Structure−Activity Relationships of α-, β1-, γ-, and δ-Tomatine and Tomatidine against Human Breast (MDA-MB-231), Gastric (KATO-III), and Prostate (PC3) Cancer Cells

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ABSTRACT: Partial acid hydrolysis of the tetrasaccharide (lycotetraose) side chain of the tomato glycoalkaloid α-tomatine resulted in the formation of four products with three, two, one, and zero carbohydrate side chains, which were separated by high-performance liquid chromatography (HPLC) and identified by thin-layer chromatography (TLC) and liquid chromatography ion-trap time-of-flight mass spectrometry (LCMS-IT-TOF). The inhibitory activities in terms of IC_{50} values (concentration that inhibits 50% of the cells under the test conditions) of the parent compound and the hydrolysates, isolated by preparative HPLC, against normal human liver and lung cells and human breast, gastric, and prostate cancer cells indicate that (a) the removal of sugars significantly reduced the concentration-dependent cell-inhibiting effects of the test compounds, (b) PC3 prostate cancer cells were about 10 times more susceptible to inhibition by α-tomatine than the breast and gastric cancer cells or the normal cells, (c) the activity of α-tomatine against the prostate cancer cells was 200 times greater than that of the aglycone tomatidine, and (d) the activity increased as the number of sugars on the aglycone increased, but this was only statistically significant at $p < 0.05$ for the normal lung Hel299 cell line. The effect of the alkaloids on tumor necrosis factor α (TNF-α) was measured in RAW264.7 macrophage cells. There was a statistically significant negative correlation between the dosage of γ- and α-tomatine and the level of TNF-α. α-Tomatine was the most effective compound at reducing TNF-α. The dietary significance of the results and future research needs are discussed.

KEYWORDS: Tomatoes, α-tomatine, hydrolysis, TLC, mass spectrometry, cancer cells, inhibition, tumor necrosis factor α, structure−function relationships, mechanisms

■ INTRODUCTION

Tomatoes, a major food source, synthesize secondary metabolites, including the glycoalkaloids α-tomatine and dehydrotomatine, which serve as natural defenses against plant fungi, viruses, bacteria, insects, and worms.[1−3] In previous studies, we reported that (a) the tomato glycoalkaloid α-tomatine and the potato glycoalkaloids α-chaconine and α-solanine were strong inhibitors of human cancer cells,[4,5] (b) the inhibition of cancer cells by green tomato extracts that contain high levels of α-tomatine was much higher than that by red tomato extracts lacking α-tomatine,[6,7] and (c) long-term feeding of small amounts of α-tomatine protected fish (trout) against dibenzo-[a,l]pyrene-induced colon cancer.[8] To our knowledge, this last-mentioned study seems to be the only one that evaluated the anticarcinogenicity of α-tomatine in vivo.

The results of our previous studies suggest that consumption of both high-tomatine unripe green and low-tomatine ripe red tomatoes containing other bioactive compounds might have an additive anticarcinogenic effect compared to that of green or red tomatoes alone.[6] Tomatoes are most commonly consumed ripe but some regional cuisines use unripe tomatoes. We previously reported that some wild-type potato cultivars also contain high amounts of α-tomatine.[9,10] Other natural sources of tomatine include an unusually high-tomatine ripe red fruit of a regional cherry tomato variant of Andean origin[11] and tomato leaves.[12,13]
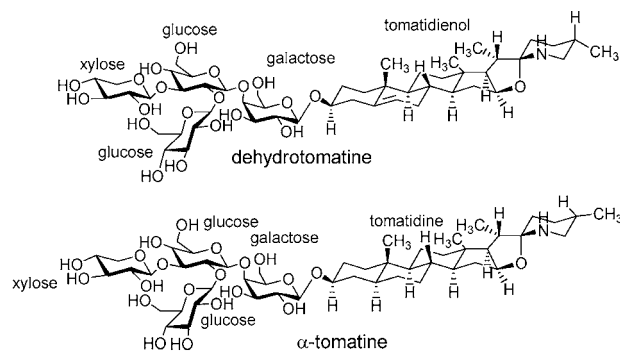
Figure 1 shows that the α-tomatine molecule consists of a steroidal part with a side chain consisting of four carbohydrates (one galactose, two glucose, and one xylose). Because one or more of the carbohydrate groups can, in principle, be cleaved (hydrolyzed) by enzyme or acid hydrolysis in the plant or in vivo after consumption, it was of interest to test the hypothesis that both the nature and number of carbohydrate groups might influence bioactivity. Therefore, the main objective of the present study was to compare the growth-inhibiting effect

dehydrotomatine: tomatidenol-3-O-[β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside]

α-tomatine: tomatidine-3-O-[β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside]

β₁-tomatine: tomatidine-3-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside]

β₂-tomatine: tomatidine-3-O-[β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside]

γ-tomatine: tomatidine-3-O-[β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside]

δ-tomatine: tomatidine-3-O-β-D-galactopyranoside

tomatidine: 5α-tomatidan-3β-ol

Figure 1. Structures of dehydrotomatine, α-tomatine, and α-tomatine hydrolysis products. Molecular weights: dehydrotomatine (tetrasaccharide), 1032.2; α-tomatine (tetrasaccharide), 1034.2; β₁-tomatine (trisaccharide), 902.05; β₂-tomatine (trisaccharide), 872.03; γ-tomatine (disaccharide), 739.90; δ-tomatine (monosaccharide), 577.75; and tomatidine (aglycone), 415.66. The chemical names shown in the figure were adapted from ref 51.

of α-tomatine and four hydrolysis products to side chains of three, two, one, and zero sugars (Figure 1) against three cancer and two normal cell lines using the 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. We also measured the effect of these compounds on the production of tumor necrosis factor α (TNF-α), a signaling molecule possibly involved in carcinogenesis, in RAW264.7 murine macrophage cells.[14]

These hydrolysis compounds were prepared by partial acid hydrolysis of commercial α-tomatine and characterized by thin-layer chromatography (TLC) and by liquid chromatography ion trap time-of-flight mass spectrometry (LCMS-IT-TOF). The results suggest that the number of carbohydrate groups attached to the aglycone seem to strongly influence the inhibition of cell growth and may also affect the expression of TNF-α by the immune system.

