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Glycoalkaloids and Metabolites Inhibit the Growth of Human Colon (HT29) and Liver (HepG2) Cancer Cells

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As part of an effort to improve plant-derived foods such as potatoes, eggplants, and tomatoes, the antiproliferative activities against human colon (HT29) and liver (HepG2) cancer cells of a series of structurally related individual compounds were examined using a microculture tetrazolium (MTT) assay. The objective was to assess the roles of the carbohydrate side chain and aglycon part of *Solanum* glycosides in influencing inhibitory activities of these compounds. Evaluations were carried out with four concentrations each (0.1, 1, 10, and 100 μg/mL) of the the potato trisaccharide glycoalkaloids α-chaconine and α-solanine; the disaccharides β_1-chaconine, β_2-chaconine, and β_2-solanine; the monosaccharide γ-chaconine and their common aglycon solanidine; the tetrasaccharide potato glycoalkaloid dehydrocommersonine; the potato aglycon demissidine; the tetrasaccharide tomato glycoalkaloid α-tomatine, the trisaccharide β_1-tomatine, the disaccharide γ-tomatine, the monosaccharide δ-tomatine, and their common aglycon tomatidine; the eggplant glycoalkaloids solamargine and solasonine and their common aglycon solasodine; and the nonsteroidal alkaloid jervine. All compounds were active in the assay, with the glycoalkaloids being the most active and the hydrolysis products less so. The effectiveness against the liver cells was greater than against the colon cells. Potencies of α-tomatine and α-chaconine at a concentration of 1 μg/mL against the liver carcinoma cells were higher than those observed with the anticancer drugs doxorubicin and camptothecin. Because α-chaconine, α-solanine, and α-tomatine also inhibited normal human liver HeLa (Chang) cells, safety considerations should guide the use of these compounds as preventative or therapeutic treatments against carcinomas.

KEYWORDS: Colon cancer cells; liver cancer cells; growth inhibition; glycoalkaloids; structure−activity relationships

INTRODUCTION

Previous studies have shown that (a) the glycoalkaloid β-solamarine present in the folk medicine *Solanum dulcamara* inhibited sarcoma tumors in mice (*1*); (b) solamargine and solasonine isolated from *Solanum sodomaeum* were effective treatments of malignant human skin tumors including basal and squamous cell carcinomas (*2*, *3*); (c) solamargine and solasonine were more toxic to human cancer cells than to other cell types (*4*); (d) solamargine from *Solanum nigrum* was cytotoxic to human solid tumor cell lines (*5*); (e) the anticarcinogenic action of solamargine (isolated from fresh berries of the Chinese herb *Solanum incanum*) on human hepatoma cells (Hep3B) is the result of cell death by apoptosis (programmed cell death) (*6*); (f) tomatidine inhibited the resistance of cancer cells to drugs (*6*); and (f) solasonine from *Solanum crinitum* and *Solanum jabrense* was cytotoxic against Ehrlich carcinoma and human K562 leukemia cells (*7*).

In previous studies (*8*−*12*), we found that the cytotoxicity of potato glycoalkaloids against frog embryos was influenced by the chemical structure of the carbohydrate, galactose, glucose, or rhamnose, the number of carbohydrate groups of the side chain attached to the 3-OH position of the aglycons, and the structure of the aglycon.

Because some of the hydrolysis products (metabolites) of glycoalkaloids are found in plant tissues and are formed during normal digestion and metabolism of the parent glycoalkaloids after consumption, it was of interest to compare the relative potencies of the glycoalkaloids with zero, one, two, three, and four sugar groups attached to the 3-position of the aglycons. The main objective of this study was to evaluate the inhibition

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of growth of human colon and liver cancer cells induced by structurally different glycoalkaloids and hydrolysis products, which may be present in edible plant foods or formed after consumption, digestion, and metabolism of the parent glycoalkaloids by animals and humans. The results should make it possible to better focus on possible relationships among structural features of the test compounds and their ability to inhibit the growth of cancer cells. For comparison, we also evaluated the inhibition of hepatoma cells by two cancer drugs and that of normal human liver cells by three glycoalkaloids.

MATERIALS AND METHODS

Materials. α-Chaconine, α-solanine, solasonine, demissidine, α-tomatine, tomatidine, solanidine, dehydrocommersonine, jervine, doxorubicin (adriamycin), and camptothecin were obtained from Sigma (St. Louis, MO). α-Solamargine was a gift of Prof. A. E. de Almeida. β_1-Chaconine, β_2-chaconines, γ-chaconine, β_2-solanine, β_1-tomatine, γ-tomatine, and δ-tomatine were isolated from a partial hydrolysis mixture of the parent glycoalkaloids and characterized by HPLC and mass spectrometry (*13*, *14*).

Cell Lines and Cell Cultures. Human hepatoma HepG2 cells and human colon carcinoma HT29 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD) and from Korean Cell Line Bank (KCLB, Seoul, Korea), respectively. The normal human HeLa (Chang) cells were a gift of Prof. Kim Jun Ae of the Division of Pharmacology, Yeungnam University, Gyonsan, Korea. The cells were maintained in an MEM medium supplemented with 10% of fetal bovine serum, 50 units/mL penicillin, and 50 mg/mL streptomycin, at 37 °C in a 5% CO_2 incubator. All cell culture reagents were obtained from GibcoBRL (Life Technologies, Cergy-Pontoise, France). Each sample was dissolved in DMSO as a stock solution of 2 mg/200 μL and stored at −4 °C.

Microculture Tetrazolium (MTT) Assay for Growth Inhibition of Cells. The MTT assay is based on the principle that MTT is a water-soluble tetrazolium salt (*15*). Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring induced by dehydrogenase enzymes present in viable cells. Dead cells do not cause this change. The following reagents and instruments were used: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl terazolium bromide; MTT reagent, 5 mg/mL in phosphate-buffered saline, protected from light, and stored −20 °C; MEM medium (containing 10% fetal bovine serum and 1% penicillin−streptomycin); microplate reader (Bio-Rad Co., Hercules, CA).

