used to place the carbonyl group at C-3 and a conjugated double bond at the C-4 and C-6 positions. Further HMBC correlations [the carbonyl carbon at δ 211.6 with H-11α and Me-18; the carbonyl carbon at δ 210.8 with the oxygenated methylene protons at C-21 (δ 4.27 and 4.58) and the methine proton at C-17 (δ, 3.30)] showed that two carbonyl carbons and one oxygenated primary carbon are located at C-12, C-20, and C-21, respectively, and the hydroxy acetyl side chain is connected at C-17. Thus, the planar structure of **1** was established as 21-hydroxypregna-4,6-diene-3,12,20-trione (**1**).

NOESY NMR experiments were used for assignment of the relative configuration of **1**. The NOESY correlations [H-2β with Me-19; Me-19 with H-8 and H-11β; H-8 and H-11β with Me-18; Me-18 with H-15β and H-16β; H-14 with H-17 and H-15α; H-15α with H-16α; H-21a with H-17; H-21b with Me-18 and H-17; H-9 with H-11α] indicated the full stereostructure of the molecule **1**. Thus, 10β-Me, 8β-H, 9α-H, 13β-Me, 14α-H, and 17β-hydroxyacetyl substituents were confirmed for **1**.

Compound **2** was assigned the elemental composition $C_{21}H_{28}O_3$, which was determined from the HRFABMS and ^1H and ^{13}C NMR spectroscopic data. Similar IR and UV data were obtained for compound **2** as compared to compound **1**. The ^{13}C NMR spectrum displayed 21 carbon signals with two carbonyl carbons located at δ 198.8 and 216.6, one hydroxylated methine carbon at δ 68.0, and four olefin carbons at δ 124.7 (d), 129.0 (d), 138.1 (d), and 161.4 (s). As judged from the DEPT and HMQC spectra, the remaining carbon resonances were three methyl carbons, five methylene carbons, four methine carbons, and two quaternary carbons. The ^1H NMR spectrum showed two singlet methyls (δ 1.18 and 1.21) and one doublet methyl (δ 1.17).

Compound **2** showed very similar ^{13}C and ^1H NMR spectra to those of **1** except in the vicinity of the side chain at C-17 (Tables 1 and 2). The connectivity of the protonated carbons determined by analysis of the $^1H-^1H$ COSY spectrum and the carbon−carbon connections through the nonprotonated carbon atoms determined by the HMBC experiment indicated that compound **2** has a pregna-4,6-diene-3,12-dione structure like **1**. The chemical shifts of C-1 through C-11, C-15, and C-19 in the ^{13}C NMR spectrum of **2** were superimposable on those of **1**, with differences observed in the values of C-20 and C-21. HMBC correlations [the carbonyl carbon at δ 216.6 with H-9, H_2-11, H-17, and Me-18; the quaternary carbon at δ 57.7 with H-11α, H-15α, H-16α, H-17, and Me-18; and the hydroxylated methine carbon at δ 68.0 with the protons of Me-21 (δ 1.17) and methine proton at C-17 (δ, 2.03)] showed that one carbonyl carbon and one hydroxylated methine carbon are located at C-12 and C-20, respectively, and that an acetyl side chain is connected at C-17. Thus, the planar structure of **2** was established as 20-hydroxypregna-4,6-diene-3,12-dione (**2**). Compound **2** showed similar NOESY correlations to those of **1** except for H-20 and Me-21 of the side chain at C-17. Thus, the 10β-Me, 8β-H, 9α-H, 13β-Me, 14α-H, and 17β-hydroxyethyl configurations in **2** could be confirmed. Additional NOESY correlations in the vicinity of H-20 [H-20 with Me-18 and H-16β; Me-21 with H-16α,β] indicated that the configuration of C-20 is *R* (Figure 1). Thus, the structure of compound **2** was determined as 20*R*-hydroxypregna-4,6-diene-3,-12-dione.