■ MATERIALS AND METHODS

Materials. Standard α-tomatine (lot 3011) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Standard tomatidine hydrochloride was obtained from Sigma (St. Louis, MO). Activated aluminum oxide and anisaldehyde were obtained from Kanto Chemical Co. (Tokyo, Japan). Silica-coated TLC plates were obtained from Merck (Darmstadt, Germany). High-performance liquid chromatography (HPLC)-grade acetonitrile and formic acid were obtained from J.T. Baker (Phillipsburg, N.J). All other compounds and reagents were obtained from Sigma.

Normal human liver (Chang) and lung (Hel299) cell lines and human prostate (PC3), breast (MDA-MB-231), and gastric adenocarcinoma (KATO-III) cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD) and from Korean Cell Line Bank (KCLB, Seoul, Korea). RAW264.7 and L929 cells used for the TNF-α studies were obtained from Riken BRC Cell Bank (Tsukuba, Japan). The cells were maintained in α-MEM or RPMI

1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in a 5% CO₂ incubator. Cell culture reagents were obtained from Gibco BRL (Life Technologies, Cergy-Pontoise, France). Each sample was dissolved in dimethyl sulfoxide(DMSO) (10%, 10 mg/mL) and stored at −4 °C.

Dulbecco's modified Eagle's medium (DMEM), actinomycin D, and murine recombinant TNF-α were purchased from Wako Pure Chemical Industries (Osaka, Japan). Eagle's minimum essential medium (MEM) was purchased from Nissui Pharmaceutical (Tokyo, Japan). FBS and RPMI 1640 medium were purchased from Gibco BRL (Grand Island, NY).

Acid Hydrolysis of Commercial α-Tomatine. A 10 mL vial with a sealed Teflon cap containing 152.3 mg of commercial α-tomatine dissolved in 1 N HCl (4 mL) was heated at 100 °C for 25 min. The cooled solution was neutralized with 1 N NH₄OH and partitioned 4 times with water-saturated n-butanol (10 mL). The combined butanol layers were evaporated to dryness on an aspirator at 45 °C, and the residue was dissolved in water-saturated n-butanol (6 mL).

Isolation of α-Tomatine Partial Hydrolysis Products. The water-saturated n-butanol solution was applied to an aluminum oxide column (30 × 1.5 cm). The compounds were eluted with water-saturated n-butanol at a flow rate of 0.5 mL/min controlled with a Hitachi L-600 pump. The eluate was collected in 5 mL fractions. The fractions were examined by TLC and by LCMS-IT-TOF for the detection of hydrolysis products.

Identification of Hydrolysis Products. TLC was performed on Merck precoated silica gel G plates, 0.25 μm thick. The plate was developed in a chamber with a mixture of chloroform/methanol/1% NH₄OH (65:35:5, v/v/v). Spots were visualized by spraying with anisaldehyde reagent or 30% sulfuric acid and heating for 5 min at 120 °C.

LCMS-IT-TOF experiments were performed on a LCMS-IT-TOF mass spectrometer with an electrospray ionization (ESI) source (Shimadzu, Kyoto, Japan). The interface voltage and current were 4.50 kV and 76 μA for positive-ion mode. The flow rate of nebulizing gas was 1.5 L/min, and the N₂ drying pressure was 0.2 M Pa. The curved desorption line and heat block temperature were both at 200 °C. The detector voltage of the TOF analyzer was 1.68 kV. Ultrahigh-purity argon was used as the collision gas for collision-induced dissociation experiments. The relative energy in collisions was 100%. The sample injection volume was 10 μL. A direct valve was set to transmit and divert the HPLC eluent to waste. Mass spectral data were collected from m/z 100−1500. Data acquisition and processing were carried out with Shimadzu LCMS solution software (version 3.41).

MTT Assay for Growth Inhibition of Cells. The MTT assay that differentiates dead from living cells was adapted from the literature.[15] The following reagents and instruments were used: MTT reagent, 5 mg/mL in phosphate-buffered saline, protected from light and stored at 20 °C, MEM cell medium (containing 10% FBS and 1% penicillin/streptomycin), and a microplate reader (Bio-Rad Co., Hercules, CA). Cell lines were seeded into a 96-well microplate (1 × 10⁴ cells/well) and incubated for 24 h. Next, cells were treated with four concentrations (1, 10, 50, and 100 μg/mL) of the test compounds for 48 h. The MTT solution (0.1 mg/mL) was then added to each well. After 4 h of incubation at 37 °C, DMSO (200 μL) was added to each well. The absorbance (A) was then read at 540 nm. The decrease in A measures the extent of decrease in the number of viable cells following exposure to the test substances calculated by using the following formula: percent inhibition of cells = $(A_{control} - A_{test\ substance})/A_{control} \times 100$.

