Cell Differentiation Inducing Steroids from *Withania somnifera* L. (Dun.)

Masanori Kuroyanagi,*[a] Kazutoshi Shibata,[b] and Kaoru Umehara[b]

School of Bioresources, Hiroshima Prefectural University,[a] 562 Nanatsukacho, Shobara-shi, Hiroshima 727—0023, Japan and School of Pharmaceutical Sciences, University of Shizuoka,[b] 52—1, Yada, Shizuoka-shi 422—8526, Japan.
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In the course of screening for cell differentiation inducers from botanical sources, the methanol extract of *Withania somnifera* L. (Dun.) showed activity. From the aerial parts of the plant, sixteen withanolides, including three new compounds, 14, 15 and 16, were isolated and their structures elucidated to be (20*S*,22*R*)-4β,5β,6α,27-tetrahydroxy-1-oxo-witha-2,24-dienolide, (20*R*,22*R*,24*S*,25*R*)-4β,20β-dihydroxy-5β,6β-epoxy-3β-methoxy-1-oxo-withanolide and 3-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]-(20*S*,22*R*)-1α,3β-dihydroxywitha-5,24-dienolide, respectively, from the spectral data. Of these withanolides, 1, 2, 3 and 4 showed potent cell differentiation inducing activity against M1 cells. The most potent compound, 3, showed more potent activity than dexamethasone, the positive control. These active compounds have the same partial structure of the AB ring part, having a 4β-hydroxy-5β,6β-epoxy-2-en-1-one moiety.

Key words *Withania somnifera*; Solanaceae; withanolide; cell differentiation inducer; M1 cell

Much attention has been given to cell differentiation inducers as a new type of antitumor agent. In the course of our investigation on the screening of cell differentiation inducing compounds from botanical sources, many kinds of cell differentiation inducing compounds, such as lignoids,[1] diterpenoids,[2] triterpenoids,[3] flavonoids[4] and steroids[5—7] have been isolated. The methanol extract of the aerial parts of *Withania somnifera* L. (Dun.) (Solanaceae) also showed potent cell differentiation inducing activity against mouse myeloid leukemia (M1) cells.[8] *W. somnifera* is well known as a folk medicine and to afford withanolides, which are steroidal derivatives having a characteristic partial structure in the A,B-ring part and the side chain of δ-lactone. To date more than 40 withanolides have been isolated from *W. somnifera*.[9—11] They have also been isolated from other solanaceous plants: *Physalis* sp.,[12] *Ancistus* sp.,[13] *Jaboroa* sp.,[14] *Datura* sp.[15] and *Dunelia* sp.[16] This paper is concerned with the isolation of withanolides from the aerial parts of *W. somnifera* and their cell differentiation inducing activity.

Results and Discussion

Methanol extract of the aerial parts of *W. somnifera* cultivated in the medicinal plant garden of the University of Shizuoka showed cell differentiation inducing activity against M1 cells. The extract was fractionated between AcOEt and water, and the water layer was extracted with butanol. The AcOEt-soluble fraction showed the most potent cell differentiation inducing activity, and this fraction was separated chromatographically as shown in the Experimental section to afford sixteen withanolides (**1**—**16**).

Compounds **1**—**4** were shown to have the same A,B-ring part, namely a 4β-hydroxy-5β,6β-epoxy-2-en-1-one residue, from the characteristic ^{1}H- and ^{13}C-NMR spectral patterns, and these isolates were deduced to be withaferin A,[17] withanolide D,[18] dihydrowithanolide D[19] and 27-hydroxywithanolide D,[20] respectively. Compound **5** showed the same NMR signal pattern as those of **1**, except for the presence of a glucopyranosyl moiety, and was deduced to be sitoinoside IX.[21] Compounds **6**—**8** showed the absence of the 2-ene group and were shown to be 3β-methoxy-2,3-dihydrowithaferin A,[22] viscosalactone B,[23] and 2,3-dihydrowithaferin A,[22] respectively. Compounds **9**—**11** showed the presence of the same A,B-ring part, having a 1α,3β-dihydroxy-5-ene residue, and their structures were established to be (20*R*,22*R*)-1α,3β,20β-trihydroxy-witha-5,24-dienolide,[24] pubesenolide,[5] and physagulin D,[26] respectively. Compounds **12** and **13** were identified as ixocarpanolide[27] and 6α-chloro-5β-hydroxywithaferin A,[28] respectively.

