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## THE CYTOTOXIC PRINCIPLES OF *SOLANUM INCANUM*<sup>1</sup>

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**ABSTRACT.**—In continuation of work on *Solanum incanum* a new steroidal alkaloid glycoside has been isolated from the fresh berries, which is named incanumine, and characterized as  $O(3)-\{\beta\text{-D-xylopyranosyl-(1}\rightarrow 3_{\text{glu}})-[\beta\text{-D-xylopyranosyl-(1}\rightarrow 4_{\text{rha}})-\alpha\text{-L-rhamnopyranosyl-(1}\rightarrow 4_{\text{glu}})]-\beta\text{-D-glucopyranosyl}\}$ -solasodine [**1**]. Solamargine, solasodine, ursolic acid, and ursolic acid derivatives (3-*O*-palmitoyl ursolic acid, 3-*O*-crotonyl ursolic acid, 3-*O*-propionyl ursolic acid) exhibited significant cytotoxic effects against human PLC/PRF/5 cells in vitro. Esterification of ursolic acid with aliphatic acids clearly enhanced the cytotoxic effects against human PLC/PRF/5 cells in vitro.

In the course of screening for cytotoxic and antihepatotoxic principles of Formosan *Solanum* plants, we have previously reported the isolation of solasodine, solamargine, solasonine, ursolic acid, carpesterol, and  $\beta$ -sitosterol from the fruits of *Solanum incanum* L. (Solanaceae). All except for  $\beta$ -sitosterol showed novel liver protective effects against  $\text{CCl}_4$ -induced liver damage (1,2). In this paper we report the isolation of stigmasterol- $\beta$ -D-glucoside, khasianine, and a new steroidal alkaloid glycoside named incanumine [**1**] from this same plant. Solamargine and solasonine possess inhibitory effects against JTC-26 cells in vitro (3). Ursolic acid showed significant cytotoxicity in the lymphocytic leukemia cells (P-388, L-1210) and human lung carcinoma cells (A-549), and marginal cytotoxicities in KB cells, human colon cells (HCT-5), and mammary tumor cells (MCF-7) (4). Esterified derivatives of ursolic acid showed equivalent or slightly increased activity against the growth of L-1210 and P-388 leukemic cells (4). Therefore, solamargine, solasodine, khasianine,

carpesterol, ursolic acid, and ursolic acid derivatives (3-*O*-benzoyl ursolic acid, 3-*O*-palmitoyl ursolic acid, 3-*O*-cinnamoyl ursolic acid, 3-*O*-crotonyl ursolic acid, and 3-*O*-propionyl ursolic acid) isolated from this plant or derived from isolates were screened for cytotoxic effects against human PLC/PRF/5 cells. Khasianine, solasodine, and ursolic acid have also been screened against KB cells in vitro.

### RESULTS AND DISCUSSION

Si gel chromatography of the tertiary base fraction obtained from the MeOH extracts of the fresh berries yielded three crystalline compounds, stigmasterol-3- $\beta$ -D-glucoside, khasianine, and incanumine [**1**]. Incanumine [**1**],  $\text{C}_{49}\text{H}_{79}\text{NO}_{19}$ , was positive to Dragendorff's reagent. Its ir spectrum (KBr) showed the presence of a hydroxyl group at  $3400\text{ cm}^{-1}$ . Acidic hydrolysis yielded glucose, rhamnose, and xylose, as detected by tlc, and solasodine, identified by comparison with an authentic sample. The ei mass spectrum of **1** showed no molecular ion but a peak at  $m/z$  576 [ $\text{aglycone} + \text{glucose} - \text{H}_2\text{O} + \text{H}^+$ ] besides typical ions at  $m/z$  114 and 138, which are characteristic of a solasodine skeleton (5). In the fab mass spectrum (positive mode) the peak of highest mass

<sup>1</sup>Part VI in the series "Studies on the Constituents of Formosan *Solanum* Species." For Part V see C.N. Lin and K.H. Gan, *Planta Med.*, **55**, 48 (1989).

number was observed at  $m/z$  986  $[M + H]^+$ , besides significant peaks at  $m/z$  882, 720, 736, 576, 442, and 397 (6) (Figure 1). The above results of mass spectra confirm the  $[M]^+$  of **1** to be 985, and it has a fragmentation pattern which is consistent with the sequential loss of four sugars from a solasodine aglycone.