The assay was carried out as follows. HT29 and HepG2 cells were seeded into a 96-well microplate (5×10^4 cells/well) and incubated for 24 h to allow them to adhere to the plates. Then, cells were treated with four concentrations each of the test compounds (0.1, 1, 10, and 100 μg/mL). MTT (final concentration = 0.5 mg/mL) was then added into each well. After incubation for various time periods at 37 °C, 200 μL of DMSO was added into each well. The optical density (OD) was then read at a wavelength of 540 nm.

Statistical Methods. The optical densities were transformed by natural logarithms prior to analyses of variance to stabilize the variance among compounds and concentrations. One-way analyses of variance (ANOVA) were used along with Dunnett's one-tailed test for decreases from the control (*16*). For the experiments with multiple compounds, the analyses were run separately for each concentration and the control was included for each run. A footnote to the tables identifies the statistical test, Dunnett's test, and the significance level.

RESULTS AND DISCUSSION

Structures of Glycoalkaloids and Metabolites. Figure 1 depicts the structures of the 18 plant-derived compounds evaluated in this study. Members of the Solanaceae family of plants synthesize secondary metabolites including glycoalkaloids to protect themselves against phytopathogens (*17*−*19*). These plants include potatoes, tomatoes, and eggplants. α-Chaconine and α-solanine are the two major glycoalkaloids in commercial potato varieties (*Solanum tuberosum*). Solamargine and solasonine are two major glycoalkaloids found in eggplants (*Solanum melongena*) and many other nonfood *Solanum* plants (*20*). Structurally, glycoalkaloids consist of two parts, a nitrogen-containing steroid (aglycon) and a carbohydrate side chain. α-Chaconine and solamargine have a chacotriose structure (a branched bis-α-L-rhamnopyranosyl-β-glucopyronose), and the side chain attached to α-solanine and solasonine has a solatriose structure (α-L-rhamnopyranosyl-β-glucopyranosyl-β-galactopyranose). The two carbohydrate side chains of the two potato glycoalkaloids are identical to the corresponding side chains in the two eggplant glycoalkaloids. Thus, the eggplant glycoalkaloids differ from those found in potatoes only in the structure of the steroidal part of the molecules. The carbohydrate side chain called a lycotetraose attached to the tomato glycoalkaloid α-tomatine consists of four sugar residues (**Figure 1**), as does the commertetraose side chain attached to the potato glycoalkaloid dehydrocommersonine present in some potato cultivars (*18*, *21*).

Cell Proliferation Tetrazolium Assay. The MTT assay we adopted for this study is based on measurements of in vitro growth in microculture wells by human cell-mediated reduction of tetrazolium. The assay is now widely used for screening the cytotoxicity of potential anticancer agents (*15*). The determined concentration of formazan produced is directly proportional to the number of viable cells.

Factors Governing Growth Inhibition. Below, we examine the time course of the inhibition and structure−inhibitory activity relationships of the test compounds.

Effect of Time. **Table 1** shows the extent of inhibition of the two cell lines after exposure to low doses (0.1, 0.5, 1, 5, and 10 μg/mL) of chaconine, α-solanine, and α-tomatine for 4, 24, and 48 h. Because inhibition of cell growth after 4 h did not significantly differ from that observed with the longer time periods, the 4 h period was used for the comparative studies of the test compounds shown in **Table 2**. Below we examine in some detail the relationship of the structure of the test compounds to inhibitory activities of the two tumor cell lines and one normal cell line.

Potato Glycoalkaloids and Hydrolysis Products. The experimental values for the growth inhibition of the two cancer cell lines are shown in **Table 2**. The table contains data for a series of experiments, each with its own control for the growth inhibition induced by four concentrations: 0.1, 1, 10, and 100 μg/mL. The differences in control values for the separate experiments do not affect the extent of inhibition of cell growth based on control values determined individually for each experiment.

The growth inhibition of the colon cells by the potato glycoalkaloid α-chaconine at 0.1, 1, 10, and 100 μg/mL was from 0, 66.4, 77.3, and 81.4%, respectively. For the liver cells, the corresponding values were 39.5, 83.7, 83.3, and 87.6%, respectively. These results show that (a) α-chaconine is a potent inhibitor of both cell lines; (b) although the inhibition of both cell lines generally increases with concentration, the increase does not appear to be a linear function of the concentration; and (c) the inhibition of the liver cells is greater than that of the colon cells. The 39.5% inhibition of the liver cells at the very low concentration of 0.1 μg/mL is striking.

Hydrolytic cleavage of one of the sugar residues from the trisaccharide side chain of α-chaconine can occur at two positions, resulting in two isomeric disaccharide derivatives, β_1-chaconine and β_2-chaconine (**Figure 1**). **Table 2** shows that compared to α-chaconine (a) both compounds exhibited similar