The molecular formula of compound **14** was determined to be $C_{28}H_{40}O_7$ from the HR-FAB-MS (*m*/*z*: 489.2852 $[MH]^+$). The ^{1}H-NMR and ^{13}C-NMR data showed the presence of a hydroxymethyl group [δ_H 4.28 (1H, d, *J*=12.5 Hz), 4.33 (1H, d, *J*=12.5 Hz) and δ_C 56.8], two secondary hydroxyl groups [δ_H 5.05 (1H, t, *J*=2.5 Hz), 4.38 (1H, dt, *J*=13.5, 3.5 Hz) and δ_C 66.9, 74.4], a tertiary hydroxyl group (δ_C 77.3), a 2-en-1-one moiety [δ_H 5.93 (1H, dd, *J*=10.5, 2.0 Hz), 6.48 (1H, dd, *J*=10.5, 2.5 Hz) and δ_C 127.6, 144.5, 201.3], three singlet methyl groups [δ_H 0.65 (3H, s, Me-19), 1.15 (3H, s, Me-18), 2.01 (3H, s, Me-28)], a doublet methyl group [δ_H 0.93 (3H, d, *J*=6.5 Hz, Me-21)] and an α,β-unsaturated δ-lactone moiety (δ_C 167.2, 153.8, 125.5). The signal pattern was almost the same as that of **13**. From these data, the structure of **14** was deduced to be (20*S*,22*R*)-4β,5β,6α,27-tetrahydroxy-1-oxo-with-2,24-dienolide. The stereochemistry of the hydroxyl group at C-6 was determined to be α-equatorial from the coupling constants of H-6 [δ_H 3.96 (dd, *J*=12.0, 5.5 Hz)]. The configurations at C-4 and C-5 of **14** were interpreted to be the same as those of **13** by comparing the ^{1}H-NMR data of **13** and **14**.

The molecular formula of compound **15** was determined to be $C_{29}H_{44}O_7$ from the HR-FAB-MS (*m*/*z*: 505.3154 $[MH]^+$). The ^{1}H-NMR and ^{13}C-NMR data (Tables 1 and 2) of **15** showed the presence of a secondary methoxy group [δ_H 3.69 (1H, ddd, *J*=6.5, 4.0, 3.0 Hz), 3.32 (3H, s) and δ_C 77.5, 56.9], a secondary hydroxyl group [δ_H 3.48 (1H, d, *J*=3.0 Hz) and δ_C 75.2], an epoxy group (δ_C 60.2, 65.0), three singlet methyl groups [δ_H 0.81 (3H, s, Me-19), 1.22 (3H, s, Me-21), 1.28 (3H, s, Me-18)], two doublet methyl groups [δ_H 1.20 (3H, d, *J*=7.0 Hz, Me-27), 1.30 (3H, d *J*=7.0 Hz, Me-28)], a non-conjugated carbonyl group (δ_C 209.9), a non-conjugated δ-lactone group (δ_C 176.0), a tertiary hydroxyl group (δ_C 75.4), and characteristic methine [δ_H 4.15 (1H, dd, *J*=12.0,

* To whom correspondence should be addressed.

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Table 1. ^{1}H-NMR Spectral Data of **14**—**16** (500 MHz)

H	**14**[a]	**15**[a]	**16**[b]
1			3.67 br s
2	5.93 dd (10.5,2.0)	2.60 dd (15.0, 4.0)	
		2.97 dd (15.0, 6.5)	
3	6.48 dd (10.5, 2.5)	3.69 ddd (6.5, 4.0, 3.0)	3.86 tt (11.5, 5.0)
4	5.05 t (2.5)	3.48 d (3.0)	
6	3.96 dd (12.0,5.5)	3.21 br s	5.37 br d (5.5)
18	1.15 s	1.28 s	0.84s
19	0.65 s	0.81 s	0.56s
21	0.93 d (6.5)	1.22 s	0.85d (6.0)
22	4.38 dt (13.5, 3.5)	4.15 dd (12.0, 3.0)	4.24 dt (13.5, 3.5)
27	4.28 d (12.5)	1.20 d (7.0)	1.80 s
	4.33 d (12.5)		
28	2.01 s	1.30 d (7.0)	1.69 s
OMe		3.32 s	
G-1			4.22 d (7.0)
G′-1			4.20 d (8.0)

a) Measured in $CDCl_3$. *b*) Measured in $CDCl_3$+CD_3OD (10 : 1).