assigned to the inner-located and outer-located terminal xylosyl carbons, respectively, by comparison with the chemical shift values of methyl- $\beta$ -D-xylopyranoside (9). Therefore, the structure of **1** was elucidated as  $O$ -(3)- $\{\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3<sub>glu</sub>)- $[\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4<sub>rha</sub>)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4<sub>glu</sub>)]-

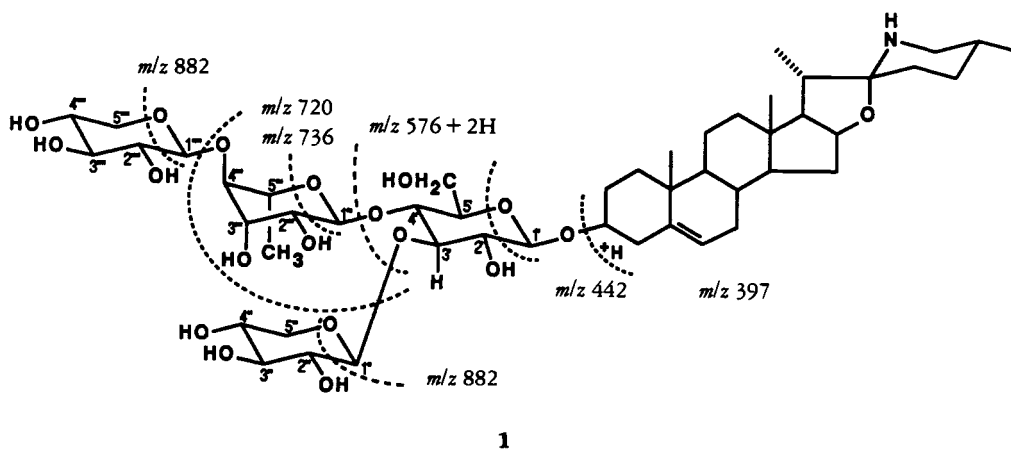


FIGURE 1. Mass spectral fragmentation of compound **1**.

The  $^1\text{H}$ -nmr spectrum of **1** showed four anomeric protons at  $\delta$  4.45 (d,  $J = 7.5$  Hz), 4.63 (d,  $J = 7$  Hz), 4.70 (br s), and 5.19 (br s), and the  $^{13}\text{C}$ -nmr spectrum of **1** (Table 1) showed four kinds of signals due to anomeric carbons at  $\delta$  100.1, 101.5, 104.6, and 104.8. In this latter spectrum, six carbon signals at  $\delta$  100.1, 74.1, 85.3, 78.2, 76.5, and 61.5 were reasonably assigned to glucosyl carbons, attached at the  $3\beta$ -hydroxyl group of solasodine, by comparison with the chemical shift values of glucosyl carbons of khasianine (7), solasodine-3- $O$ - $\beta$ -lycotetraoside (8), and methyl- $\alpha$ -L-rhamnopyranoside (9): six carbon signals at  $\delta$  101.5, 72.2, 73.3, 83.3, 71.1, and 18.0 were assigned to the rhamnosyl carbons by comparison with the chemical shift values of rhamnosyl carbons of desmonoterpenyl gleditsia saponin G (10) and methyl- $\alpha$ -L-rhamnopyranoside (9): and five carbon signals at  $\delta$  104.6, 74.1, 75.1, 70.5, and 69.2, and five carbon signals at  $\delta$  104.8, 76.7, 77.5, 71.3, and 69.3 were

$\beta$ -D-glucopyranosyl}-solasodine [1]. The cytotoxic activities of the isolates, solamargine, carpesterol, solasodine, ursolic acid, ursolic acid derivatives (3- $O$ -benzoyl ursolic acid, 3- $O$ -palmitoyl ursolic acid, 3- $O$ -cinnamoyl ursolic acid, 3- $O$ -crotonyl ursolic acid, and 3- $O$ -propionyl ursolic acid), and khasianine against human PLC/PRF/5 cells in vitro, and solasodine, ursolic acid, and khasianine against KB cells in vitro, were studied (11,12), and the results are listed in Table 2. Solamargine, solasodine, ursolic acid, and certain ursolic acid derivatives (3- $O$ -palmitoyl ursolic acid, 3- $O$ -crotonyl ursolic acid, and 3- $O$ -propionyl ursolic acid) exhibited significant inhibition of the human hepatoma PLC/PRF/5 cells, and solasodine and ursolic acid exhibited significant inhibition of KB cells. The data in Table 2 clearly indicate that the esterification of ursolic acid with aliphatic acids enhanced the cytotoxic effects against the human hepatoma PLC/PRF/5 cells in vitro.

