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Kim-Hong Gan, Chun-Nan Lin, and Shen-Jeu Won

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CYTOTOXIC PRINCIPLES AND THEIR DERIVATIVES OF FORMOSAN *SOLANUM* PLANTS[1]

KIM-HONG GAN, CHUN-NAN LIN,

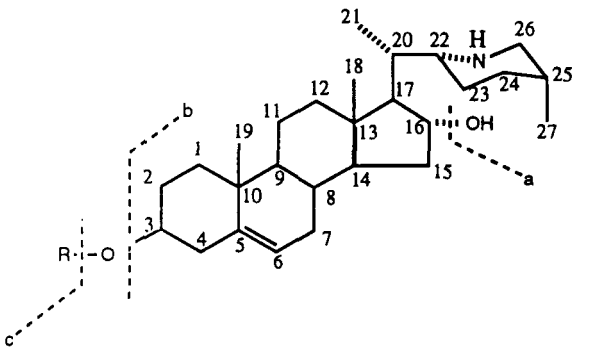
Natural Products Research Center, Kaohsiung Medical College, Kaohsiung, Taiwan 807, Republic of China

and SHEN-JEU WON

Department of Microbiology, Medical College, National Cheng Kung University, Tainan, Taiwan 701, Republic of China

ABSTRACT.—The new steroidal alkaloid capsimine-3-*O*-β-D-glucoside [**1**] was isolated from the root bark of *Solanum capsicastrum*, and carpesterol [**2**], 3β-(*p*-hydroxy)-benzoyloxy-22α-hydroxy-4α-methyl-5α-stigmast-7-en-6-one [**3**], and a new steroidal glycoside named indioside A [**4**] were isolated from the fruit of *Solanum indicum*. Indioside A was characterized as 3β-*O*-{α-L-rhamnopyranosyl-(1→2), β-D-glucopyranosyl-(1→4), β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-diosgenin. Khasianine, dihydrosolasodine, capsimine, and capsimine-3-*O*-β-D-glucoside exhibited strong activity against liver damage induced by CCl_4. Capsimine and narigenin exhibited significant cytotoxic effect against human PLC/PRF/5 and KB cells in vitro, and capsicastrine and etioline exhibited significant cytotoxicity against human PLC/PRF/5 cells in vitro.

Continued work on Formosan *Solanum* (Solanaceae) plants has yielded a new steroidal alkaloid, capsimine-3-*O*-β-D-glucoside [**1**], from the root bark of *Solanum capsicastrum* Link and carpesterol [**2**], 3β-(*p*-hydroxy)-benzoyloxy-22α-hydroxy-4α-methyl-5α-stigmast-7-en-6-one [**3**], narigenin, a mixture of stigmasterol and β-sitosterol, β-sitosterol-3-*O*-β-D-glucoside, and a new steroidal glycoside, indioside A [**4**], from the fruit of *Solanum indicum* L. In this paper, we report the isolation and characterization of **1** and **4**, the ^{13}C-nmr spectral assignments of **2** and **3**, and the configurations at C-22 and C-24 of **3** (2). Since the steroidal alkaloids showed antihepatotoxic and cytotoxic effects, khasianine (3), dihydrosolasodine (the reduced product of solasodine), capsimine (1), and **1** have been screened for their protective effect against CCl_4-induced hepatic damage, and *N*-methylsolasodine (*N*-methylated from solasodine), capsimine (1), solasonine (4), narigenin, etioline (5), and capsicastrine (5) have been screened for cytotoxic effects.



1 R=glucosyl

[1]Part VII in the series, "Studies on the Constituents of Formosan *Solanum* species." For part VI see C.N. Lin, C.M. Lu, M.K. Cheng, K.H. Gan, and S.J. Won, *J. Nat. Prod.*, **53**, 513 (1990).

RESULTS AND DISCUSSION

Compound **1**, $C_{33}H_{55}NO_7$, mp 214–215°, was recrystallized from MeOH. Its ir spectrum (KBr) showed the presence of hydroxyl (3400 cm^{-1}). Acidic hydrolysis yielded glucose, as evidenced by tlc, and capsimine, identified by comparison with an authentic sample (1). The eims of **1** showed no molecular ion but had an $[M-2H]^+$ peak (*m/z* 575) and typical ions at *m/z* 559 $[M-18]^+$, 397 $[b-2H]^+$, 380 $[M-glucose-a]^+$, and 18 $[a+H]^+$. A base peak at *m/z* 98 appeared as the result of C-20/C-22 bond fission (6). The ion at *m/z* 162 $[c]^+$ indicated the sugar moiety (7). In the fabms (positive mode) the peak of the highest mass number was observed at *m/z* 578 $[M+H]^+$, indicating that the $[M]^+$ of **1** was 577.