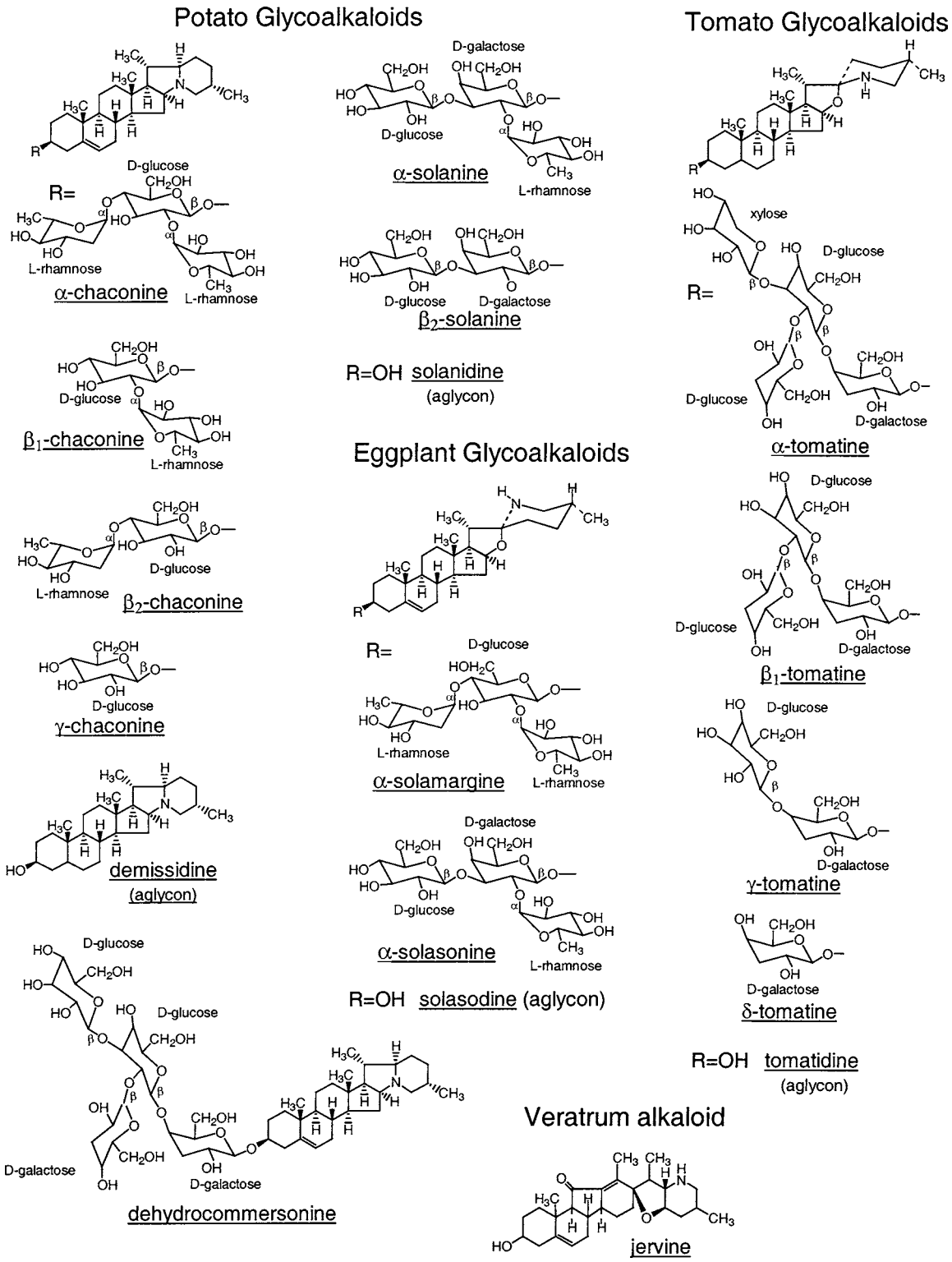

Figure 1. Structures of the 18 compounds evaluated in this study. See text.

activity against both cell lines at the 100 μg/mL concentration, (b) the activity at the lower concentrations was much lower against both cell lines, and (c) β_1- and β_2-chaconines were inactive at the 0.1 and 1 μg/mL levels.

Hydrolytic removal of two carbohydrate residues from α-chaconine results in the formation of the monosaccharide γ-chaconine. **Table 2** shows that (a) the inhibition by γ-chaconine of the colon cells was 0% at both the 0.1 and 1 μg/mL levels and 12.8 and 19.0% at the 10 and 100 μg/mL levels, respectively; and (b) the corresponding inhibition values of the liver cells by the four concentrations are 17.1, 20.5, 76.3, and 80.7%, respectively. These results indicate that γ-chaconine exhibits low activity against the colon cells in contrast to the high activity against the liver cells. The activity of γ-chaconine against the liver cells is greater than those mentioned for β_1-chaconine and β_2-chaconine and approaches that of α-chaconine.

Potatoes contain a second trisaccharide glycoalkaloid, called α-solanine. Both compounds share the same aglycon but differ in the nature of the carbohydrate side chains attached to the aglycon (**Figure 1**). **Table 2** shows that compared to α-chaco-

Table 1. Effect of Time on Growth Inhibition of HepG2 Carcinoma Cells by Low-Dose Concentrations of α-Chaconine, α-Solanine, and α-Tomatine

		4 h		24 h		48 h	
sample	concn (μg/mL)	OD[a]	growth inhibition (%)	OD[a]	growth inhibition (%)	OD[a]	growth inhibition (%)
control		1.44 ± 0.107		0.99 ± 0.055		0.96 ± 0.018	
α-chaconine	10	0.20 ± 0.003	86.1	0.17 ± 0.007	82.8	0.12 ± 0.001	87.5
	5	0.41 ± 0.05	71.5	0.15 ± 0.003	84.8	0.13 ± 0.003	86.5
	1	0.70 ± 0.02	51.4	0.19 ± 0.005	80.8	0.13 ± 0.002	86.5
	0.5	1.56 ± 0.065*	–8.3	1.06 ± 0.061*	–7.1	1.13 ± 0.114*	–17.7
	0.1	1.54 ± 0.03*	–6.9	1.12 ± 0.032*	–13.1	1.13 ± 0.114*	–17.7
α-solanine	10	0.18 ± 0.019	87.5	0.18 ± 0.003	81.8	0.14 ± 0.004	85.4
	5	0.37 ± 0.04	74.3	0.16 ± 0.003	83.8	0.13 ± 0.002	86.5
	1	1.54 ± 0.009*	–6.9	1.08 ± 0.036*	–9.1	1.02 ± 0.094*	–6.3
	0.5	1.56 ± 0.062*	–8.3	1.06 ± 0.026*	–7.1	1.08 ± 0.03*	–12.5
	0.1	1.55 ± 0.064*	–7.6	1.01 ± 0.003*	–2.0	0.99 ± 0.038*	–3.1
α-tomatine	10	0.15 ± 0.003	89.6	0.13 ± 0.005	86.9	0.13 ± 0.004	86.5
	5	0.20 ± 0.021	86.1	0.14 ± 0.006	85.9	0.14 ± 0.005	85.4
	1	0.55 ± 0.037	61.8	0.22 ± 0.024	77.8	0.17 ± 0.009	82.3
	0.5	0.65 ± 0.014	54.9	0.31 ± 0.019	68.7	0.2 0± 0.019	79.2
	0.1	1.07 ± 0.034	25.7	0.39 ± 0.004	60.6	0.33 ± 0.045	65.6

