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Transformation of Bioactive Compounds by *Fusarium sacchari* Fungus Isolated from the Soil-Cultivated Ginseng

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Ginsenoside bioactive compounds, namely, compound K (C-K), compound Mx (C-Mx), and ginsenoside Mc (G-Mc), were the metabolites of ginsenosides Rb_1, Rb_2, Rb_3, and Rc by intestinal microflora of humans or rats, microorganisms, and enzymes, and C-K showed beneficial effects *in vitro* and *in vivo* as an antitumoral agent. The objective of this work was to explore an efficient procedure for biotransformation of these bioactive compounds. Thus, a filamentous fungus, *Fusarium sacchari*, was first obtained from the soil-cultivated ginseng, which was verified to possess a potent capacity of transformation of C-K, C-Mx, and G-Mc. The optimal biotransformation conditions of *F. sacchari* with C-K, C-Mx, and G-Mc were obtained as follows: transforming temperature, 30 °C; transforming time, 6 days; rotary speed, 160 rpm; pH of the medium, 5.5. HPLC analysis indicated that these three bioactive compounds were key metabolites and their structures were confirmed by ^{1}H and ^{13}C NMR analysis. Moreover, the *in vitro* antitumor activities of C-K, C-Mx, and G-Mc and the *in vivo* antitumor activities of the transformed product mainly containing these compounds were also evaluated. Among C-K, C-Mx, and G-Mc, C-K exhibited the most potent antitumor activities. The *in vivo* study showed that the transformed products by *F. sacchari* had much more antitumor activity than those of commonly used ginsenoside Rg_3 and Paclitaxel.

KEYWORDS: *Fusarium sacchari*; compound K; compound Mx; ginsenoside Mc; transformation; bioactive compound; antitumor activity

INTRODUCTION

Panax notoginseng, a famous traditional Chinese medicine which is now cultivated on a large scale in China, has been used in China for many years due to its beneficial effects on blood circulation and antiinflammatory properties (*1, 2*). The saponins of *P. notoginseng*, obtained from the roots, leaves, and seeds, have been regarded as the principal components responsible for the pharmaceutical and biological effects. Various naturally occurring dammarane-type saponins and hydrolydrates have been isolated (*3–7*).

More than 30 dammarane-type saponins have been obtained from *P. notoginseng*, and the main constituents are ginsenosides that contain an aglycon with a dammarane skeleton. These include protopanaxadiol-type saponins, such as notoginsenoside R_1 and ginsenosides Rb_1, Rb_2, Rb_3, Rc, and Rd, and protopanaxatriol-type saponins, such as ginsenosides Re, Rg_1, and Rg_2 (*8–10*). Ginsenosides Rb_1, Rb_2, Rb_3, and Rc are metabolized to compound K (C-K), compound Mx (C-Mx), and ginsenoside Mc (G-Mc) by intestinal bacteria and crude snailase (*7, 11–20*).

There are many studies showing that C-K, C-Mx, and G-Mc exhibited antitumor activities *in vitro* as well as *in vivo* and were responsible for the main pharmacological activities of ginseng (*7, 14, 17, 24–30*).

In our previous work, several bioactive dammarane-type saponins and enzymatic conversion products had been characterized (*31–34*). The objective of this work was to explore an efficient procedure for transforming saponins of *P. notoginseng* leaves (*PNLS*) to bioactive compounds (i.e., C-K, C-Mx, and G-Mc). For this purpose, an efficient filamentous fungus, *Fusarium sacchari* (*F. sacchari*), was first selected from the soil-cultivated ginseng, which showed high capability for such transformation under the optimized conditions. Furthermore, quantification and structural elucidation of the bioactive compounds (i.e., C-K, C-Mx, and G-Mc) were performed, and the

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Table 1. Calibration Curves and Conversion Equations for C-K, C-Mx, and G-Mc[a]

analyte	acetonitrile/water (v/v)	standard curves	r^2	test range (μg/mL)	conversion equations
C-K	60:40	$Y = 12093.48 + 333821.39X$	0.999	0.54–7.02	$X = (Y - 12093.48)V/(333.82vm)$
G-Mc	40:60	$Y = 164748.16X - 2755.41$	0.999	0.39–5.07	$X = (Y + 2755.41)V/(164.75vm)$
C-Mx	48:52	$Y = 21142.11 + 129057.97X$	0.999	1.10–8.80	$X = (Y - 21142.11)V/(129.06vm)$

[a] Y, peak area ratio (analyte/standard); X, concentration of compound in the transformed product (μg/mL).