The ^{1}H-nmr spectrum of **1** in CD_3OD showed two singlets (3H each) at δ 0.80 and 1.00 for C-18 and C-19 angular methyl groups, one doublet (6H, $J=5.5$ Hz) at δ 1.02 corresponding to two secondary methyl groups at C-20 and C-25, one doublet (1H, $J=7.5$ Hz) at δ4.38 for the anomeric H-1 sugar proton indicating the β configuration of the glycosidic linkage (8), and one broad singlet at δ 5.38 for the vinylic H-6. The ^{1}H-nmr resonance of the C-27 methyl doublet at δ 1.02 in **1** indicated axial orientation of the C-27 methyl group (6). The monoglycosidic nature of **1** was also confirmed by its ^{13}C-nmr data (Table 1), in which all 33 carbon atoms were assigned by ^{1}H- decoupling data, DEPT pulse, and comparison with the published data of capsimine (1). The shift values for carbon atoms of **1** (except for C-2 to C-4) and glucosyl carbons corresponded well with those of capsimine (1) (Table 1). A characteristic shift for C-2 to C-4 of **1** indicated that glycosylation occurred at the C-3 hydroxyl group of **1** (5). These correlations indicated that the glycosyl function was located at the 3β position and suggested that glycoalkaloid **1** was (22*R*,25*R*)-22,26-epiminocholest-5-ene-3β,16α-diol-3-*O*-β-D-glucoside.

The ^{13}C-nmr spectral assignments of **2** (9,10) are shown in Table 1. The shift values for carbon atoms of **3** (2) (except for C-1′ to C-6′) corresponded very well with those of **2** (Table 1). In **3**, the presence of a hydroxyl group at C-4′ causes a high field shift for C-2′, C-3′, and C-5′ and a low field shift for C-2′ and C-6′ (11), indicating the presence of a hydroxyl group at the para position of the benzoate moiety.

The chemical shift values for C-22 and C-24 of **3** corresponded well with those of **2**, indicating that the side chain stereochemistry of **3** was the same as that of **2**. Therefore, the configuration at C-22 and C-24 of **3** should be *R*.

Compound **4** gave a positive Liebermann-Burchard test. Its ir (KBr) showed the presence of a hydroxyl group at 3400 cm^{-1}. Acidic hydrolysis yielded glucose and rhamnose, as detected by tlc, and diosgenin, identified by ir, nmr, and ms. In the fabms (positive mode) the peak of highest mass number was observed at *m/z* 1193 $[M+H]^+$, along with significant peaks as shown in the Experimental (12). These results confirm the $[M]^+$ of **4** to be 1192, and it has a fragmentation pattern consistent with the sequential loss of five sugars from a diosgenin aglycone. The fabms (positive mode) of **4** acetate showed peaks due to the peracetylated terminal hexose (*m/z* 331) and methylpentose (*m/z* 273, base peak) (13). Also, **4** possessed a sugar sequence as shown in the structure.

The ^{1}H-nmr spectrum (CD_3OD) of **4** showed five anomeric protons at δ 4.30 (d, $J=7.5$ Hz), 4.42 (d, $J=7.5$ Hz), 4.50 (br s), 5.14 (d, $J=7.5$ Hz), and an anomeric proton signal may overlap with signal of D_2O at δ 4.78, and the ^{13}C nmr of **4** (Table 1) showed five signals due to anomeric carbons at δ 100.4, 102.3, 102.5, 103.0, and 105.4. In this latter spectrum, six carbon signals, at δ 102.5, 78.0, 88.2, 70.7, 76.6, and 62.0, were assigned to glucosyl carbons, attached at the 3β-hydroxyl group of diosgenin, by comparison with the chemical shift values of the corresponding glucosyl carbons of enzymic hydrolytic, methylated, and reduced product of 26-*O*-β-D-gluco-

pyranosyl(22ξ,25*R*)-3β,22,26-trihydroxyfurost-5-ene-3-*O*-L-rhamnosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (13); six carbon signals at δ 103.0, 78.6, 72.4, 79.3, 77.3, and 62.6 were reasonably assigned to the inner glucosyl car-