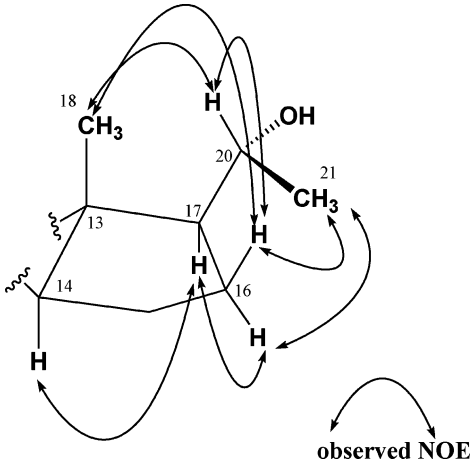

Figure 1. NOE correlations of the D ring and C-17 side chain of compound **2**.

Compound **3** was assigned the molecular composition $C_{21}H_{26}O_4$, determined by HREIMS and from the ^1H and ^{13}C NMR spectroscopic data. Similar IR and UV data were obtained for compound **3** as compared to compounds **1** and **2**. The ^1H and ^{13}C NMR spectra of **3** were also similar to those of **1**, with differences observed in the values for H- and C-12, 16, 17, and 21. HMBC correlations [the hydroxylated methine carbon at C-12 (δ 72.2) with H_2-11, H-14, and Me-18; the quaternary carbon at C-13 (δ 49.7) with H-8, H-11α, H-14, H_2-15, and Me-18; the oxygenated tertiary carbon at C-17 (δ 74.8) with H-15β, Me-18, and Me-21; the oxygenated methine carbon at C-16 (δ 67.1) with H-14 and H-15β; the carbonyl carbon at δ 208.2 with Me-21 (acetyl methyl)] were used to place one hydroxyl group at C-12, one carbonyl group at C-20, and an epoxy group between the C-16 and C-17 positions. Thus, the planar structure of **3** was assigned as 16,17-epoxy-12-hydroxypregna-4,6-diene-3,20-dione (**3**). Compound **3** showed very similar NOESY correlations to those of **1** except for H-12, H-16, and Me-21 of the side chain at C-17. NOESY correlations [H-11α for H-12; H-14 with H-12 and H-15α; H-15α with H-16; H-16 with Me-21] indicated the full stereostructure of **3**. Thus, the structure of compound **3** was determined as 16β,17β-epoxy-12β-hydroxypregna-4,6-diene-3,20-dione.

The in vitro anti-inflammatory activity of the isolated **2**−**4** was estimated by inhibition of the induction of ICAM-1[6−9] using human cultured cell line A549, an in vitro human endothelial cell model, and the results are expressed as IC_{50} values. Cell viability was measured by an MTT assay (Table 3). Compound **4** showed potent inhibitory activity against the induction of ICAM-1 with weak inhibitory activity against cell growth of A549 cells (Figure 2).

The cytotoxic activities of compounds **1**−**5** were evaluated against three human cell lines, WI-38, VA-13, and HepG2. Compound **4** exhibited significant growth inhibition of VA-13 and HepG2 cells. It is interesting to note that the cytotoxicity of **4** to

Table 2. ^{1}H NMR Spectroscopic Data of Compounds **1**−**3** (500 MHz, δ in ppm and J in Hz, $CDCl_3$)