[a] Values are means ± SD ($n = 3$). An asterisk (*) indicates no significant difference at the 5% level from respective control using Dunnett's one-tailed test for a decrease in the OD value (number of cells).

nine (a) the inhibitory activity of α-solanine at the 100 μg/mL level was similar for both cell lines and (b) the inhibition of both cell lines by α-solanine at the lower concentrations was lower. These results suggest that the nature of the carbohydrate can affect cytotoxicity.

Hydrolytic removal of one glucose residue from α-solanine results in the formation of the disaccharide β_2-solanine. **Table 2** shows that compared to α-solanine, the inhibitory activity of the disaccharide was about the same at the 100 μg/mL concentration for both cell lines and about 40% lower at the 10 μg/mL level with the liver cells and 84% lower for the colon cells.

Hydrolytic removal of all three sugar residues from α-chaconine results in the formation of the aglycon solanidine, which lacks a carbohydrate side chain (**Figure 1**). **Table 2** shows that (a) the inhibition of the colon cells by solanidine was 0% at both the 0.1 and 1 μg/mL levels and 32.5 and 80.9% at the 10 and 100 μg/mL levels, respectively, and (b) the corresponding inhibition of the liver cells with the four concentrations was higher, 0, 9.5, 71.5, and 82.2%, respectively. These results indicate that the activity of solanidine at the 100 μg/mL level was within the range observed with α-chaconine. At the lower concentrations, the inhibition by solanidine was similar to that of β_1- and β_2-chaconines. The data on structure−activity relationships of series also demonstrate that although the nature and number of sugar residues of the side chains influence activities, the aglycon without a carbohydrate side chain is also active against both cell lines at concentrations >1 μg/mL. It is also relevant to note that solanidine, but not the glycoalkaloids, exhibited in vitro estrogenic activity (*22*).

Dehydrocommersonine, Demissidine, and Jervine. To assess possible relationships between chemical structure and inhibitory activity, we included in this study the potato glycoalkaloid dehydrocommersonine present in some wild potato cultivars (*19, 23*), the aglycon demissidine, derived from the glycoalkaloid demissine also present in some potato varieties (*24*), and the nonsteroidal *Veratrum* alkaloid jervine (*25*). **Table 2** shows that dehydrocommersonine was highly active in the assay, demissidine less so, and the activity of jervine was low. These observations suggests that the steroidal moieties of both glycoalkaloids and aglycons are key structural features governing activities.

Tomatine and Hydrolysis Products. The tomato glycoalkaloid α-tomatine has a tetrasaccharide side chain attached to the aglycon tomatidine (**Figure 1**). Commercial tomatine used in this study is a ~10:1 mixture of α-tomatine and dehdyrotomatine (*26*, *27*). Tomatine appears to be the strongest inhibitor of both cell lines, as evidenced by the inhibition of colon cancer cells ranging from 38.0 to 81.5%. The corresponding values for the inhibition of the liver cancer cells are greater at each concentration, ranging from 46.3 to 89.2%.

Hydrolytic removal of sugar groups from the tetrasaccharide results in the formation of β_1-tomatine, γ-tomatine, and δ-tomatine, with three, two, and one sugar, respectively (*14*, *17*). **Table 2** shows that the activities of the three hydrolysis products were less than that of the parent glycoalkaloid at the lower concentrations. Activities at the highest concentration of 100 μg/mL approached that of tomatine.

Table 2 also shows that the inhibitory activity of the aglycon tomatidine is significantly lower than that of α-tomatine, ranging from 0 to 19.7% for the colon carcinoma cells and from 0.1 to 28.6% for the liver carcinoma cells.

These results imply that in vivo acid- or enzyme-catalyzed partial hydrolysis of tomatine sugar moieties may result in retention of most of the activity against cancer cells. However, this may not be the case if the glycolysis proceeds to completion to form the aglycon tomatidine.

Solamargine, Solasonine, and Solasodine. Eggplants and numerous so-called medicinal nonfood plants as well as herbs contain the glycoalkaloids solamargine and solasonine (*28*). The trisaccharide moieties of these two glycoalkaloids attached to their common aglycon solasodine are the same as those present in α-chaconine and α-solanine, respectively (**Figure 1**).

Table 2 shows that (a) the inhibitory activity of solamargine against both cell lines at the 1, 10, and 100 μg/mL concentrations is generally similar to that of solasonine; (b) inhibition induced by 0.1 μg/mL of solamargine was 0% for both cell lines compared to the 34.7 and 25.6% inhibition by solasonine at this low concentration with the colon and liver cells, respectively; and (c) the activity of the aglycon solasodine is the same