Table 2. ^{13}C-NMR Spectral Data of **14**—**16** (125 MHz)

C	**14**[a]	**15**[a]	**16**[b]
1	201.3	209.9	72.1
2	127.6	39.6	36.3
3	144.5	77.5	74.0
4	66.9	75.2	37.8
5	77.3	65.0	137.3
6	74.4	60.2	124.6
7	36.6	31.3	31.7
8	33.7	28.8	31.5
9	44.9	42.8	41.1
10	56.8	50.5	42.6
11	22.6	21.9	20.2
12	39.1	39.7	39.3
13	43.0	42.9	41.3
14	55.6	56.6	56.1
15	24.0	23.9	24.2
16	27.2	21.5	27.1
17	51.8	54.6	51.8
18	10.0	15.6	11.4
19	11.8	14.2	19.1
20	38.7	75.4	38.7
21	13.2	21.3	13.1
22	78.7	80.3	78.6
23	29.8	31.1	29.4
24	153.8	31.1	150.2
25	125.5	40.5	121.4
26	167.2	176.0	167.8
27	56.8	13.4	12.0
28	20.0	20.5	19.9
OMe		51.9	
G-1			101.4
2			73.3
3			76.0
4			69.8
5			75.1
6			68.3
G-1′			101.4
2′			73.3
3′			76.3
4′			69.9
5′			75.9
6′			61.2

a) Measured in $CDCl_3$. *b*) Measured in $CDCl_3$–CD_3OD (10 : 1).

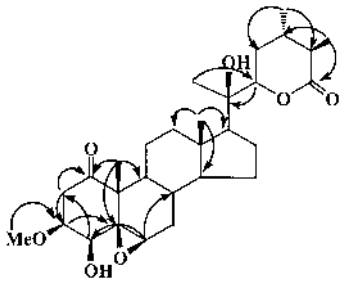

Fig. 1. Selected HMBC Correlations of **15**

3.0 Hz, H-22)] and methylene groups [δ_H 2.60 (1H, dd, *J*=15.0, 4.0 Hz, H-2), 2.97 (1H, dd, *J*=15.0, 6.5 Hz, H-2)]. From these data and the ^{1}H–^{1}H correlation spectroscopy (COSY) of **15**, the presence of an A,B-ring unit having a 4β-hydroxy-3β-methoxy-5β,6β-epoxy-1-one structure and side chain moiety having a hydroxyl group at C-20 and a non-conjugated δ-lactone were indicated. The identical ^{13}C-NMR chemical shifts of the δ-lactone moiety of **15** with those of **3** indicated that **15** had the same structure, including stereochemistry, as that of **3**. The position of the methoxy group was confirmed by difference nuclear Overhauser effect (NOE), in which irradiation at the methoxy group gave correlations with H-3 and H-4, and irradiation at H-2α and H-4 gave correlation with the methoxyl group. The structure of **15** was further confirmed by the detection of C–H long-range correlations in the HMBC experiment. The proton signal of methoxy group at δ_H 3.32 showed a cross peak with the carbon signal at δ_C 77.5 (C-3). The proton signal at δ_H 3.69 (H-3) showed a cross peak with the carbon signal at δ_C 65.0 (C-5), and the proton signal at δ_H 3.48 (H-4) showed a cross peak with the carbon at δ_C 39.6 (C-2). The proton signal of a methyl group at δ_H 0.85 (Me-19) showed cross peaks with the carbonyl carbon at δ_C 209.9 (C-1) and an epoxyl carbon at δ_C 65.0 (C-5). Further HMBC correlations were also detected as shown in Fig. 1. These HMBC data indicated the positions of a methoxyl group, a carbonyl group, hydroxyl groups and an epoxy group. The identical CD spectrum of **15** at 290 nm based on n$\rightarrow\pi^*$ of a saturated ketone as that of **6** indicated that the absolute configuration of AB-ring part of **15** was the same as that of **6**.