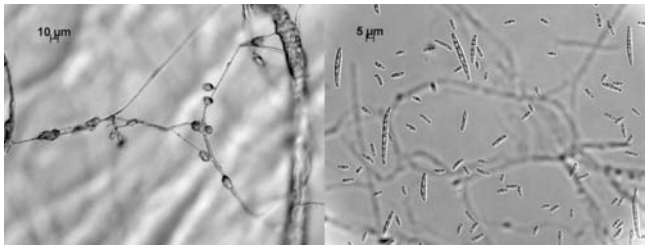


Figure 1. Microcharacteristic of *F. sacchari* with spores.

in vitro antitumor activities of C-K, C-Mx, and G-Mc and *in vivo* antitumor activities of the transformed product were also evaluated.

MATERIALS AND METHODS

General. TLC analysis was carried out on precoated silica gel GF_{254} plates. The plates were developed with the solvent $CHCl_3$–MeOH–H_2O (7:3:1 v/v/v, lower phase), sprayed with 10% (v/v) H_2SO_4 in ethanol, and heated at 105–110 °C for 10 min until visualization. Melting points were determined on a Yanaco MP-S3 micro-melting point apparatus (uncorrected). ^1H and ^{13}C NMR spectral data were obtained in pyridine-d_5 on a Bruker APX-300 with TMS as internal standard.

Table 2. Amount of C-K, C-Mx, and G-Mc by *F. sacchari* on Different Media

medium	C-K ($mg \cdot g^{-1}$)	C-Mx ($mg \cdot g^{-1}$)	G-Mc ($mg \cdot g^{-1}$)
PDA	146.93	233.90	93.44
wort	87.09	152.76	55.79
martin	111.62	198.31	78.66
zpek	47.45	66.20	15.35

Metabolites were analyzed by a high-performance liquid chromatography (HPLC) system (Hitachi L-7100) equipped with a quaternary gradient pump, a UV–visible detector coupled to a data processing computer (Zhejiang University N2000), a thermostat controlling the column temperature, and a manual injection valve. Other conditions were as follows: column, Kromasil ODS C18 (150 mm × 4.6 mm i.d., 5 μm); column temperature, 30 °C; flow rate, 1 mL/min; injection volume, 5 μL; detection wavelength, 203 nm; isocratic elution with CH_3CN–H_2O (55:45 v/v) during 18 min, followed by washing and reequilibrating the column to the initial conditions.

Materials and Chemicals. *P. notoginseng* leaves were obtained from Yunan Province (P. R. China) and identified by Liaoning University of Chinese Traditional Medicine. *F. sacchari* was screened from the soil-cultivated ginseng (Xinbin, Liaoning, P. R. China) and identified by the Institute of Microbiology, Chinese Academy of Sciences. Paclitaxel ($C_{47}H_{51}NO_{14}$, purity >99%, HPLC) was purchased from Sigma Chemical Co. (St. Louis, MO); ginsenosides C-K and G-Mc ($C_{36}H_{62}O_8$ and $C_{41}H_{70}O_{12}$, purity >97%, HPLC) were isolated by Dr. J. Binhui et al. (*31*, *32*); ginsenoside Rg_3 ($C_{42}H_{72}O_{13}$, purity >98%, HPLC) and C-Mx ($C_{41}H_{70}O_{12}$, purity >96%, HPLC) were obtained by silica gel column chromatography and preparative HPLC (C18 column) as described (*35*).