position	1	2	3
1	α 1.76 (1H, m)	α 1.74 (1H, ddd, 13.9, 13.5, 5.4)	α 1.75 (1H, m)
	β 1.89 (1H, ddd, 13.2, 5.4, 2.2)	β 1.88 (1H, ddd, 13.5, 5.4, 2.2)	β 2.00 (1H, ddd, 13.4, 5.4, 2.2)
2	α 2.47 (1H, m)	α 2.47 (1H, m)	α 2.45 (1H, brdd, 18.1, 4.4)
	β 2.60 (1, ddd,	β 2.58 (1H, ddd,	β 2.56 (1H, ddd,
	13.9, 13.9, 5.4)	13.9, 12.7, 5.4)	18.1, 14.4, 5.4)
4	5.75 (1H, s)	5.75 (1H, s)	5.68 (1H, s)
6	6.21 (1H, dd, 9.8, 2.7)	6.21 (1H, dd, 9.8, 2.7)	6.12 (1H, dd, 9.8, 2.7)
7	6.12 (1H, brd, 9.8)	6.14 (1H, dd, 9.8, 1.5)	5.93 (1H, dd, 9.8, 1.7)
8	2.65 (1H, brt, 10.0)	2.65 (1H, brt, 10.8)	2.19 (1H, m)
9	1.65 (1H, ddd, 13.6, 10.0, 4.4)	1.64 (1H, m)	1.32 (1H, ddd, 13.3, 9.6, 4.2)
11	α 2.33 (1H, dd, 13.7, 4.4)	α 2.37 (1, dd, 14.2, 4.4)	α 1.91 (1H, ddd, 13.2, 4.4, 4.2)
	β 2.56 (1H, dd, 13.7, 13.6)	β 2.56 (1H, dd, 14.2, 13.9)	β 1.57 (1H, ddd, 13.2, 13.2, 11.0)
12			α 4.29 (1H, br dd, 11.0, 4.4)
14	1.68 (1H, m)	1.62 (1H, dd, 12.0, 6.1)	1.94 (1H, dd, 12.7, 5.6)
15	α 2.07 (1H, m)	α 1.99 (1H, m)	α 2.15 (1H, m)
	β 1.69 (1H, m)	β 1.58 (1H, m)	β 1.71 (1H, m)
16	α 1.84 (1H, m)	α 1.84 (1H, m)	3.91 (1H, s)
	β 2.31 (1H, m)	β 1.34 (1H, m)	
17	3.3 (1H, dd, 9.3, 9.5)	2.03 (1H, dd, 9.8, 9.8)	
18	1.07 (3H, s)	1.18 (3H, s)	0.99 (3H, s)
19	1.20 (3H, s)	1.21 (3H, s)	1.09 (3H, s)
20		3.55 (1H, m)	
21	4.27 (1H, d, 19.8)	1.17 (3H, d, 6.11)	2.04 (3H, s)
	4.58 (1H, d, 19.8)		

Table 3. Effect of Compounds **2**−**4** on Induction of ICAM-1 and on Cell Viability

	2	3	4
ICAM-1 IC_{50} (μM)[a]	>300	>100	7.0
MTT IC_{50} (μM)[b]	>300	>100	53.1

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

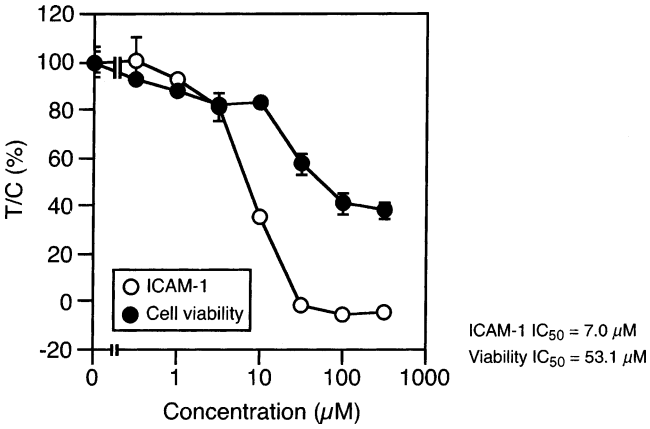

Figure 2. Effect of compound **4** on the induction of ICAM-1 in response to IL-1α. Open circles represent the ICAM-1 expression (mean ± SD of triplicate cultures). Cell viability was measured by a MTT assay (filled circles). Data points represent mean ± SD of triplicate cultures.

the parental normal WI-38 cells (4.9 μg/mL) was less than that to VA-13 (Table 4).