The molecular formula of compound **16** was determined to be $C_{40}H_{62}O_7$ from the HR-FAB-MS (*m*/*z* 789.4045 $[MNa]^+$ and *m*/*z*: 767.4218 $[MH]^+$). The molecular formula and the ^{13}C-NMR spectrum of **16** suggested the presence of two glucopyranosyl moieties. The ^{1}H-NMR and ^{13}C-NMR spectra of **16** showed the presence of the characteristic AB-ring part having a 1α-hydroxy-3β-oxy-5-ene structure [δ_H 3.67 (1H, br s, H-1), 3.86 (1H, tt, *J*=11.5, 5.0 Hz, H-3), 5.37 (1H, br d, *J*=5.5 Hz, H-6), and δ_C 72.1 (C-1), 74.0 (C-3), 137.3 (C-5), 124.6 (C-6)], two olefinic methyl groups [δ_H 1.69 (3H, s, Me-28), 1.80 (3H, s, Me-27)], a doublet methyl group [δ_H 0.85 (3H, d, *J*=6.0 Hz)], two singlet methyl groups [δ_H 0.56 (3H, s, Me-19), 0.84 (3H, s, Me-18)], the characteristic H-22 proton [δ_H 4.24 (1H, dd, *J*=13.5, 3.5 Hz)] and an α,β-unsaturated δ-lactone moiety. The connected position of the first glucopyranosyl moiety was determined to be at C-3 from the glycosylation shift of C-3 (+Δ7.9 ppm) compared with that of **10**. The position of the second glucopyranosyl moiety was determined to be at C-6 of the first glucopyra-

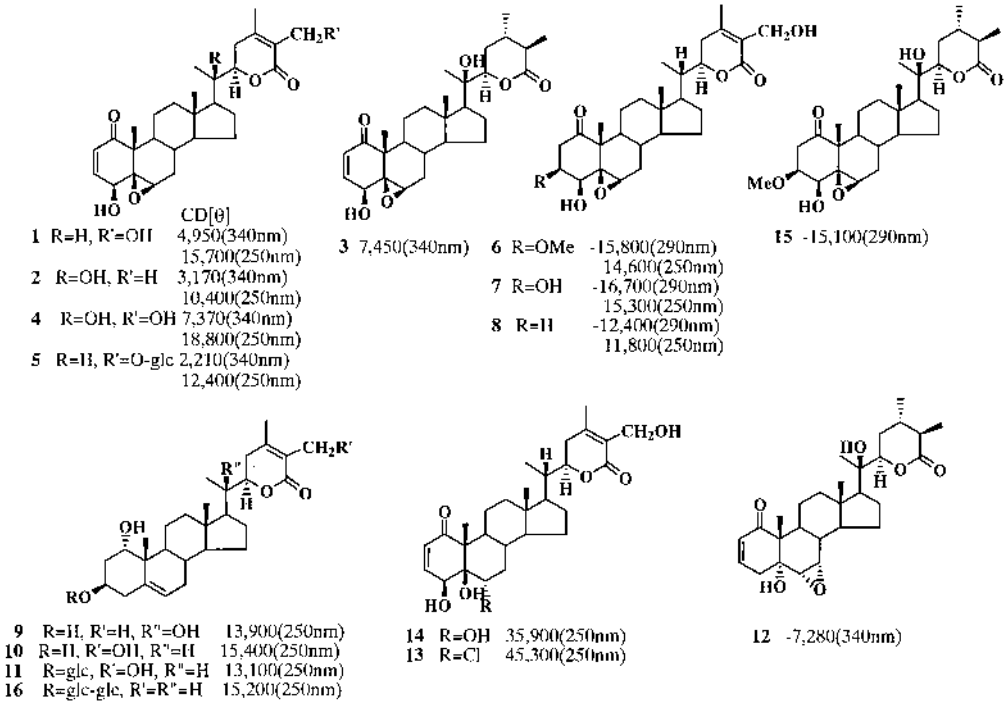

Fig. 2. Structures of the Withanolides and Their CD Data in MeOH

nosyl moiety from the glycosylation shifts of G-6 ($+\Delta$ 6.4 ppm) and G-5 ($-\Delta$1.4). From these data, the structure of **16** was established to be 3-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]-(20*S*,22*R*)-1α,3β-dihydroxywitha-5,24-dienolide.