All chemicals and solvents were of analytical or HPLC grade. Cell culture media, fetal bovine serum (FBS), phosphate-buffered saline

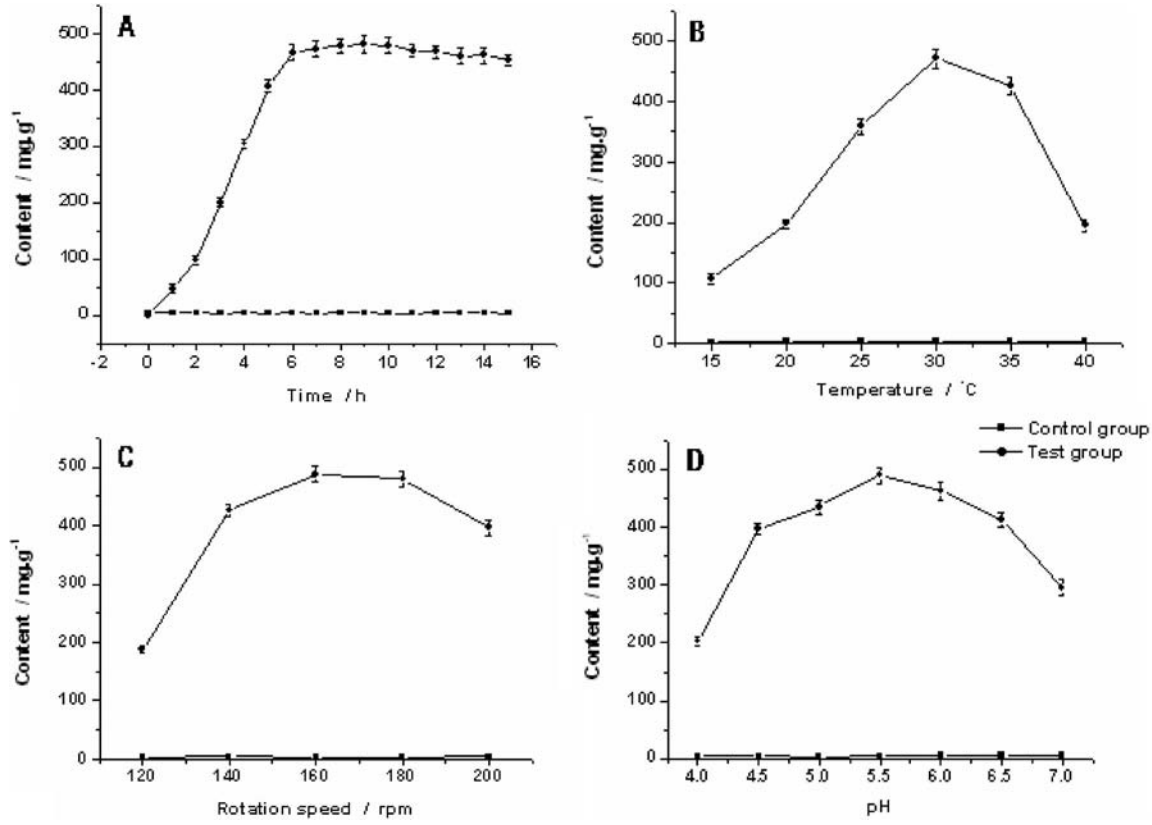


Figure 2. Effects of different transforming conditions on the amount of transformed product: (**A**) time; (**B**) temperature; (**C**) rotating speed; (**D**) pH.