TNF-α Production in RAW264.7 Cells Measured in L929 Cells. *Cell Culture for TNF-α Assay.* Murine leukemia-induced macrophage-like monocyte RAW264.7 cells were cultured in 75 cm² plastic flasks (Falcon, NJ) in DMEM supplemented with 10% heat-inactivated FBS (56 °C, 30 min), L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL). Cell cultures were maintained in a humidified 5% CO₂ incubator at 37 °C. Murine fibrosarcoma L929 cells were cultured in 75 cm² plastic flasks (Falcon, NJ) in MEM supplemented with FBS (10%), L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL). Cell cultures were maintained in a humidified 5% CO₂ incubator at 37 °C.

Stimulation of TNF-α in RAW264.7 Cells. Murine macrophage RAW264.7 cells (0.5×10^6 cells/well) were re-incubated with a fresh RPMI 1640 medium for 2 h to replace the former medium, DMEM. The RAW264.7 cells were then stimulated with six concentrations (0, 0.1, 1, 4, 8, and 10 μM) of the test sample for 24 h at 37 °C and 5% CO_2. At the end of the incubation, the culture supernatant was collected by centrifugation at 2000g for 5 min. The supernatant was assayed for TNF-α using the L929 cell assay.

Assay of TNF-α. The assay was adapted from the literature.[16−19] TNF-α was measured by means of a cytolytic assay with actinomycin-D-treated L929 cells, using murine rTNF-α as the standard. L929 cells (2.3×10^5 cells/mL) were plated in 96-well microplates in RPMI 1640 medium that included FBS (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL) and cultured for 2 h. Supernatant samples (50 μL) obtained from macrophages stimulated with the tested samples (above) and RPMI 1640 medium containing actinomycin D (50 μL; 4 μg/mL) were added to the microplates, which were then cultured for 20 h at 37 °C and 5% CO_2. After incubation, the plates were washed and cell lysis was determined by staining with 0.1% crystal violet in ethanol/formaldehyde for 15 min at room temperature. After washing with water and drying, the cells were dissolved in ethanol/phosphate-buffered saline (PBS) (100 μL; 1:1, v/v). The absorbance of the cell lysate in each well was measured using a SH-1000 Lab microplate reader (Corona Electric, Hitachinaka, Japan) at 570 and 630 nm.

Statistical Analysis. Statistical analysis of the effect of the alkaloid on TNF-α was performed using Student's *t* test. Data are expressed as the mean ± standard error (SE). Statistical significance is defined as $p < 0.05$. Statistical differences between alkaloid treatments of cells were determined by analysis of variation (ANOVA) followed by Holm−Sidak tests using the Sigma Plot 11 software (Systat, Chicago, IL).

■ RESULTS AND DISCUSSION

Characterization of Acid Hydrolysis Products of α-Tomatine. In an effort to prepare all theoretically possible hydrolysis products of the tetrasaccharide α-tomatine with zero, one, two, and three carbohydrate groups, the tomato glyco-alkaloid was subjected to partial hydrolysis in 1 N HCl at 100 °C for 25 min. These conditions are similar to those that we previously used to determine the structure of the related tomato glycoalkaloid dehydrotomatine.[20] A water-saturated *n*-butanol solution of the neutralized sample was fractionated on an aluminum oxide column, and the eluted fractions were then characterized by TLC and mass spectrometry.

Table 1 shows that the hydrolysate contained four compounds with R_f values ranging from 0.46 to 0.77. The percentage of each isolate relative to that of the original α-tomatine (100%) ranged from 5.78 to 10.70. The structures of the hydrolysis products were determined with the aid of LCMS-IT-TOF. Figure 2 depicts the mass spectra of the four hydrolysis products and two standards, α-tomatine and tomatidine. On the basis of the values of the MS $[M + 1]^+$ molecular (parent) ion peaks summarized in Table 1 and fragmentation patterns shown in Figure 2, we assigned the following structures to the fractions separated on the aluminum oxide column: fraction 1 (20−26), tomatidine; fraction 2 (30−39), δ-tomatine; fraction 3 (48−52), γ-tomatine; and fraction 4 (54−61), $β_1$-tomatine. The mass fragmentation patterns of tomatidine in the hydrolysate were identical to those of standard tomatidine. The molecular parent ion peak of α-tomatine shown in Figure 2 is identical to its known molecular weight.

Figure 1 shows that, in principle, partial hydrolysis of α-tomatine can form two trisaccharide derivatives, $β_1$-tomatine, described above, and $β_2$-tomatine, which has a different trisaccharide side chain. Although $β_2$-tomatine is formed naturally in the tomato plant by enzymatic hydrolysis of α-tomatine by

tomatinase,[21,22] we could not find it in the acid hydrolysate evaluated in the present study. Because tomatinase was not available to us, we attempted to prepare $β_2$-tomatine by enzymatic hydrolysis with a β-glycosidase enzyme derived from almonds (Wako Pure Chemicals, Osaka, Japan). TLC analysis showed that this attempt was also unsuccessful (results not shown).

Cell Growth Inhibiting Effects of α-Tomatine and Four Hydrolysis Products. *Structure−Activity Relationships.* The four isolated hydrolysis products and commercial α-tomatine were then evaluated for their ability to inhibit the growth of three cancer and two normal human cell lines as described below. Table 2 shows the inhibitory effects of four concentrations (1, 10, 50, and 100 μg/mL) of each test substance against two normal and three cancer cell lines evaluated by the MTT cell viability assay. The data in the table show that, except for the negative tomatidine values for the normal liver cells, the inhibition of all other cells was concentration-dependent. Negative values represent cell growth, and positive values represent inhibition of growth.