Table 2. Inhibitory Effect of Glycoalkaloids and Hydrolysis Products (Metabolites) on the Growth of HT29 Colon and HepG2 Liver Carcinoma Cells after 4 h

	colon HT29 cancer cells		liver HepG2 cancer cells	
sample	cell growth[a] (OD)	growth inhibition (%)	cell growth[a] (OD)	growth inhibition (%)
control	1.247 ± 0.066		1.037 ± 0.043	
α-chaconine				
100 μg/mL	0.232 ± 0.013	81.4	0.129 ± 0.001	87.6
10 μg/mL	0.283 ± 0.008	77.3	0.173 ± 0.001	83.3
1 μg/mL	0.419 ± 0.025	66.4	0.169 ± 0.005	83.7
0.1 μg/mL	1.337 ± 0.003*	–7.1	0.628 ± 0.049	39.5
β_1-chaconine				
100 μg/mL	0.213 ± 0.004	82.9	0.141 ± 0.006	86.4
10 μg/mL	0.642 ± 0.082	48.5	0.581 ± 0.017	44.0
1 μg/mL	1.204 ± 0.074*	3.5	0.860 ± 0.032	17.1
0.1 μg/mL	1.290 ± 0.039*	–3.4	0.746 ± 0.026	28.1
β_2-chaconine				
100 μg/mL	0.218 ± 0.005	82.5	0.142 ± 0.002	86.3
10 μg/mL	0.579 ± 0.030	53.6	0.645 ± 0.032	37.8
1 μg/mL	1.265 ± 0.053*	–1.4	0.959 ± 0.067*	7.5
0.1 μg/mL	1.259 ± 0.073*	–0.9	1.081 ± 0.029*	–4.2
γ-chaconine				
100 μg/mL	1.011 ± 0.038	19.0	0.200 ± 0.044	80.7
10 μg/mL	1.087 ± 0.048	12.8	0.246 ± 0.036	76.3
1 μg/mL	1.277 ± 0.050*	–2.4	0.825 ± 0.095	20.5
0.1 μg/mL	1.251 ± 0.030*	–0.3	0.860 ± 0.038	17.1
solanidine				
100 μg/mL	0.239 ± 0.017	80.9	0.185 ± 0.011	82.2
10 μg/mL	0.842 ± 0.058	32.5	0.296 ± 0.024	71.5
1 μg/mL	1.259 ± 0.028*	–1.0	0.939 ± 0.099	9.5
0.1 μg/mL	1.324 ± 0.089*	–6.1	1.024 ± 0.090*	1.3
control	1.509 ± 0.092		1.227 ± 0.060	
α-solanine				
100 μg/mL	0.230 ± 0.011	84.7	0.135 ± 0.011	89.0
10 μg/mL	0.276 ± 0.020	81.7	0.234 ± 0.014	80.9
1 μg/mL	0.870 ± 0.094	42.4	0.958 ± 0.036	21.9
0.1 μg/mL	1.511 ± 0.069*	–0.1	1.283 ± 0.016*	–4.5
control	1.260 ± 0.189		1.013 ± 0.179	
β_2-solanine				
100 μg/mL	0.347 ± 0.007	72.4	0.176 ± 0.007	82.6
10 μg/mL	0.613 ± 0.008	51.4	0.878 ± 0.029*	13.3
1 μg/mL	1.178 ± 0.097*	6.5	1.055 ± 0.091*	–4.1
0.1 μg/mL	1.087 ± 0.1*	13.7	0.996 ± 0.038*	1.7
control	1.26 ± 0.189		1.013 ± 0.179	
dehydrocommersonine				
100 μg/mL	0.362 ± 0.018	71.2	0.168 ± 0.004	83.4
10 μg/mL	0.555 ± 0.018	56.0	0.212 ± 0.01	79.1
1 μg/mL	0.678 ± 0.022	46.2	0.222 ± 0.005	78.1
0.1 μg/mL	1.217 ± 0.198*	3.4	1.096 ± 0.219*	–8.2
control	1.049 ± 0.073		0.820 ± 0.029	
demissidine				
100 μg/mL	0.731 ± 0.045	30.3	0.355 ± 0.030	56.8
10 μg/mL	0.887 ± 0.051	15.5	0.427 ± 0.008	48.0
1 μg/mL	0.913 ± 0.077	13.0	0.787 ± 0.028*	4.1
0.1 μg/mL	0.922 ± 0.108*	12.1	0.713 ± 0.058	13.1
control	1.266 ± 0.058		1.227 ± 0.060	
α-tomatine				
100 μg/mL	0.234 ± 0.003	81.5	0.132 ± 0.000	89.2
10 μg/mL	0.360 ± 0.015	71.6	0.178 ± 0.005	85.5
1 μg/mL	0.508 ± 0.036	59.9	0.243+0.040	80.2
0.1 μg/mL	0.785 ± 0.029	38.0	0.658 ± 0.031	46.3
control	1.26 ± 0.189		1.013 ± 0.179	
β_1-tomatine				
100 μg/mL	0.377 ± 0.014	70.1	0.171 ± 0.003	83.1
10 μg/mL	0.613 ± 0.034	51.3	0.194 ± 0.005	80.9
1 μg/mL	1.174 ± 0.093*	6.9	0.884 ± 0.103*	12.7
0.1 μg/mL	1.166 ± 0.125*	7.5	1.044 ± 0.013*	–3.1
γ-tomatine				
100 μg/mL	0.536 ± 0.036	57.5	0.29 ± 0.031	71.4
10 μg/mL	1.039 ± 0.018	17.5	0.833 ± 0.102	17.8
1 μg/mL	1.153 ± 0.197*	8.5	1.264 ± 0.117*	–24.8
0.1 μg/mL	1.223 ± 0.15*	3.0	1.053 ± 0.243*	–3.9