One mechanism underlying multidrug resistance (MDR) in mammalian tumor cells has been ascribed to enhanced removal of drugs due to overexpression of efflux transporter proteins, such as P-glycoprotein (Pgp), a multidrug-resistance protein (MRP).[10] Thus, agents that inhibit the function of this protein could overcome the MDR effect. Calcein derived from calcein AM by endogenous esterase is used as an easily operated functional fluorescent probe for this drug efflux protein.[11−13] Compounds **1**, **2**, and **5** showed significant MDR reversal activity toward these cells by comparison with a control (Table 4). Since compounds **1**, **2**, and **5** showed very weak or no cytotoxic activities, they have promise as lead compounds as modulators of MDR (Table 5).

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 Shimadzu FTIR-4200 infrared spectrometer. UV spectra were measured using a JASCO V-530 UV/vis 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, HMQC, and HMBC experiments. HRFABMS were recorded on a JEOL JMS-HX110 instrument, and HREIMS were recorded on JEOL GC mate-BU20 and JEOL JMS-HX110 instruments. 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. The bark and twigs of *Nerium oleander* 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. The air-dried bark and twigs (19.5 kg) were combined and extracted with MeOH (85 L) for 20 days. The MeOH extract was concentrated to 4 L and extracted with hexane (8 × 1.0 L). Water (1.3 L) was added to the MeOH layer, extracted with EtOAc (3 × 3.0 L), dried (Na_2SO_4), and concentrated to give an oily material (96.5 g). This was separated by column chromatography [silica gel (1.1 kg), gradient mixture of hexane, EtOAc, and MeOH] into five fractions, A−E. Fraction B [hexane−EtOAc (1:1), EtOAc], fraction C (EtOAc), and fraction D [EtOAc−MeOH (1:1)] gave on drying viscous oils, weighing 29.58, 23.33, and 32.14 g, respectively. Fraction B was dissolved in EtOAc (110 mL), stirred for 1 h, filtered, and concentrated to give a viscous oil (30 g). This was separated into nine fractions (B1−B9) by column chromatography [silica gel (1.5 kg), gradient mixture of hexane, EtOAc, and MeOH]. Fraction B5 [hexane−EtOAc (4:6)] gave on drying a viscous oil (0.670 g). Fractions B6 and B7 [EtOAc (100%)] gave on drying additional viscous oils (B6, 13.39 g;

Table 4. Effects of Compounds **1**−**5** on the Accumulation of Calcein in MDR 2780 AD Cells and Cytotoxic Activities of Compounds against WI-38, VA-13, and HepG2 Cells

	calcein accumulation (% of control)[a,b]			cytotoxicity IC_{50} (μg/mL)[c]		
compound	0.25 μg/mL	2.5 μg/mL	25 μg/mL	WI-38	VA-13	HepG2
1	110	105	140	48.1	87.7	94.9
2 (1)	100	122	136	66.4	57.4	49.2
2 (2)	116	127	138			
3	103	108	99	51.1	73.1	62.1
4	109	92	107	4.9	0.68	2.5
5	110	154	148	73.5	>100	>100

[a] The amount of calcein accumulated in multidrug-resistant human ovarian cancer 2780 AD cells was determined relative to a control in the presence of 0.25, 2.5, and 25 μg/mL of each test compound. [b]Values are the relative amount of calcein accumulated in the cell compared with the control experiment and represent the means of triplicate determinations. [c]IC_{50} values represent the means of duplicate determinations.