The data in Table 2 were used to calculate IC_{50} values in terms of μg/mL and μM shown in Table 3 and depicted visually in Figure 3. In the table, statistically significant differences between the compounds were calculated within the cell lines. In the figure, statistically significant differences between the cell lines were calculated for each compound. The observations indicate that (a) the test compounds are active against human cells and (b) activities generally decrease progressively as the number of carbohydrate groups attached to the steroidal side chain decrease from four (α-tomatine), three ($β_1$-tomatine), two (γ-tomatine), one (δ-tomatine), and zero (tomatidine). Prostate cancer (PC3) cells are the most susceptible to inhibition by the tomatines. The IC_{50} value for the inhibition of PC3 cells by α-tomatine of 0.003 mM is exceptionally low (i.e., very high activity), about 10 times greater than for the normal cells. Also, for PC3, the activity of α-tomatine is 200 greater than that of tomatidine. The corresponding values for the hydrolysates relative to tomatidine are as follows: $β_1$-tomatine, 6.6; γ-tomatine, 4.3; and δ-tomatine, 3.4. These observations imply that the carbohydrate moiety seems more significant than the steroidal part of the test compounds in the cell inhibition process.

Figure 3 shows the decreasing trend of the IC_{50} values with an increasing number of sugars on the side chain. The differences between the cell lines are not consistent between the different compounds. For example, PC3 and KATO-III cell lines responded similarly when exposed to α- and $β_1$-tomatine. However, KATO-III cells were less inhibited by γ-tomatine, δ-tomatine, and especially, tomatidine than PC3 cells. Overall, α-tomatine is the most inhibitory of the compounds tested, and PC3 cells are the most susceptible to inhibition by all compounds.

TNF-α Production. TNF-α is a cell-signaling cytokine produced primarily by immune system macrophages that is involved in systemic inflammation. It has both apoptotic and anti-apoptotic activities governed by different pathways. In an effort to determine whether tomatine and hydrolysis products can affect the immune-system-mediated response to tumorigenesis, leukemia-virus-induced macrophage-like mouse monocytes (RAW264.7) were exposed to tomatines and tomatidine. The resulting reduction in TNF-α levels provides a measure of this effect. Results from dosing the cells with variable levels of the test compounds showed that all of the compounds, except

Table 1. TLC Retention Values (R_f), Yield, and MS $[M + 1]^+$ Ions of Four Peaks Eluted from an Aluminum Oxide Column Obtained by Partial Hydrolysis of α-Tomatine (1 N HCl at 100 °C for 25 min) Compared to Commercial Standards

compound or column fraction (elution time in min)	R_f value[a]	yield (mg)[b]	MS $[M + 1]^+$	identification
fraction 1 (20−26)	0.77	8.8 (5.78)[c]	416.3446	tomatidine
fraction 2 (30−39)	0.62	16.3 (10.70)	578.3981	δ-tomatine
fraction 3 (48−52)	0.52	13.7 (8.99)	740.4519	γ-tomatine
fraction 4 (54−61)	0.46	10.0 (6.57)	902.5018	β_1-tomatine
standard α-tomatine	0.44		1034.5399	
standard tomatidine	0.78		416.3452	

[a]TLC conditions: solvent, chloroform/methanol/1% NH$_4$OH (65:35:5, v/v/v); detection, anisaldehyde spray followed by heating at 120 °C for 5 min. [b]Each compound is expressed as milligrams produced from the acid hydrolysis of α-tomatine (152.3 mg). [c]The percentage of each compound based on the original weight of α-tomatine is shown in parentheses.
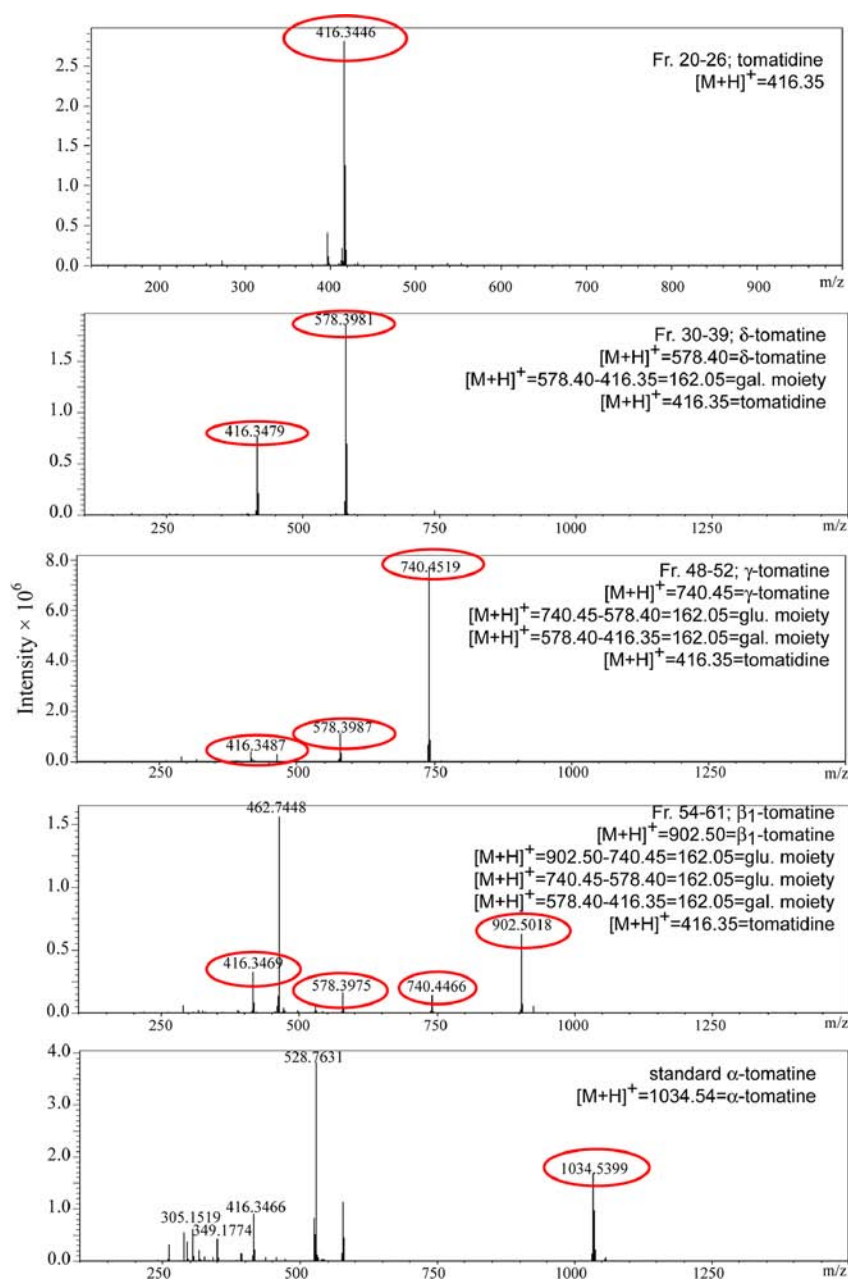
Figure 2. LCMS-IT-TOF spectra of four fractions obtained from acid hydrolysis of standard α-tomatine. Note parent ion molecular peaks.