Table 2. (Continued)

sample	colon HT29 cancer cells: cell growth[a] (OD)	colon HT29 cancer cells: growth inhibition (%)	liver HepG2 cancer cells: cell growth[a] (OD)	liver HepG2 cancer cells: growth inhibition (%)
δ-tomatine				
100 μg/mL	0.484 ± 0.031	61.6	0.173 ± 0.006	82.9
10 μg/mL	0.929 ± 0.214	26.3	0.478 ± 0.03	52.8
1 μg/mL	1.325 ± 0.055*	–5.2	1.068 ± 0.176*	–5.5
0.1 μg/mL	1.356 ± 0.105*	–7.6	1.225 ± 0.056*	–20.9
control	1.151 ± 0.063		1.124 ± 0.0680	
tomatidine				
100 μg/mL	0.924 ± 0.011	19.7	0.802 ± 0.144	28.6
10 μg/mL	1.019 ± 0.103	11.4	0.800 ± 0.011	28.8
1 μg/mL	1.182 ± 0.026*	–2.7	1.109 ± 0.002*	1.3
0.1 μg/mL	1.244 ± 0.026*	–8.1	1.117 ± 0.074*	0.6
control	1.049 ± 0.073		0.820 ± 0.029	
solamargine				
100 μg/mL	0.189 ± 0.021	82.0	0.134 ± 0.003	83.6
10 μg/mL	0.296 ± 0.003	71.8	0.152 ± 0.004	81.4
1 μg/mL	0.746 ± 0.049	28.9	0.329 ± 0.048	59.9
0.1 μg/mL	1.035 ± 0.059*	1.3	0.777 ± 0.033*	5.2
solasonine				
100 μg/mL	0.194 ± 0.003	81.5	0.150 ± 0.002	81.7
10 μg/mL	0.331 ± 0.009	68.4	1.170 ± 0.003	79.3
1 μg/mL	0.661 ± 0.026	37.0	0.646 ± 0.026	21.3
0.1 μg/mL	0.685 ± 0.095	34.7	0.611 ± 0.013	25.6
solasodine				
100 μg/mL	0.241 ± 0.008	77.0	0.178 ± 0.007	78.3
10 μg/mL	0.472 ± 0.023	55.0	0.174 ± 0.004	78.3
1 μg/mL	0.763 ± 0.085	27.2	0.379 ± 0.016	53.8
0.1 μg/mL	0.846 ± 0.086	19.4	0.682 ± 0.004	16.9
control	1.26 ± 0.189		1.013 ± 0.179	
jervine				
100 μg/mL	1.063 ± 0.07	15.6	0.667 ± 0.063	34.1
10 μg/mL	1.033 ± 0.072	18.0	0.858 ± 0.088*	15.3
1 μg/mL	1.21 ± 0.113*	4.0	1.15 ± 0.109*	–13.5
0.1 μg/mL	1.32 ± 0.106*	–4.8	1.297 ± 0.094*	–28.1

[a] Values are means ± SD ($n = 3$) except for solasonine and α-tomatine ($n = 6$). An asterisk (*) indicates no significant difference at the 5% level from respective control using Dunnett's one-tailed test for a decrease in the OD value (number of cells).

as the activities of the glycoalkaloid solasonine at all four concentrations. Evidently, unlike the potato glycoalkaloids, the trisaccharide side chain of these glycosides does not appear to be required for the inhibition.

Comparison with Doxorubicin and Camtothecin. The drugs doxorubicin and camptothecin are currently used to treat cancer patients (*29*, *30*). It was therefore of interest to compare the potencies of these two drugs to the plant-derived compounds. A preliminary study showed that the inhibitory effects of both drugs on growth of the liver hepatoma cells at the concentration of 1 μg/mL (46.8 and 61.0%, respectively) were lower than those observed with α-chaconine and α-tomatine (80.8 and 77.8%, respectively). Because the two classes of compounds exert their effects by different mechanisms (the glycoalkaloids disrupt cell membranes and the anticancer drugs intercalate with DNA), combinations of anticancer drugs with plant glycoalkaloids may exert synergistic effects in cells and tissues.

Growth Inhibition of Normal Human Liver HeLa (Chang) Cells. To find out whether the inhibition of cell growth by the glycoalkaloids is selective for cancer cells, we also examined the effect of exposing a normal liver cell line to α-chaconine, α-solanine, and α-tomatine for 48 h. The data in **Table 3** show that these compounds also inhibited the growth of the normal cells. The lack of an apparent differential effect on carcinoma and normal liver cells implies that in addtion to efficacy, safety considerations should govern possible therapeutic uses of the plant compounds. Normal colon cells were not available to us for comparison.

Table 3. Inhibitory Effects of α-Tomatine, α-Solanine, and α-Chaconine on the Growth of Normal HeLa (Chang) Human Liver Cells after 48 h

sample	concn (μg/mL)	OD[a]	growth inhibition (%)
control		1.432 ± 0.034	
α-tomatine	100	0.119 ± 0.005	91.7
	10	0.200 ± 0.007	86.1
	1	0.379 ± 0.022	73.6
	0.1	1.124 ± 0.105	21.5
α-solanine	100	0.115 ± 0.001	92.0
	10	0.184 ± 0.007	87.1
	1	1.253 ± 0.035	12.5
	0.1	1.267 ± 0.021	11.5
α-chaconine	100	0.113 ± 0.002	92.1
	10	0.216 ± 0.013	84.9
	1	0.482 ± 0.042	66.4
	0.1	1.459 ± 0.07*	–1.9

[a] Values are means ± SD ($n = 3$) except for control ($n = 6$). An asterisk (*) indicates no significant difference at the 5% level from respective control using Dunnett's one-tailed test for a decrease in the OD value (number of cells).

Significance for Medicine and Agriculture. Our results demonstrate that the potato glycoalkaloids α-chaconine and

α-solanine, the eggplant glycoalkaloids solamargine and solasonine, the tomato glycoalkaloid α-tomatine, and some of their hydrolysis products are potent inhibitors of human colon and liver carcinoma cells. In terms of relative potency, α-tomatine appears to be the most active compound, especially at the lower concentrations, among those evaluated in this study. Moreover, other studies have shown that orally consumed α-tomatine appears to be relatively nontoxic (it is ~20 times less toxic to mice than are the other glycoalkaloids) (*17*), that it lowers plasma low-density lipoprotein cholesterol and triglycerides in hamsters (*31*, *32*), and that it enhances the immune response by inducing cytokines in immunized animals (*33*). Tomatine and other glycoalkaloids are also reported to exhibit antibiotic activities against bacteria, fungi, and viruses (*17*, *34*−*37*). A key question for the possible use of the compounds evaluated in this study in cancer prevention and treatment should be the ratio of effective therapeutic to toxic dose for each of the plant-derived compounds (*2*, *22*, *38*, *39*). Studies are needed to define this ratio.