TABLE 1. ^{13}C-nmr Chemical Shift Values and Assignments (TMS as internal standard) of **1–4**.[a]

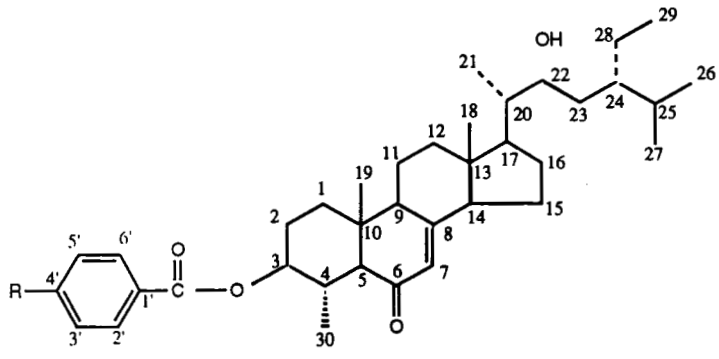
Carbon	Compound			
	1[b]	2[c]	3[d]	4[b]
C-1	37.9	38.9	38.9	38.0
C-2	30.7	22.6	22.9	30.7
C-3	80.0	79.0	78.6	79.0
C-4	39.7	31.9	32.4	39.3
C-5	142.4	60.1	59.9	141.9
C-6	122.8	200.0	199.5	122.6
C-7	32.8	123.7	124.1	33.2
C-8	32.8	160.9	161.2	32.7
C-9	51.6	51.1	50.8	51.7
C-10	38.5	39.3	39.3	38.6
C-11	21.8	21.8	21.9	21.9
C-12	40.8	36.3	36.1	39.5
C-13	45.6	45.1	45.1	41.9
C-14	55.5	54.9	54.8	57.7
C-15	36.6	26.2	26.7	32.8
C-16	76.4	27.1	27.4	80.5
C-17	64.1	53.2	53.4	62.6
C-18	13.7	11.9	12.1	16.8
C-19	19.9	20.5	20.7	19.9
C-20	32.8	41.5	41.6	not observed
C-21	18.7	12.6	13.1	14.6
C-22	64.1	71.1	69.9	111.8
C-23	26.1	30.2	30.3	32.7
C-24	32.8	42.8	43.6	30.7
C-25	27.2	28.9	29.3	30.7
C-26	52.3	17.8	18.1	67.1
C-27	16.1	17.5	17.6	17.9
C-28		23.6	23.9	
C-29		12.3	12.3	
C-30		14.7	14.6	
C-1′	102.7	130.6	122.7	102.5
C-2′	75.3	129.6	132.4	78.0
C-3′	78.2	128.4	116.2	88.2
C-4′	71.8	132.9	163.6	70.7
C-5′	78.0	128.4	116.2	76.7
C-6′	62.8	129.6	132.4	62.0
C_6H_5-*C*=O		166.4	166.4	
C-1″				100.4
C-2″				72.1[e]
C-3″				72.3[e]
C-4″				73.9
C-5″				69.9
C-6″				17.8
C-1‴				103.0
C-2‴				78.6
C-3‴				72.4[e]
C-4‴				79.3
C-5‴				77.3
C-6‴				62.6

TABLE 1. Continued.