β_1-tomatine, produced a significant decrease in TNF-α production at the highest 10 μM dose. The lowest dose of

β_1-tomatine increased TNF-α production. The ANOVA test between compounds showed that, other than for β_1-tomatine,

Table 2. Inhibitory Effect by α-Tomatine and Four Hydrolysis Products Isolated after Separation on an Aluminum Oxide Column against Normal Liver Cells (Chang), Normal Lung Cells (Hel299), Prostate Cancer Cells (PC3), Breast Cancer Cells (MDA-MB-231), and Gastric Adenocarcinoma (KATO-III) Determined by the MTT Assay[a]

test product	concentration (µg/mL)	growth inhibition (%)				
		normal liver (Chang)	normal lung (Hel299)	prostate cancer (PC3)	breast cancer (MDA-MB-231)	gastric adenocarcinoma (KATO-III)
tomatidine	1	−18.6 ± 6.6	5.4 ± 0.6	2.9 ± 0.8	1.5 ± 1.2	2.2 ± 1.5
	10	−11.6 ± 4.2	8.8 ± 1.0	8.3 ± 1.8	2.1 ± 0.9	4.8 ± 1.2
	50	−1.7 ± 0.8	14.9 ± 3.2	19.8 ± 2.7	4.0 ± 0.7	8.1 ± 0.6
	100	2.1 ± 0.4	22.0 ± 1.2	22.6 ± 2.2	15.6 ± 2.4	9.8 ± 2.1
δ-tomatine	1	−19.9 ± 6.6	2.7 ± 0.7	1.2 ± 1.3	1.9 ± 0.9	1.4 ± 0.3
	10	−9.7 ± 6.2	6.0 ± 0.5	3.8 ± 0.6	5.2 ± 0.9	6.1 ± 1.0
	50	5.1 ± 1.4	7.6 ± 0.7	41.9 ± 0.4	8.8 ± 1.2	7.4 ± 2.6
	100	23.7 ± 1.2	15.5 ± 2.9	50.0 ± 1.0	59.2 ± 1.4	33.8 ± 3.7
γ-tomatine	1	−21.2 ± 1.6	3.2 ± 0.4	−5.7 ± 2.8	2.3 ± 1.2	1.9 ± 2.9
	10	8.5 ± 0.4	8.5 ± 2.9	2.0 ± 0.1	3.3 ± 0.8	4.4 ± 0.2
	50	19.5 ± 1.4	13.1 ± 4.3	22.8 ± 5.6	4.2 ± 1.5	11.3 ± 2.1
	100	46.7 ± 0.4	65.1 ± 0.4	47.8 ± 2.6	36.6 ± 7.7	32.6 ± 0.4
β₁-tomatine	1	−3.8 ± 0.3	8.5 ± 2.5	4.6 ± 0.8	−6.1 ± 1.9	3.4 ± 1.2
	10	2.2 ± 1.1	20.7 ± 1.7	5.1 ± 1.0	2.0 ± 0.2	4.3 ± 1.0
	50	8.8 ± 2.3	23.0 ± 2.3	27.0 ± 2.6	22.8 ± 6.7	23.1 ± 2.8
	100	36.3 ± 3.1	74.8 ± 1.7	62.3 ± 2.0	64.5 ± 3.2	64.0 ± 4.0
α-tomatine (std)	1	−29.5 ± 7.8	5.5 ± 1.1	41.1 ± 1.0	2.3 ± 1.6	30.8 ± 3.0
	10	48.5 ± 5.8	52.9 ± 0.7	80.9 ± 0.6	42.8 ± 3.8	74.0 ± 3.4
	50	88.9 ± 0.7	92.0 ± 0.1	92.8 ± 0.3	94.2 ± 0.2	91.9 ± 0.2
	100	89.3 ± 0.2	94.3 ± 0.2	92.9 ± 0.3	94.6 ± 0.1	91.9 ± 0.1