The CD spectra of the sixteen withanolides were measured and the data are shown in Fig. 2. The 4β-hydroxy-5β,6β-epoxy-2-en-1-one structure of the A,B-ring part gave a positive Cotton effect at 340 nm based on the n→π* transition of an α,β-unsaturated ketone. The α,β-unsaturated δ-lactone having a 22*R* configuration gave a strong positive Cotton effect at 250 nm based on the n→π* transition of the α,β-unsaturated δ-lactone. The 3β,4β-dihydroxy-5β,6β-epoxy-1-one structure gave a strong Cotton effect at 290 nm based on the non-conjugated ketone, and the 5α-hydroxy-6α,7α-epoxy-2-en-1-one structure gave a negative Cotton effect at 340 nm based the on n→π* transition of an α,β-unsaturated ketone. The 4β,5β-dihydroxy-2-en-1-one structure gave no Cotton effect. These CD data should be useful for further discussions of the stereochemistry of withanolides.

Ten of these compounds, **1**—**4**, **6**—**10** and **14**, were examined for their cell differentiation inducing activity against M1 cells. The activity was determined by observation of the inducibility of phagocytosis of M1 cells according to reported methods,[3] and the results are shown in Table 3. Of the tested isolates, compounds **1**—**4** showed very potent activity. Compound **3** showed the most potent activity and was more potent than dexamethasone used as a positive control. The active compounds **1**—**4** have the same 4β-hydroxy-5β,6β-epoxy-2-en-1-one A,B-ring structure, but different side chains. Compound **14**, in which the 5β,6β-epoxy ring was opened to a 5,6-diol, showed a slight activity, and compounds **6**—**10**, having different A,B-ring structures, showed weak activity. From these facts, the 4β-hydroxy-5β,6β-epoxy-2-en-1-one structure was regarded as essential for the activity, but 20-hydroxy group and the α,β-unsaturated δ-lactone were not. The level of the cell differentiation inducing activity of **3** makes it one of the most active natural products from plant sources.

Table 3. Cell Growth and Phagocytosis of M1 Cells Treated with Withanolides

Compound	Conc. (μM)	G.R.[a] (%)	Phago. activity
Cont.[b]		100	3.0±1.7
Dex.[c]	1	53	52.3±3.1
1	10	0	n.d.[d]
	2	7	60.0±0
	1	36	20.7±3.1
2	10	0	n.d.
	2	1	n.d.
	1	9	42.2±6.4
3	10	0	n.d.
	2	6	30.7±3.8
	1	11	70.7±9.0
4	10	2	n.d.
	2	9	60.7±4.7
6	10	50	10.3±4.0
	2	64	5.7±1.5
7	10	72	14.0±4.0
	2	69	5.0±1.0
8	10	99	4.7±1.2
	2	76	19.0±7.0
9	10	85	25.3±4.6
	2	62	2.3±2.3
10	10	89	4.7±3.5
	2	98	4.0±2.6
14	10	78	20.3±4.2
	2	100	3.0±2.0

a) Growth rates. *b*) Control. *c*) Dexamethasone. *d*) Not detected.

3-Methoxy derivatives, **6** and **15**, were assumed to be MeOH adducts of **1** and **3**, respectively, because of the treatment with MeOH in the process of extraction and purifica-

tion of these compounds.[22)]

Experimental

General Procedures ^{1}H-NMR and ^{13}C-NMR were measured with a JEOL GSX-500 FT NMR spectrometer, and the chemical shifts are given in ppm from TMS as an internal standard. FAB-MS were recorded on a JEOL JMX-SX-102 mass spectrometer using *m*-nitrobenzyl alcohol as a matrix. UV spectra were recorded with a Hitachi U3410 spectrometer. CD spectra were recorded on a JASCO J-20A spectropolarimeter. HPLC was carried out on a YMC ODS-7 packed column using an acetonitrile–water solvent system monitored at 210 nm.