Table 5. Effect of Compounds **2** and **5** on the Accumulation of Calcein in Multidrug-Resistant 2780 AD Cells

	calcein accumulation[a]					
compound	concentration (μg/mL)	average[b] (count/well)	% of control[c]	activity[d]	verapamil (%)[e]	evaluation max. verapamil % concentration
2 (2)	0.25	3937	116	+	120	P[f]
	2.5	4327	127	+	117	120
	25	4679	138	++	93	0.25 μg/mL
5	0.25	3743	110	±	113	P[f]
	2.5	5240	154	+++	141	141
	25	5019	148	++	100	2.5 μg/mL
verapamil	0 (control)	3395	100			
	0.25	3298	97	±	100	
	2.5	3701	109	±	100	
	25	5034	148	++	100	

[a] The amount of calcein that accumulated in multidrug-resistant human ovarian cancer 2780 AD cells was determined relative to the control in the presence of 0.25, 2.5, and 25 μg/mL of the pregnanes. [b]Values represent the means of triplicate determinations. [c]Values are the relative amount of calcein accumulated in the cell compared with the control experiment. [d] Indices are expessed on a scale of four by the range of the relative amount of calcein accumluation as compared with a control experiment (%): +++, >151%; ++, 131−150%; +, 111−130%; ±, 91−110%; −, <90%. [e]Values are expressed as the relative amount of calcein accumulation in the cell as compared with that of verapamil. [f]P, positive: the activity was more potent than that of verapamil (verapamil % > 100%).

B7, 2.61 g). Fraction B5 afforded compound **3** (34.2 mg, 0.00018%) by separation using HPLC [silica gel, hexane−EtOAc (4:6)], and further separation of the second fraction gave 404 mg by HPLC [ODS, MeOH−MeCN−H_2O (1:9:10)]. Fraction B6 was subjected to silica gel column chromatography [silica gel (700 g), gradient of hexane, EtOAc, and MeOH] to give seven fractions, B61−B67 [hexane−EtOAc (1:1), B64 (11.5 g)], and B64 was separated using HPLC [ODS, MeOH−MeCN−H_2O (1:6:9)] to give subfraction B642 (827 mg). This subfraction was further separated by HPLC [ODS, MeOH−MeCN−H_2O (1:9:10)] to give B6422 (283 mg), which in turn was purified by HPLC [ODS, MeOH−MeCN−H_2O (1:6:9.3)] to give B64222 (180 mg) and then by additional HPLC [ODS, MeOH−MeCN−H_2O (1:9:10)] to give **4** (131.4 mg, 0.00067%). Fraction C was separated by HPLC [silica gel, hexane−EtOAc (1:59)] into six fractions, C1−C6, and C3 (9.32 g) was separated by HPLC [silica gel, hexane−EtOAc (3:7)] into four further fractions, C31−C34. Fraction C33 (4.10 g) was separated by HPLC [ODS, MeOH−MeCN−H_2O (1:6:9)] to give 13 fractions (C33-1−C33-13), with **1** (55.9 mg, 0.00029%) obtained from C33-4 without further purification. Fraction C33-9 (950 mg) was further separated by HPLC [ODS, MeOH−MeCN−H_2O (4:4:9)] to give **2** (43.0 mg, 0.00022%). Fraction D (30.66 g) was dissolved in EtOAc (75 mL) and filtered. The filtrate gave on drying a viscous oil (17.06 g), which was separated by column chromatography [silica gel, 620 g; gradient of $CHCl_3$ and MeOH; $CHCl_3$−MeOH (98:2) to MeOH(100%)] to give 12 fractions (D1−D12). Fraction D4 [$CHCl_3$−MeOH (98:2), 1.66 g] was further separated by HPLC [silica gel, EtOAc (100%)] to give six fractions (D41−D46). Fraction D45 (667 mg) was separated by HPLC [ODS, MeOH−MeCN− H_2O (4:4:10)] to afford six additional fractions (D451−D456), and D452 gave **5** (164.5 mg, 0.00084%).