[a]The listed value is expressed as the average ± standard deviation (SD) (n = 3).

the TNF-α levels at the 10 µM dose were not significantly different from each other. α-Tomatine produced a significant decrease at the next to highest 8 µM dose. These results indicate that α-tomatine is more effective than its hydrolysis products in reducing the production of TNF-α. These results complement the reported stimulation of the immune system by an α-tomatine adjuvant in an experimental malaria vaccine.[23]

Mechanistic Aspects. *Cell Interactions with Glycoalkaloids and Aglycones.* To facilitate a better understanding of the possible mechanisms that govern tomatine-induced inhibition of cancer cells discussed below, we will first briefly mention several published studies on glycoalkaloid−cell interactions on a variety of cells. Glycoalkaloids are produced by plants in defense against phytopathogens.[24] The glycosides are usually more active than the aglycones or hydrolysates.[2] A strategy used by some pathogens to overcome α-tomatine phytotoxicity is to hydrolyze the tetrasaccharide by the tomatinase enzyme to the less toxic β, γ, or δ forms or completely to tomatidine.[25]

Trisaccharide potato glycoalkaloids, such as α-chaconine and α-solanine, and the tetrasaccharide tomato glycoalkaloid, α-tomatine, are known to disrupt cell membranes.[26] Their mode of action seems to be initiated by complexation with membrane sterol components.[27] The loss of toxicity of hydrolysates against fungi was better correlated with reduced complex formation with cholesterol than with reduced surfactant properties.[28] α-Tomatine forms stronger sterol complexes *in vitro* than either the potato glycoalkaloids[29] or the tomatine hydrolsates.[28]

The mechanism governing tomatidine activity against cells may be different from that of α-tomatine. Tomatidine does not bind with sterols[29] yet inhibited some but not all fungal cells.[25] Some fungi are able to detoxify α-tomatine by producing enzymes that hydrolyze the molecule into β₁-tomatine[30] or tomatidine[31] and to subsequently convert the tomatidine into the dehydro form, called tomatidenol.[32] α-Tomatine but not

tomatidine reduced the sodium-active transport in frog skin, although both increased the permeability of frog embryo membranes, with α-tomatine being more effective than tomatidine.[33] Surprisingly, tomatidine caused greater leakage from membranes of some plant cells than α-tomatine.[34] Tomatidine also exhibited greater fungicidal activity against *Saccharomyces cerevisiae* than any of the glycosides. Its fungicidal activity was associated with the disruption of ergosterol biosynthesis and not with the permeabilization of cell membranes.[35] The susceptibility of small colony variants of *Staphylococcus aureus* to tomatidine was attributed to their dysfunctional electron-transport system.[36] Susceptibility in normal *S. aureus* strains could be induced by inhibiting the electron transport system. Macromolecular biosynthesis was reduced in tomatidine-treated cells, particularly incorporation of radiolabeled leucine into proteins. This indicates that a primary cellular target of tomatidine is the bacterial protein biosynthesis machinery.

α-Chaconine and α-tomatine but not the corresponding aglycones solanidine and tomatidine inactivated the herpes simplex virus.[37] Because viruses do not contain sterols, the authors suggest that viral inactivation probably results from insertion of the glycoalkaloids into the viral envelope.

The cited studies indicate that α-tomatine and tomatidine seem to exhibit different modes of action against plant, fungal, yeast, viral, bacterial, and animal cells and that the nature of the carbohydrate side chain affects bioactivity.

Human Cancer Cells. Structural features of α-tomatine and its hydrolysis products as well as noncovalent and hydrogen-bonding interactions may influence relative affinities to membranes.[38] Because of the strong affinity of α-tomatine for sterols,[29] it is likely that the relative susceptibilities of different cancer cell lines to inhibition may be related to the nature and content of cancer membrane sterols and phospholipids.[26,39,40]

Table 3. Inhibitory Effect (IC$_{50}$) by Commercial Standard α-Tomatine and Four Hydrolysis Products Isolated after Separation on an Aluminum Oxide Column against Normal Liver Cells (Chang), Normal Lung Cells (Hel299), Prostate Cancer Cells (PC3), Breast Cancer Cells (MDA-MB-231), and Gastric Adenocarcinoma (KATO-III) Determined by the MTT Assay[a]

	IC$_{50}$									
products	normal liver (Chang)		normal lung (Hel299)		prostate cancer (PC3)		breast cancer (MDA-MB-231)		gastric adenocarcinoma (KATO-III)	
	µg/mL	mM	µg/mL	mM	µg/mL	mM	µg/mL	mM	µg/mL	mM
tomatidine	413.9 ± 18.1	0.996 ± 0.044	265.5 ± 9.1	0.639 ± 0.022 a	248.9 ± 11.2	0.599 ± 0.027	336.5 ± 7.9	0.810 ± 0.019	623.0 ± 7.9	1.499 ± 0.019
δ-tomatine	170.7 ± 16.1	0.295 ± 0.028	369.0 ± 7.1	0.639 ± 0.012 a	100.5 ± 5.0 a	0.174 ± 0.009	84.5 ± 6.6 a	0.146 ± 0.011	150.4 ± 11.3 a	0.260 ± 0.020
γ-tomatine	101.4 ± 5.8	0.137 ± 0.008 a	76.0 ± 11.9 a	0.103 ± 0.016	103.2 ± 16.6 a	0.139 ± 0.022	137.8 ± 16.6	0.186 ± 0.022	156.0 ± 8.4 a	0.211 ± 0.011
β$_1$-tomatine	134.4 ± 10.1	0.149 ± 0.011 a	60.8 ± 12.1 a	0.067 ± 0.013	82.5 ± 9.6 a	0.091 ± 0.011	82.3 ± 11.0 a	0.091 ± 0.012	77.1 ± 13.3	0.085 ± 0.015
α-tomatine	33.9 ± 2.2	0.033 ± 0.002	26.2 ± 4.4	0.025 ± 0.004	3.0 ± 0.3	0.003 ± 0.000	26.4 ± 3.6	0.026 ± 0.003	16.4 ± 10.0	0.016 ± 0.010