Plant Material and Isolation Seeds of *W. somnifera* were obtained from Tsukuba Medicinal Plants Research Center of the National Institutes of Health Sciences, and were cultivated at the Medicinal Plant Garden of the University of Shizuoka. The plants were harvested in November, 1992. Their aerial parts (4 kg) were extracted with MeOH under reflux to give a MeOH extract (640 g), which was fractionated between AcOEt and water to give an AcOEt soluble fraction (220 g) and an aqueous layer. The aqueous layer was extracted with *n*-BuOH to give a *n*-BuOH-soluble fraction (70 g). The AcOEt-soluble fraction showed potent cell differentiation inducing activity and was chromatographed on a silica gel column using a $CHCl_3$–MeOH gradient solvent system to give 8 combined fractions, fractions 1—8, according to their TLC patterns. Fraction 2 (30 g) was chromatographed on a silica gel column using a hexane–AcOEt solvent system and HPLC, successively using an ODS column (YMC Co. Ltd.) and a CH_3CN–H_2O solvent system, to give **1** (2 g), **2** (20 mg), **3** (75 mg), **6** (70 mg), **7** (30 mg), **8** (200 mg), **12** (16 mg), **13** (14 mg) and **15** (17 mg). Fraction 4 (40 g) was also purified in the same way as above to give **1** (250 mg), **4** (20 mg), **6** (90 mg), **8** (80 mg), **9** (25 mg) and **10** (30 mg). Fraction 5 (12 g) was purified by HPLC using a ODS column to give **1** (600 mg). Fraction 6 (6 g) gave **8** (200 mg). Fraction 7 (4 g) gave **5** (30 mg) and **11** (47 mg). Fraction 8 (13 g) gave **1** (150 mg), and **16** (120 mg).

(20*S*,22*R*)-4β,5β,6β,27-Tetrahydroxy-1-oxo-witha-2,24-dienolide (**14**): Amorphous powder. HR-FABMS; *m*/*z*: 489.2852 $[MH]^+$ (Calcd for $C_{28}H_{41}O_7$: 489.2852). UV λ_{max} 215 nm (ε 13050). ^{1}H-NMR data in Table 1. ^{13}C-NMR data in Table 2. CD data in Fig. 2.

(20*R*,22*R*,24*S*,25*R*)-4β,20β-Dihydroxy-5β,6β-epoxy-3β-methoxy-1-oxowithanolide (**15**): Amorphous solid. HR-FABMS; *m*/*z*: 505.3154 $[MH]^+$ (Calcd for $C_{29}H_{45}O_7$: 505.3165). ^{1}H-NMR data in Table 1. ^{13}C-NMR data in Table 2. CD data in Fig. 2.

3-*O*-[β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl]-(20*S*,22*R*)-1α,3β-dihydroxy-witha-5,24-dienolide (**16**): Amorphous powder. HR-FABMS; *m*/*z*: 789 $[MNa]^+$ (Calcd for $C_{40}H_{62}O_{14}Na$: 789.4037), *m*/*z*: 767.4218 $[MH]^+$ (Calcd for $C_{40}H_{63}O_{14}$: 767.4218). ^{1}H-NMR data in Table 1. ^{13}C-NMR data in Table 2. CD data in Fig. 2.

Measurement of Phagocytosis Experiments for cell differentiation inducing activity were carried out by measuring the inducibility of phagocytosis according to a reported method.[3)] Cells were cultured at a concentration of 2×10^5 cells/ml into culture medium (2 ml) and incubated with 20 μl sample solution diluted with ethanol. After 48 h, the cells were washed and incubated for 4 h with a suspension of polystyrene latex particles (2 μl/ml serum free medium). Then the cells were washed thoroughly 3 or 4 times with phosphate buffered saline and the percentage of phagocytic cells was determined.