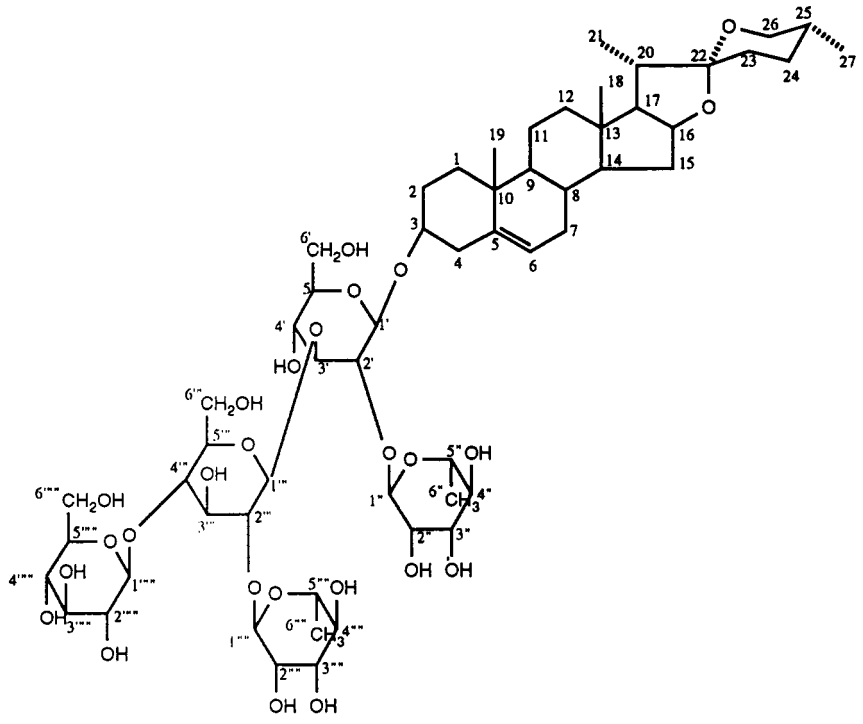
Carbon	Compound			
	1[b]	2[c]	3[d]	4[b]
C-1‴				102.3
C-2‴				72.2[e]
C-3‴				72.4
C-4‴				74.8
C-5‴				69.9
C-6‴				18.1
C-1⁗				105.4
C-2⁗				74.8
C-3⁗				78.0
C-4⁗				70.9
C-5⁗				77.3
C-6⁗				64.5

[a]The number of directly attached protons to each carbon was verified with the DEPT pulse sequence.
[b]Spectra recorded in CD_3OD.
[c]Spectra recorded in $CDCl_3$.
[d]Spectra recorded in pyridine-d_5.
[e]Assignments with the same sign are interchangeable.

bons by comparison with the chemical shift values of the corresponding glucosyl carbons of solamargine (14), a mixture of (25ξ)-solanidan-3β,23β-diol and (25ξ)-Δ^5-solanidan-3β,23β-diol (15), and kaempferol-3-*O*-β-D-glucopyranosyl-(1↦4)-β-D-glucopyranoside (16); and six carbon signals at δ 105.4, 74.8, 78.0, 70.9, 77.3, and 64.5, six carbon signals at δ 100.4, 72.1, 72.3, 73.9, 69.9, and 17.8, and six carbon signals at δ 102.3, 72.2, 72.4, 74.8, 69.9, and 18.1 were assigned to the terminal glucosyl carbons, inner, and outer rhamnosyl carbons, respectively, by comparison with the chemical shift values of methyl-β-D-glucopyranoside and methyl-α-L-rhamnopyranoside (17). Therefore, the structure of **4** was characterized as 3β-*O*-{α-L-rhamnopyranosyl-(1↦2), β-D-glucopyranosyl-(1↦4), β-D-glucopyranosyl-(1↦3)-[α-L-rhamnopyranosyl-(1↦2)]-β-D-glucopyranosyl}-diosgenin. The nmr spectra of **4** acetate also supported structure **4**. The protective effect of khasianine (3), dihydrosolasodine (the reduced product of solasodine), capsimine, and **1** against CCl_4-induced hepatic damage, and the cytotoxic effects of *N*-methylsolasodine, capsimine, sol-



2 R=H
3 R=OH



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asonine (4), narigenin, etioline (5), and capsicastrine (5) against human PLC/PRF/5 and KB cells in vitro (18, 19) were studied and the results were listed in Tables 2 and 3, respectively. Khasianine, dihydrosolasodine, capsimine, and **1** exhibited strong activity against liver damage induced by CCl_4. Capsimine and narigenin exhibited significant inhibition of human hepatoma PLC/PRF/5 and KB cells in vitro, and capsicastrine and etioline exhibited significant inhibition of PLC/PRF/5 cells in vitro.

The increment of dose (mg/kg) of tested compounds does not enhance liver protec-

TABLE 2. Effect of Some Compounds and Selected Derivatives of *Solanum capsicastrum* and *Solanum indicum* on CCl_4 (0.1 ml/kg)-induced Hepatotoxicity in Mice.