[a]The listed value is expressed as the average ± SD ($n = 3$). Values with a common letter within a column are not significantly different ($p < 0.05$).
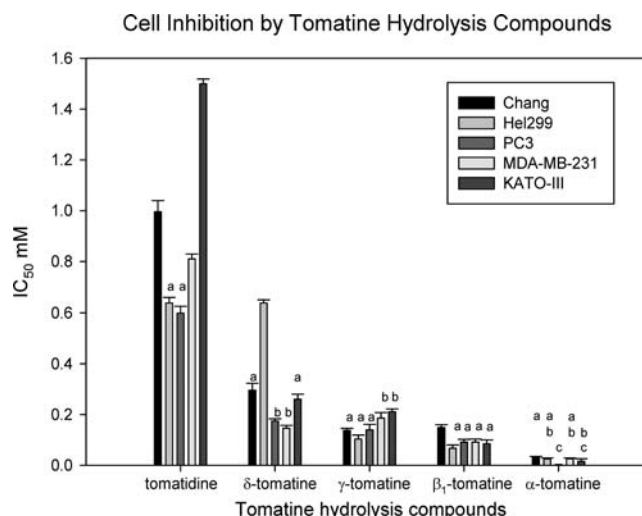

Figure 3. Effect of tomatine hydrolysis compounds on cell inhibition. The lower the bar graph, the greater the activity. Cell lines sharing the same letter within a group are not significantly different ($p < 0.05$).

In previous studies,[6,7] we compared the growth inhibitory activities of the pure tomato glycoalkaloids α-tomatine and dehydrotomatine, the corresponding aglycones tomatidine and tomatidenol, and crude glycoalkaloid extracts from green and red tomatoes against different human cancer cell lines. We found that different cell lines differ in relative susceptibilities to inactivation. Cell inhibition correlated (−0.80, $p < 0.05$) with the α-tomatine content of the crude extracts, but it seems that other unknown components of the red and green extracts may also exert an inhibitory and/or stimulatory effect. It is striking that dehydrotomatine, whose structure differs from that of α-tomatine only by the presence of a double bond in ring B of the aglycone moiety (Figure 1), exhibited a significantly lower inhibiting activity than α-tomatine, presumably because its affinity for cholesterol is also lower.

In addition to the above-mentioned cytotoxic effects, cancer cell growth or inhibition may also be guided by cell signaling. Shih et al.[41] investigated the mechanism of the antimetastatic effect of α-tomatine in non-small cell human lung adenocarcinoma A549 cells. They found that, at noncytotoxic concentrations, (a) the glycoalkaloid inhibited cell invasion and migration and phosphorylation of Akt and extracellular signal-regulated kinases 1 and 2, (b) α-tomatine did not affect phosphorylation of c-Jun N-terminal kinase (JNK) and the p38 gene, (c) α-tomatine decreased nuclear levels of nuclear factor κB (NF-κB), c-Fos, and C-jun, and (d) the glycoalkaloid inhibited the binding abilities of NF-κB and activator protein-1 (AP-1). These and related molecular events suggest that inhibition of metastasis occurs by reducing the activities of matrix metalloproteinases MMP-2 and MMP-9 and urokinase plasminogen activator through the suppression of the phosphoinositide 3-kinase/Akt or extracellular signal-regulated kinase signaling pathway and inhibition of NF-κB and AP-1 binding activities. The authors suggest that these results indicate that α-tomatine might have a therapeutic value in the treatment of lung cancer.

A related study by Shieh et al.[42] confirmed that α-tomatine suppressed invasion and migration of human non-small cell lung cancer NCI-H460 cells through inactivation of the focal adhesion kinase/phosphoinositide 3-kinase/Akt signaling pathway and by lowering the binding activity of NF-κB. The proposed mechanism of the antimetastatic effect occurs through

inactivation of the signaling pathway and enhancement of IkBα protein expression to reduce NF-κB DNA binding activity, resulting in the downregulation of MMP-7 expression. Additional events that contribute to the inhibition of cell migration and invasion include interference with the rearrangement of the actin cytoskeleton by decreasing the expression of *p*-focal adhesion kinase. The observation that TNF-α treatment enhances the motility and invasiveness of prostatic cancer cells suggests that this pro-inflammatory protein contributes to prostate cancer metastasis[43] and that α-tomatine-induced reduction in macrophage production of TNF-α (Figure 4) could help overcome these molecular events.
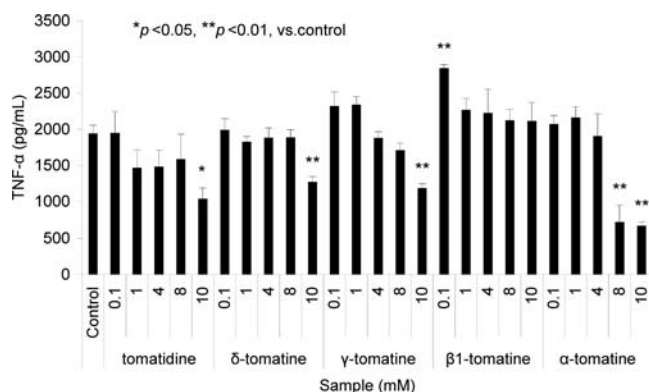

Figure 4. Effect of α-tomatine and four hydrolysis products on TNF-α production by RAW264.7 macrophage-like cells.