TABLE 1.  $^{13}\text{C}$ -nmr Chemical Shifts (ppm) of Solamargine, Khasianine, and Compound 1.

Carbon	Solamargine <sup>a,b</sup>	Khasianine <sup>b</sup>	1 <sup>c</sup>
C-1	37.5	37.3	37.5
C-2	30.1	30.0	30.5
C-3	77.8	78.2	78.2
C-4	40.6	41.3	39.0
C-5	140.8	140.8	141.3
C-6	121.7	121.7	121.3
C-7	32.6	32.2	32.5
C-8	32.3	31.5	31.5
C-9	50.3	50.2	51.2
C-10	37.1	37.3	37.3
C-11	21.1	21.0	21.3
C-12	40.0	40.0	41.2
C-13	40.7	40.5	41.3
C-14	56.6	56.6	57.1
C-15	31.7	32.3	32.5
C-16	78.8	78.7	78.9
C-17	64.0	63.4	63.4
C-18	16.4	16.5	16.8
C-19	19.4	19.7	19.3
C-20	41.7	41.5	41.3
C-21	15.7	15.6	15.1
C-22	98.3	98.3	<sup>d</sup>
C-23	34.5	34.6	32.6
C-24	31.0	31.0	30.5
C-25	31.7	31.5	30.6
C-26	47.7	48.0	47.5
C-27	19.6	19.3	19.3
C-1'	100.2	102.6	100.1
C-2'	78.1	75.4	74.1
C-3'	76.8	77.0	85.3
C-4'	77.8	78.7	78.2
C-5'	76.8	76.6	76.5
C-6'	61.3	61.2	61.5
C-1''	101.9	102.4	104.6
C-2''	72.7	72.7	74.3
C-3''	72.4	72.7	75.1
C-4''	73.8	73.9	70.5
C-5''	69.4	70.3	69.2
C-6''	18.4	18.5	
C-1'''	102.8		101.5
C-2'''	72.4		72.2
C-3'''	72.4		73.3
C-4'''	74.1		83.3
C-5'''	70.4		71.1
C-6'''	18.6		18.0
C-1''''			104.8
C-2''''			76.6
C-3''''			77.5
C-4''''			71.3
C-5''''			69.3

<sup>a</sup>Data in this column are from Murakami *et al.* (8).

<sup>b</sup>Measured in pyridine-*d*<sub>5</sub>.

<sup>c</sup>Measured in CD<sub>3</sub>OD + CDCl<sub>3</sub>.

<sup>d</sup>Not observed.

TABLE 2. Cytotoxicities<sup>a</sup> of *Solanum incanum* Compounds and Their Derivatives Against Various Tumor Cells [ED<sub>50</sub> (ug/ml), N = 4].

Compound	Cell line	
	PLC/PRF/5	KB
Solamargine	1.53	
Carpesterol	8.47	
Khasianine	8.60	
Solasodine	3.75	3.74
Ursolic acid	3.89	3.76
3-O-Benzoyl ursolic acid	6.89	
3-O-Palmitoyl ursolic acid	3.54	
3-O-Cinnamoyl ursolic acid	7.24	
3-O-Crotonyl ursolic acid	2.59	
3-O-Propionyl ursolic acid	2.54	

<sup>a</sup>For significant activity of the pure compound, an ED<sub>50</sub> ≤ 4.0 μg/ml is required (13).

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—All mps are uncorrected. Ft-nmr spectra were recorded on a VXR-300/51 Superconducting High Resolution FT NMR System, ir spectra on a Hitachi model 260-30; ms on a JMS-HX 110 Mass Spectrometer, and optical rotation on a Jasco model dip-181 digital polarimeter.