Substance	Dose (mg/kg)	Serum (Mean ± SD)	
		SGPT (%)	SGPT (%)
PEG	0.1 ml/kg	14.89 ± 9.19	10.07 ± 5.54
Control	0.1 ml/kg	100.00 ± 57.07	100.00 ± 42.40
Khasianine	0.1	14.28 ± 12.20[a]	8.89 ± 7.67[b]
	3.0	17.13 ± 26.08[a]	8.39 ± 11.14[b]
Dihydrosolasodine	0.1	5.87 ± 1.13[a]	3.46 ± 0.23[a]
	3.0	7.06 ± 2.48[a]	3.89 ± 1.21[b]
Capsimine-3-*O*-β-D-glucoside [**1**]	0.1	4.60 ± 0.85[a]	4.82 ± 16.64[a]
	3.0	14.65 ± 10.60[a]	4.99 ± 1.88[a]
Capsimine	0.1	19.40 ± 14.25[a]	9.20 ± 9.60[b]
	3.0	9.92 ± 7.00[a]	6.87 ± 0.70[a]

[a]Significantly different from control, $p<0.01$.
[b]Significantly different from control, $p<0.001$.

TABLE 3. Cytotoxicity[a] of Capsimine, *N*-Methylsolasodine, Solasonine, Narigenin, Etioline, and Capsicastrine Against PLC/PRF/5 and KB Cells In Vitro.

Compound	ED_{50}[b] (μg/ml) (n = 8/group)	
	PLC/PRF/5	KB
Capsimine	1.97	1.35
N-Methylsolasodine	8.02	6.00
Solasonine	7.88	11.40
Narigenin	2.47	2.71
Etioline	2.67	11.23
Capsicastrine	1.78	—

[a]For significant activity of the pure compound, an ED_{50}<4.0 μg/ml is required (20).

[b]Concentration in μg/ml affording 50% inhibition of cell growth of the control.

tion in mice (except for capsimine), but glycosylation of capsimine (1) showed significantly stronger activity in mice than that of the aglycone at doses 0.1 mg/kg (Table 2). The *N*-methylated solasodine did not enhance the cytotoxic effect (3) (Table 3). Based on the above results, further studies of the bioactive principles from these *Solanum* plants appears justified.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's are uncorrected. Ft-nmr spectra were taken on a VXR-300/51 superconducting High Resolution FT system; ir spectra on a Hitachi model 260-30; ms on an MS-JMS-HX 110 Mass Spectrometer; and optical rotation on a Jasco model dip-181 digital polarimeter.

EXTRACTION AND SEPARATION.—Air-dried root bark of *S. capsicastrum* (20 kg) was collected at Tainan, Taiwan, in February 1987, chipped, and then treated as described previously (6). A voucher specimen is deposited in our laboratory. The precipitate from 3% HOAc-soluble base was chromatographed on Si gel. The column was eluted with $CHCl_3$ to afford a mixture of stigmasterol and β-sitosterol, and with $CHCl_3$-MeOH (3:1) to afford **1**. Fresh berries (9.2 kg) of *S. indicum* were collected at Kaohsiung Hsien, Taiwan, in December 1989, and a voucher specimen is deposited in our laboratory. The fresh berries were extracted several times with MeOH, and the combined extracts were chromatographed on Si gel. Elution with cyclohexane-EtOAc (4:1) gave narigenin and a mixture of stigmasterol and β-sitosterol. Elution with CH_2Cl_2-MeOH (4:1) gave β-sitosterol-3-*O*-β-D-glucoside, **2**, and **3**. Elution with EtOAc-MeOH (9:1) gave **4**.

Compound **2**, narigenin, and β-sitosterol-3-*O*-D-glucoside were identified by comparison of $[\alpha]_D$, ir, nmr, and ms with those of authentic samples. Compound **3** was identified from physical and spectral data and by chemical reaction (2).