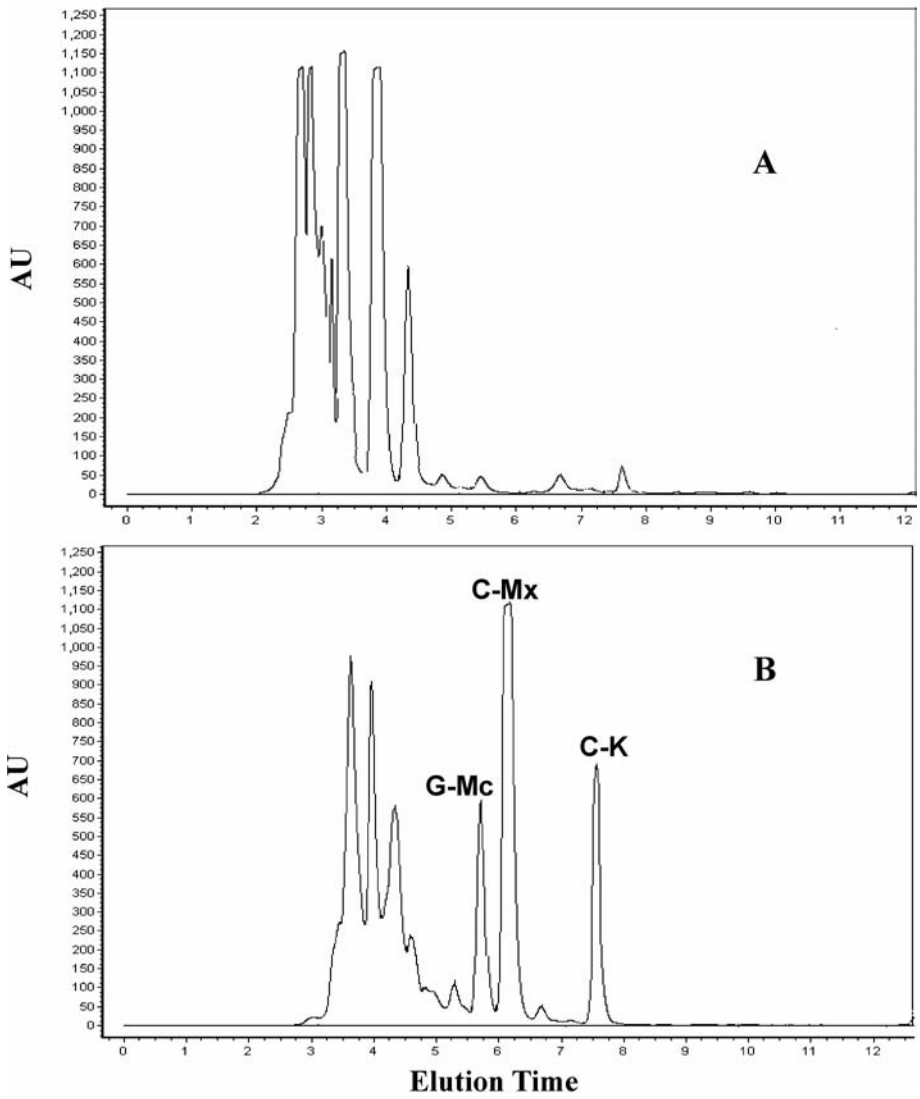


Figure 3. Typical HPLC chromatogram (203 nm) of *PNLS* (**A**) and the transformed product (**B**).

(PBS), sodium pyruvate, nonessential amino acids, penicillin–streptomycin, and other cell culture supplies were obtained from the Media Preparation Shared Facility of Shenyang Pharmaceutical University. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sino-American Biotechnology (Beijing, China).

Extraction of Tatol Saponins from *PNLS*. Air-dried *P. notoginseng* leaves (5 kg) were extracted twice with 70% ethanol. The combined extracts were evaporated under vacuum at 30 °C and then applied on a macro-reticular absorption resin (D101, 1.5 kg, supplied by Tianjin Chemical, Tianjin, P. R. China) column preconditioned with distilled water. Elution began with water to remove impurities and then with 70% ethanol to isolate the saponin fraction, which was dried with a spray dryer (YPG-100; Jiangyin Instrument Co., Ltd.) to yield total saponins (*PNLS*, 150 g).

Optimizing Conditions of the Transformation of C-K, C-Mx, and G-Mc by *F. sacchari*. Conical flasks (100 mL) containing 40 mL of medium were inoculated with *F. sacchari*. After 3 day incubation on a rotary shaker (160 rpm) at 28–30 °C, 1 mL of *PNLS* solution of 50 mg mL^{-1} was added to the substrate. Controls were composed of sterile medium to which the *PNLS* was added and under identical conditions incubated without *F. sacchari*. The reaction mixture was inoculated with *F. sacchari* and four media (martin, PDA, wort, and zpek) at different incubation times (1–15 days), temperature (15, 20, 25, 30, 35, and 40 °C), rotary speed of shaker (120, 140, 160, 180, and 200 rpm), and pH (4.0, 5.0, 5.5, 6.0, 7.0, and 8.0). The reaction mixture was extracted with 1-butanol, and the 1-butanol fraction was concentrated and dried under vacuum to yield the transformed product.

Quantification of C-K, C-Mx, and G-Mc. The contents of C-K, C-Mx, and G-Mc in the transformed product were determined by HPLC analysis. The three calibration curves were constructed with standard samples in duplicate. Every calibration curve consisted of six samples. Calibration curves and conversion equations for C-K, C-Mx, and G-Mc are shown in **Table 1**.