**EXTRACTION AND SEPARATION.**—Fresh berries (10 kg) of *S. incanum* were collected at Tainan, Taiwan, in July 1986, chipped and extracted several times with MeOH. A voucher specimen is deposited in our laboratory. The combined MeOH extracts were concentrated to dryness under reduced pressure, and the residue was chromatographed on Si gel. Elution with CHCl<sub>3</sub>-MeOH (9:1) yielded stigmaterol-β-D-glucoside. Elution with CHCl<sub>3</sub>-MeOH (5:1) gave khasianine. Elution with CHCl<sub>3</sub>-MeOH (1:1) gave 1. Stigmaterol-β-D-glucoside and khasianine were identified by [α]<sub>D</sub>, ir, nmr, ms, hydrolysis, and comparison of the mmp and spectral data of aglycones with those of the authentic samples.

**INCANUMINE [1].**—Colorless powder (MeOH): mp > 300°, [α]<sub>D</sub><sup>20</sup> + 78° (c = 0.1, MeOH), eims *m/z* (rel. int.) 576 (3), 414 (9), 296 (6), 258 (9), 138 (12), 114 (10), 18 (100); fabms positive mode *m/z* (rel. int.) 986 (11), 940 (11), 904 (61), 882 (87), 742 (17), 736 (6), 720 (29), 576 (3), 442 (7), 397 (9), 165 (100), 139 (75), 114 (14); ir ν max KBr cm<sup>-1</sup> 3400; <sup>1</sup>H nmr (CD<sub>3</sub>OD + CDCl<sub>3</sub>) δ 0.81 (3H, s, 18-Me), 0.86 (3H, d, *J* = 6 Hz, 27-Me), 1.00 (3H, d, *J* = 8 Hz, 21-Me), 1.01 (3H, s, 19-Me), 1.19 (3H, d, *J* = 7.5 Hz, rhamnosyl Me), 4.45 (1H, d, *J* = 7.5 Hz, anomeric H), 4.63 (1H, d, *J* = 7.5 Hz, anomeric H), 4.70 (1H, br s, anomeric H), 5.19

(1H, br s, anomeric H), 5.38 (1H, m, 6H);  $^{13}\text{C}$  nmr ( $\text{CD}_3\text{OD} + \text{CDCl}_3$ ) see Table 1. Compound **1** was hydrolyzed to give solasodine as colorless needles, mp 201–202°; the mmp, ir, nmr, ms were identified as those of authentic solasodine. The sugar portion was examined by tlc [solvent  $\text{CHCl}_3\text{-MeOH-Me}_2\text{CO-H}_2\text{O}$  (3:3:3:1) on Si gel] to detect methyl glucopyranoside ( $R_f$  0.59), methyl rhamnopyranoside ( $R_f$  0.81), and methyl  $\alpha,\beta$ -D-xylopyranoside ( $R_f$  0.42, 0.47).

3-O-BENZOYL URSOLIC ACID, 3-O-PALMITOYL URSOLIC ACID, 3-O-CINNAMOYL URSOLIC ACID, 3-O-CROTONYL URSOLIC ACID, AND 3-O-PROPIONYL URSOLIC ACID.—These compounds were prepared by treatment of ursolic acid with benzoyl chloride, palmitoyl chloride, cinnamoyl chloride, crotonyl anhydride, and propionyl anhydride, respectively, in pyridine, and worked up in the usual way. All the products were identified by ir and  $^{13}\text{C}$  nmr spectra.

BIOLOGICAL ASSAYS.—PLC/PRF/5 cells were established from human hepatoma and are known to produce hepatitis B surface antigen continuously in culture fluids (11). The cells were grown as continuous cultures in a growth medium consisting of Dulbecco's modified Eagle medium (DMEM, GIBCO, Grand Island, NY), 10% fetal bovine serum (FBS, GIBCO), 100 IU/ml streptomycin and 2 mM L-glutamine. The KB cells were maintained on DMEM (GIBCO) containing 10% FBS, L-glutamine, and antibiotics. For microassays, the growth medium was supplemented further with 10 mM HEPES buffer, pH 7.3. The microassay for anticellular effect was performed as previously described (12).

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