Lee et al.[44] also investigated the molecular mechanism of the antiproliferative effect of α-tomatine against human prostatic adenocarcinoma PC-3 cells, which, as discussed earlier, are highly susceptible to α-tomatine. They found that (a) cytotoxicity against the PC-3 cells occurred after 1 h of treatment, (b) cytotoxicity against normal liver and prostate cells was lower than against the PC-3 cells, and (c) cytotoxicity was mainly due to the induction of apoptosis, as evidenced by decreased mitochondrial membrane potential and increased nuclear condensation, polarization of F-actin potential, cell membrane permeability and cytochrome *c* expression, induction of activation of caspase-3, caspase-8, and caspase-9, inhibition of NF-κB nuclear translocation, and decreases in NF-κB/p50 and NF-κB/p65 in the nuclear fraction. These observations imply that both intrinsic and extrinsic pro-apoptosis pathways are involved and that α-tomatine could protect against prostate cancer development and progression.

Published studies describe efforts to delineate mechanisms that might govern the anticarcinogenic and anti-cystic-fibrosis effects of the aglycone tomatidine (Figure 1), which is much less active against cancer cells than the parent glycoalkaloid α-tomatine (Tables 2 and 3 and Figure 3). In a similar study, Chiu and Lin[45] compared the anti-inflammatory effects of tomatidine and solasodine, the aglycone of solamargine present in some eggplants, in lipopolysaccharide-stimulated macrophages. Tomatidine decreased inducible nitric oxide synthase and cyclooxygenase-2 expression through the suppression of I-kBα phosphorylation, NF-κB nuclear translocation, and JNK activation. The results imply that the anti-inflammatory effect seems to be associated with the blockage of NF-κB and JNK signaling. Solasodine, the structure of which is identical to that of tomatidine, except that it lacks a double bond in the 5,6

position of the B ring, was less potent. We previously reported that dietary tomatidine was less toxic than solasodine in pregnant and nonpregnant mice and that it did not alter mouse liver weights.[46,47]

Finally, Lavie et al.[48] reported that tomatidine acted as a potent and effective chemosensitizer in multidrug-resistant tumor cells, sensitizing the cells to the cytotoxic action of chemotherapeutic drugs adriamycin and vinblastine. The authors suggest that tomatidine could serve in combination chemotherapy with cytotoxic drugs for treating multidrug-resistant cancer.

In summary, the results of the present study show that the tetrasaccharide side chain associated with α-tomatine is a key structural feature of the molecule that influences the inhibition of both normal and cancer cells. Systematic removal of one, two, or three sugar residues from α-tomatine results in the formation of compounds with significantly reduced activity. The results also suggest that the aglycone part of the molecule, tomatidine, contributes to the overall activity, because authentic tomatidine also inhibited the cells but at a significantly lower rate than the carbohydrate-containing molecules.

The results also indicate that tomatine and some of its hydrolyzed compounds reduced macrophage expression of TNF-α *in vitro* and that α-tomatine was the most effective form. Because TNF-α is pleiotropic, it can both induce and inhibit cell apoptosis; therefore, further studies are required to understand the net consequences of these effects.

Of particular interest is the observed high activity of α-tomatine against the prostate cancer cells. This observation suggests that α-tomatine administered orally or by injection into tumor tissues merits further study to determine whether it has the potential to treat prostate and other cancers in humans, including those that may be associated with viral infections.[49,50]

Because we do not know whether α-tomatine undergoes *in vivo* acid or enzyme hydrolysis in the digestive tract or after absorption into blood and tissues, we do not know whether the efficacy of orally consumed α-tomatine will be reduced by its hydrolysis into the less effective β-, γ-, and δ-tomatines or the aglycone, tomatidine. The possible relationship between disruptions of cancer cell membranes by the test compounds to growth inhibition, as well as the bioavailability and metabolism of α-tomatine and hydrolysis products, merits further study. Reported *in vivo* dietary studies of tomatidine,[46,47] tomatine,[8,52] high-tomatine green tomatoes,[53] and lycopene[54] could serve as animal models for evaluating bioavailability and cancer chemopreventive effects of tomatine and α-chaconine[4,55] hydrolysis products.

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Notes
The authors declare no competing financial interest.

■ ABBREVIATIONS USED

A, absorbance; AP-1, activator protein-1; CID, collision-induced dissociation; DMEM, Dulbecco's modified Eagle's medium; ESI, electrospray ionization; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; IC$_{50}$, dose-dependent concentration that inhibited 50% of the cells; JNK, c-Jun N terminal kinase; LCMS-IT-TOF, liquid chromatography ion trap time-of-flight mass spectrometry; MEM, Eagle's minimum

essential medium; NF-κB, nuclear factor κB; MTT, 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide; TLC, thin-layer chromatography; TNF-α, tumor necrosis factor α

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