21-Hydroxypregna-4,6-diene-3,12,20-trione (1): colorless microcrystals; mp 161−163 °C; $[\alpha]^{24}_D$ +90.6 (*c* 0.223, MeOH); UV ($CHCl_3$) λ_{max} (log ϵ) 265 (4.23) nm; IR ($CHCl_3$) ν_{max} 3500, 1709, 1663, 1630, 1618 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HREIMS *m*/*z* 342.1832 (calcd for $C_{21}H_{26}O_4$ 342.1831); HRFABMS *m*/*z* 343.1906 $[M + H]^+$ (calcd for $C_{21}H_{27}O_3$, 343.1909).

20*R*-Hydroxypregna-4,6-diene-3,12-dione (2): colorless microcrystals; mp 167−170 °C; $[\alpha]^{20}_D$ +85.3 (*c* 0.346, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 278 (4.19) nm; IR ($CHCl_3$) ν_{max} 3416, 1694, 1663, 1653, 1618 cm^{-1}; ^1H ($CDCl_3$) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HRFABMS *m*/*z* 329.2133 $[M + H]^+$ (calcd for $C_{21}H_{29}O_3$, 329.2117).

16β,17β-Epoxy-12β-hydroxypregna-4,6-diene-3,20-dione (3): colorless microcrystals; mp 169−172 °C; $[\alpha]^{20}_D$ −2.4 (*c* 0.415, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 276 (3.98) nm; IR (KBr) ν_{max} 3440, 1694, 1650, 1615 cm^{-1}; ^1H ($CDCl_3$) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HREIMS *m*/*z* 342.1831 (calcd for $C_{21}H_{26}O_4$ 342.1831).

12β-Hydroxypregna-4,6,16-triene-3,20-dione (neridienone A, 4). The structure of compound **4** was confirmed by analysis of its NMR spectra (^1H NMR, ^{13}C NMR, H−H COSY, DEPT, HMQC, HMBC, and NOESY), and the physical and spectroscopic data were in general agreement with those reported in the literature.[2] The ^{13}C NMR data of **4** have been reported[4] with some incorrect assignments: ^{13}C NMR ($CDCl_3$, 125 MHz) δ 199.4 (C-3), 198.9 (C-20), 162.7 (C-5), 155.2 (C-17), 149.0 (C-16), 138.6 (C-7), 129.1 (C-6), 124.3 (C-4), 73.2 (C-12), 52.8 (C-13), 51.6 (C-14), 49.0 (C-9), 36.1 (C-10), 34.8 (C-8), 33.9 (C-2), 33.6 (C-1), 31.7 (C-15), 28.6 (C-11), 26.8 (C-21), 16.2 (C-19), 11.5 (C-18).

20*S*,21-Dihydroxypregna-4,6-diene-3,12-dione (neridienone B, 5). The structure of compound **5** was confirmed by analysis of its NMR spectra (^1H NMR, ^{13}C NMR, H−H COSY, DEPT, HMQC, HMBC, and NOESY), and the physical and spectroscopic data of **5** were in good agreement with those reported in the literature.[2] The ^{13}C NMR data of **5** have not yet been reported: ^{13}C NMR ($CDCl_3$, 125 MHz) δ 216.8 (C-12), 198.7 (C-3), 161.2 (C-5), 137.9 (C-7), 129.1 (C-6), 124.7 (C-4), 72.1 (C-20), 65.9 (C-21), 57.6 (C-13), 52.5 (C-9), 51.0 (C-14), 44.9 (C-17), 37.3 (C-11), 36.3 (C-8), 36.2 (C-10), 33.6 (C-2), 33.5 (C-1), 23.8 (C-16), 22.9 (C-15), 15.9 (C-19), 12.2 (C-18).

Inhibitory Activity on Induction of ICAM-1. Experimental details have been described in previous papers.[3,8,9,14]

Cell Growth Inhibitory Activity to WI-38, VA-13, and HepG2 in Vitro. Experimental details have been described in previous papers.[3,14,15]

Cellular Accumulation of Calcein. Experimental details have been described in previous papers.[14,15]

References and Notes

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