Informal guidelines recommend a maximum glycoalkaloid content of 20 mg/100 g of fresh potatoes (*19*). However, the glycoalkaloid content of most commercial potato varieties is much lower, ranging between ~2 and ~5 mg/100 g of fresh weight (*40*). Consumption of ~500 g of potatoes will therefore result in a dietary intake of 10−25 mg of glycoalkaloids. Whether these amounts can benefit human health is not known.

The content of tomato glycoalkaloids depends on the maturity of the tomatoes (*27*, *41*). Because the glycoalkaloids are enzymatically degraded as the tomato fruit matures on the vine, the content of widely consumed red tomatoes is low, ranging from ~1 to 3 mg/100 g. By contrast, the values range between ~20 and ~50 mg/100 g for sparsely consumed green tomatoes (e.g., pickled green tomatoes; Southern fried green tomatoes) (*42*). These considerations imply that it may benefit human health to create through plant breeding or molecular biology techniques red tomatoes with a high tomatine content.

NOTE ADDED AFTER ASAP

The page numbers for ref *27* were incorrect in the original posting of April 16, 2004. These were corrected on April 20, 2004.

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LITERATURE CITED

(1) Kupchan, S. M.; Barboutis, S. J.; Knox, J. R.; Lau Cam, C. A. *β*-Solamarine tumor inhibitor isolated from *Solanum dulcmara*. *Science* **1965**, *150*, 1827−1829.

(2) Cham, B. E.; Gilliver, M.; Wilson, L. Antitumor effects of glycoalkaloids isolated from *Solanum sodomaeum*. *Planta Med.* **1987**, *53*, 34−36.

(3) Cham, B. E. Solasodine glycosides as anti-cancer agents: preclinical and clinical studies. *Asia Pac. J. Pharmacol.* **1994**, *9*, 113−118.

(4) Daunter, B.; Cham, B. E. Solasodine glycosides. In vitro preferential toxicity for human cancer cells. *Cancer Lett.* **1990**, *55*, 209−220.

(5) Hu, K.; Kobyashi, H.; Dong, A.; Jing, Y.; Iwasaki, S.; Yao, X. Antineoplastic agents III: steroidal glycosides from *Solanum nigrum*. *Planta Med.* **1999**, *65*, 35−38.

(6) Kuo, K. W.; Hsu, S. H.; Li, Y. P.; Lin, W. L.; Liu, L. F.; Chang, L. C.; Lin, C. C.; Lin, C. N.; Sheu, H. M. Anticancer activity evaluation of the *Solanum* glycoalkaloid solamargine. Triggering apoptosis in human hepatoma cells. *Biochem. Pharmacol.* **2000**, *60*, 1865−1873.

(7) Esteves-Souza, A.; Sarmento da Silva, T. M.; Alves, C. C. F.; de Carvalho, M. G.; Braz-Filho, R.; Echevarria, A. Cytotoxic activities against Ehrlich carcinoma and human K562 leukemia of alkaloids and flavonoid from two *Solanum* species. *J. Braz. Chem. Soc.* **2002**, *13*, 838−842.

(8) Rayburn, J. R.; Bantle, J. A.; Friedman, M. Role of carbohydrate side chains of potato glycoalkaloids in developmental toxicity. *J. Agric. Food Chem.* **1994**, *42*, 1511−1515.

(9) Blankemeyer, J. T.; Atherton, R.; Friedman, M. Effect of potato glycoalkaloids α-chaconine and α-solanine on sodium active transport in frog skin. *J. Agric. Food Chem.* **1995**, *43*, 636−639.

(10) Blankemeyer, J. T.; White, J. B.; Stringer, B. K.; Friedman, M. Effect of α-tomatine and tomatidine on membrane potential of frog embryos and active transport of ions in frog skin. *Food Chem. Toxicol.* **1997**, *35*, 639−646.

(11) Blankemeyer, J. T.; McWilliams, M. L.; Rayburn, J. R.; Weissenberg, M.; Friedman, M. Developmental toxicology of solamargine and solasonine glycoalkaloids in frog embryos. *Food Chem. Toxicol.* **1998**, *36*, 383−389.

(12) Friedman, M.; Craig, C. F.; Butchko, C. A.; Blankemeyer, J. T. Folic acid protects against potato glycoalkaloid α-chaconine-induced disruption of frog embryo cell membranes and developmental toxicity. *J. Agric. Food Chem.* **1997**, *45*, 3991−3994.

(13) Friedman, M.; McDonald, G. M. Acid-catalyzed partial hydrolysis of carbohydrate groups of the potato glycoalkaloid α-chaconine in alcoholic solutions. *J. Agric. Food Chem.* **1995**, *43*, 1501−1506.

(14) Friedman, M.; Kozukue, N.; Harden, L. A. Preparation and characterization of acid hydrolysates of the tomato glycoakaloid α-tomatine. *J. Agric. Food Chem.* **1998**, *46*, 2096−2101.

(15) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589−601.

(16) SAS. *SAS/STAT User's Guide*, version 6; Statistical Analysis Institue: Cary, NC, 1990.

(17) Friedman, M. Tomato glycoalkaloids: role in the plant and in the diet. *J. Agric. Food Chem.* **2002**, *50*, 5751−5780.

(18) Friedman, M.; McDonald, G. M. Steroidal glycoalkaloids. In *Naturally Occurring Glycosides: Chemistry, Distribution, and Biological Properties*; Ikan, R., Ed.; Wiley: New York, 1999; pp 311−343.