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References

1) Umehara K., Sugawa A., Kuroyanagi M., Ueno A., Taki T., *Chem. Pharm. Bull*., **41**, 1774—1779 (1993).
2) Matsuda T., Kuroyanagi M., Sugiyama S., Umehara K., Ueno A., Nishi K., *Chem. Pharm. Bull*., **42**, 1216—1225 (1994).
3) Umehara K., Takagi R., Kuroyanagi M., Ueno A., Taki T., Chen Y.-J., *Chem. Pharm. Bull*., **40**, 401—405 (1992).
4) Sugiyama S., Umehara K., Kuroyanagi M., Ueno A., Taki T., *Chem. Pharm. Bull*., **41**, 714—719 (1993).
5) Sunayama R., Kuroyanagi M., Umehara K., Ueno A., *Phytochemistry*, **34**, 529—533 (1993).
6) Umehara K., Endoh M., Miyase T., Kuroyanagi M., Ueno A., Taki T., *Chem. Pharm. Bull*., **42**, 611—616 (1994).
7) Umehara K., Sumi N., Sato H., Miyase T., Kuroyanagi M., Ueno A., *Chem. Pharm. Bull*., **43**, 1565—1568 (1995).
8) Ichikawa Y., *Journal of Cell Physiology*, **74**, 223—234 (1969).
9) Kirson I., Glotter E., Lavie D., Abraham A., *J. Chem. Soc*. (*C*), **1971**, 2032—2044.
10) Velde V. V., Lavie D., *Phytochemistry*, **20**, 1359—1364 (1981).
11) Bessalle R., Lavie D., *Phytochemistry*, **31**, 3648—3651 (1992).
12) Shingu K., Yahara S., Okabe H., Nohara T., *Chem. Pharm. Bull*., **40**, 2448—2451 (1992).
13) Nittala S. S., Lavie D., *Phytochemistry*, **20**, 2735—2739 (1981).
14) Tschesche R., Baumgarth M., Welzel P., *Tetrahedron*, **24**, 5169—5179 (1968).
15) Shingu K., Furusawa Y., Nohara T., *Chem. Pharm. Bull*., **37**, 2132—2135 (1989).
16) Lischeswki M., Hang N. T. B., Porzel A., Adam G., Massiot G.,Lavaud C., P*hytochemistry*, **31**, 939—942 (1992).
17) Lavie D., Glotter E., Shvo Y. *J. Org. Chem*., **30**, 1774—1778 (1965).
18) Lavie D., Kirson I., Glotter E., *Israel J. Chem*., **6**, 671—678 (1968).
19) Kirson I., Glotter E., Abraham A., Lavie D., *Tetrahedron*, **26**, 2209—2219 (1970).
20) Nittala S. S., Lavie D., *Phytochemistry*, **20**, 2741—2748 (1981).
21) Ghoasal S., Kaur R., Sirvastva S., *Indian J. Nat. Prod*., **40**, 2088—2092 (1992).
22) Kupchan S. M., Andeson W. K., Bollinger P., Doskotch R. W., Smith R. M., Saenz R. J. A., Schenoes H. K., Burlingame A. L., Smith D. H., *J. Org. Chem*., **34**, 3858—3866 (1969).
23) Pelletier S. W., Gebeyehu G., Nowacki J., Mody N. V., *Heterocycles*, **15**, 317—320 (1981).
24) Lischewski M., Hang N. T. B., Porzel A., Adam G., Massiot G., Lavaud C., *Phytochemistry*, **30**, 4184—4186 (1991).
25) Sahai M., *J. Nat. Prod*., **48**, 474—476 (1985).
26) Shingu K., Yahara S., Nohara T., Okabe H., *Chem. Pharm. Bull*., **40**, 2088—2091 (1992).
27) Abdullaev N. D., Vasina O. E., Maslennikova V. A., Abibakirov N. K., *Khim. Prir. Soedin*., **22**, 326—332 (1986).
28) Nittala S. S., Velde V. V., Frolow F., Lavie D., *Phytochemistry*, **20**, 2547—2552 (1981).