Capsimine-3-O-β-D-*glucopyranoside* [**1**].—Colorless powder (MeOH): mp 214–216°; $[\alpha]^{20}_D$ −68.8° [*c* = 0.05, cyclohexane-EtOAc-MeOH (1:1:2)]; eims *m/z* (rel. int.) $[M-2H]^+$ 575 (5), $[M-18]^+$ 559 (51), $[b-2H]^+$ 397 (12), $[M-\text{glucose}-a]^+$ 380 (20), $[c]^+$ 162 (12), 138 (40), 99 (18), 98 (100), $[a+H]^+$ 18 (17); fabms (positive mode) *m/z* (rel. int.) $[M+H]^+$ 578 (53), 560 (4), 444 (2), 416 (4), 398 (10), 138 (40), 125 (67), 115 (100); ir ν max (KBr) 3400 (OH) cm^{-1}; ^1H nmr see text; ^{13}C nmr (CD_3OD) see Table 1. Compound **1** was hydrolyzed to yield capsimine whose identity was confirmed by direct comparison of mp, ir, nmr, and ms spectra with those of an authentic sample. The sugar portion was examined by tlc [$CHCl_3$-MeOH-Me_2CO-H_2O (3:3:3:1) on Si gel] to detect methylglucopyranoside (R_f 0.59).

Indioside A [**4**].—White granules (MeOH): mp 160–165°; $[\alpha]^{20}_D$ −22.67 (*c* = 0.075, MeOH); fabms (positive mode) *m/z* (rel. int.) $[M+H]^+$ 1193 (6), 959 (8), $[1193-118\times2]^+$ 957 (4), 937 (10), 923 (18), 909 (8), 883 (3), 851 (3), 823 (2), 633 (2), 601 (6), 575 (4), 543 (4), 487 (9), 443 (7), 414 (4), 398 (6), 397 (11); ir ν max (KBr) 3400 (OH) cm^{-1}; ^1H nmr δ 4.30 (d, *J* = 7.5 Hz, anomeric proton), 4.42 (d, *J* = 7.5 Hz, anomeric proton), 4.50 (br s, anomeric proton), 5.14 (d, *J* = 7.5 Hz, anomeric proton), 1.01 (3H, d, *J* = 7.5 Hz, H-21), 0.99 (3H, s, H-19), 0.90 (3H, d, *J* = 7.0 Hz, H-27), 0.67 (3H, s, H-18); ^{13}C nmr see Table 1.

Indioside A acetate.—White granules (MeOH): mp 68–75°, eims *m/z* (rel. int.) no molecular ion,

1536 (0.6), 1219 (2), 777 (9), 727 (2), 663 (1), 512 (2), 453 (9), 393 (20), 331 (18), 273 (100), 153 (70), 111 (57), 43 (49); ^{1}H nmr ($CDCl_3$) δ 0.97 (3H, d, J = 7.5 Hz), 0.96 (3H, s, H-19), 0.77 (3H, s, H-18), 0.76 (3H, d, J = 7.0 Hz).

N-*Methylsolasodine*.—A solution of solasodine in MeOH was added to MeI and KOH, and the mixture was stirred at 40°. After the reaction, the precipitate (KI) was collected by filtration and washed with MeOH. The filtrate and washings were combined and evaporated to dryness. The residue was purified by chromatography on Si gel and crystallized from Me_2CO to give needles, identified as methylsolasodine from physical and spectral data.

Dihydrosolasodine.—Solasodine was dissolved in MeOH, $NaBH_4$ was added, and the mixture was stirred overnight. Cold H_2O was added, and the aqueous phase was extracted several times with $CHCl_3$. The $CHCl_3$ extract was concentrated, the residue was purified by chromatography on Si gel, and the eluate was recrystallized from a mixture of MeOH, $CHCl_3$, and Me_2CO to give colorless needles, identified from physical and spectral data.

BIOLOGICAL ASSAY.—PLC/PRF/5 cells were established from human hepatoma and are known to produce hepatitis B surface antigen continuously in culture fluids (18). The cells were grown as continuous cultures in a growth medium consisting of Dulbecco's modified Eagle medium (DMEM, GIBCO, NY), 10% fetal bovine serum (FBS, GIBCO), 100 IU/ml streptomycin, 2 mM L-glutamine, and antibiotics. For microassays, the growth medium was supplemented further with 10 nM HEPES buffer, pH 7.3. The microassay for anticellular effect was performed as reported previously (19).

ANTIHEPATOTOXIC SCREEN.—Protective effects of isolated compounds and compound derivatives were evaluated for protection against CCl_4-induced hepatotoxicity in ICR male mice (18–20 g) using 6–10 mice for each experiment. The protective effect was assessed by determination of SGOT and SGPT values (18), and liver damage was induced with a fresh mixture of equal volumes of CCl_4 and olive oil given in one injection in doses of 0.1 ml/kg of CCl_4 by the ip route. The test compound was administered and experiments conducted as described in Lin and Gan (1).

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