(19) Friedman, M.; McDonald, G. M. Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. *Crit. Rev. Plant Sci.* **1997**, *16*, 55−132.

(20) de Almeida, A. E.; Rocca, M. A. Glycoalkaloids from the fruits of *Solanum flaccidum*. *Rev. Cienc. Farm. (Sao Paulo)* **1995**, *16*, 111−118.

(21) Osman, S. F.; Herb, S. F.; Fitzpatrick, T. J.; Sinden, S. L. Commersonine, a new glycoalkaloid from two *Solanum* species. *Phytochemistry* **1976**, *15*, 1065−1067.

(22) Friedman, M.; Henika, P. R.; Mackey, B. E. Effect of feeding solanidine, solasodine, and tomatidine to non-pregnant and pregnant mice. *Food Chem. Toxicol.* **2003**, *41*, 2964−2973.

(23) Kozukue, N.; Misoo, S.; Yamada, T.; Kamijima, O.; Friedman, M. Inheritance of morphological characters and glycoalkaloids in potatoes of somatic hybrids between dihaploid *Solanum acaule* and tetraploid *Solanum tuberosum*. *J. Agric. Food Chem.* **1999**, *47*, 4478−4483.

(24) Osman, S. F.; Sinden, S. L. Analysis of mixtures of solanidine and demissidine glycoalkaloids containing identical carbohydrate units. *J. Agric. Food Chem.* **1977**, *25*, 955−957.

(25) Williams, J. A.; Guicherit, O. M.; Zaharian, B. I.; Xu, Y.; Chai, L.; Wichterle, H.; Kon, C.; Gatchalian, C.; Porter, J. A.; Rubin, L. L.; Wang, F. Y. Identification of a small molecule inhibitor of the hedgehog signalling pathway: effects on basal cell carcinoma-like lesions. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4616−4621.

(26) Friedman, M.; Levin, C. E. Dehydrotomatine content in tomatoes. *J. Agric. Food Chem.* **1998**, *46*, 4571−4576.
(27) Kozukue, N.; Han, J.-S.; Lee, K.-R.; Friedman, M. Dehydrotomatine and *a*-tomatine content in tomato fruits and vegetative plant tissues. *J. Agric. Food Chem.* **2004**, *52*, 2079−2083.
(28) Aubert, S.; Dunay, M. C.; Pochard, E. Saponins and steroidal alkaloids of eggplant (*Solanum melongena*). II. Effects of cultural conditions, genotypes, and parthenocarpy. *Agronomie (Paris)* **1989**, *9*, 751−758.
(29) Pilati, P.; Mocellini, S.; Rossi, C. R.; Scalerta, R.; Alaggio, R.; Giacomelli, L.; Geroni, C.; Nitti, D. M. Doxorubicin activity is enhanced by hyprthermia in a model of *ex vivo* vascular perfusion of human colon carcinoma. *World J. Surg.* **2003**, *27*, 640−646.
(30) Pantazis, P.; Han, Z.; Balan, K.; Wang, W.; Wyche, J. H. Camptothecin and 9-nitrocamptothecin as anti-cancer, anti-HIV cell diffrentatiation agents. Development of resistance, enhancement of induced activities, and combination treatments in cell and animal models. *Anticancer Res.* **2003**, *23*, 3623−3638.
(31) Friedman, M.; Fitch, T. E.; Yokoyama, W. E. Lowering of plasma LDL cholesterol in hamsters by the tomato glycoalkaloid tomatine. *Food Chem. Toxicol.* **2000**, *38*, 549−553.
(32) Friedman, M.; Fitch, T. E.; Levin, C. E.; Yokoyama, W. H. Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. *J. Food Sci.* **2000**, *65*, 897−900.
(33) Taylor-Robinson, A. W.; Morrow, J. W. Tomatine as an adjuvant in malaria vaccine development. *Drugs Future* **2002**, *27*, 391−402.
(34) Gubarev, M. I.; Enioutina, E. Y.; Taylor, J. L.; Visic, D. M.; Daynes, R. A. Plant-derived glycoalkaloids protect mice against lethal infection with *Salmonella typhimurium*. *Phytother. Res.* **1998**, 79−88.
(35) Fewell, A. M.; Roddick, J. G.; Weissenberg, M. Interactions between the glycoalkaloids solasonine and solamargine in relation to inhibition of fungal growth. *Phytochemistry* **1994**, *37*, 1007−1011.
(36) Fewell, A.; Roddick, J. Potato glycoalkaloid impairment of fungal development. *Mycol. Res.* **1997**, *101*, 597−603.
(37) Thorne, H. V.; Clarke, G. F.; Skuce, R. The inactivation of herpes simplex virus by some *Solanaceae* glycoalkaloids. *Antiviral Res.* **1985**, *5*, 335−343.
(38) Friedman, M. Composition and safety evaluation of potato berries, potato and tomato seeds, potato and tomato alkaloids. In *Assessment of Food Safety*; Finley, J. W., Robinson, S. F., Armstrong, A., Eds.; American Chemical Society: Washington, DC, 1992; pp 429−462.
(39) Friedman, M.; Henika, P. R.; Mackey, B. E. Feeding of potato, tomato and eggplant alkaloids affects food consumption and body and liver weights in mice. *J. Nutr.* **1996**, *126*, 989−999.
(40) Friedman, M.; Roitman, J. N.; Kozukue, N. Glycoalkaloid and calystegine contents of eight potato cultivars. *J. Agric. Food Chem.* **2003**, *51*, 2964−2973.
(41) Kozukue, N.; Friedman, M. Tomatine, chlorophyll, β-carotene and lycopene content in tomatoes during growth and maturation. *J. Sci. Food Agric.* **2003**, *83*, 195−200.
(42) Friedman, M.; Levin, C. E. α-Tomatine content in tomato and tomato products determined by HPLC with pulsed amperometric detection. *J. Agric. Food Chem.* **1995**, *43*, 1507−1511.

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