Isolation of C-K, C-Mx, and G-Mc from the Transformed Product. The transforming product (4.79 g) was subjected to chromatography on a silica gel column (250 g) and eluted in a stepwise manner with a $CHCl_3$–MeOH mixture (30:1, 20:1, 10:1, 5:1) to give 100 fractions (each of 50 mL).The fractions from 22–31 were collected and separated by Sephadex LH-20 and to yield C-K. Fraction 36–60 was rechromatographed by Sephadex LH-20 and a preparative ODS C18 column and eluted with MeOH–H_2O (88:22 v/v) with a flow rate of 3.0 mL/min and detection at 203 nm to afford successively C-Mx and G-Mc.

Cell Lines and Cell Culture. The human leukemia cell line HL-60, the human colon cancer cell line Colon205, the human gastric cancer cell line HGC-27, and the human prostate cancer cell line Du145 were prepared and maintained at 37 °C in RPMI 1640 containing 10% fetal bovine serum, 0.2% DMSO, and gentamycin sulfate (80 μg/mL).

C-K, C-Mx, and G-Mc were dissolved in DMSO at a suitable concentration and diluted with the growth medium. For drug exposure experiments, tumor cells were inoculated into 96-well plates containing serial 1:2 dilutions of C-K, C-Mx, and G-Mc in the presence or absence of C-K, C-Mx, and G-Mc in a final volume of 100 mL of growth medium and incubated at 37 °C in a humidified atmosphere of 5%

Table 3. ^{13}C NMR Data for C-K, C-Mx, and G-Mc[a]

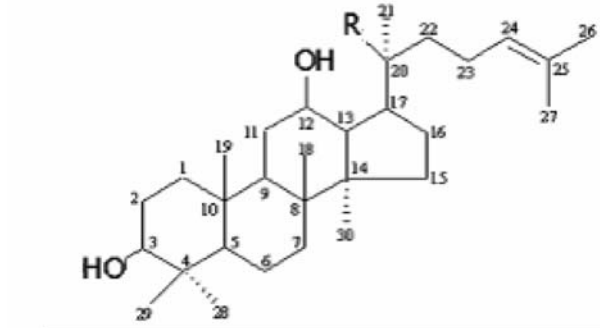
carbon	C-K	C-Mx	G-Mc
	Aglycon Moiety		
1	39.4	39.4	39.5
2	27.8	28.3	28.3
3	79.3	78.1	78.2
4	39.4	39.6	39.6
5	56.3	56.4	56.5
6	18.7	18.8	18.0
7	35.2	35.2	35.3
8	40.2	40.1	40.1
9	50.5	50.3	50.4
10	37.6	37.4	36.3
11	31.4	30.9	30.8
12	71.3	70.3	70.2
13	48.3	49.5	49.5
14	50.0	51.4	51.5
15	31.7	30.9	30.8
16	26.9	26.7	26.7
17	53.9	51.7	51.7
18	16.5	16.3	16.1
19	16.1	16.1	16.1
20	74.9	83.4	83.6
21	26.1	22.4	22.3
22	34.9	36.2	36.3
23	23.2	23.2	23.2
24	125.9	126.1	126.1
25	130.9	131.0	131.1
26	25.8	25.8	25.9
27	17.8	17.9	17.5
28	28.7	28.7	28.3
29	16.4	16.4	16.4
30	17.4	17.5	17.5
	20-Sugar Moieties		
1′	98.3	98.1	98.1
2′	75.2	75.1	74.9
3′	79.4	79.3	79.4
4′	71.7	72.2	71.6
5′	78.1	76.6	77.0
6′	62.9	68.6	70.2
1″		110.2	105.9
2″		83.4	74.2
3″		78.9	77.0
4″		86.1	71.1
5″		62.7	67.0

[a] All spectra were recorded in pyridine-d_5. Chemical shifts in ppm are relative to internal TMS. The spectra were recorded at 300 MHz.

CO_2 air for 24 h. After incubation, 10 μL of MTT solution (0.25 mg/mL) was added to each well and incubated for a further 4 h. At the end of the incubation, the growth medium was removed and replaced with 100 mL of DMSO (at room temperature). After agitation on a vortex for 5 min, the absorbance at 492 nm was determined on a Bio-Rad (model 550) microplate reader to calculate the 50% inhibition concentration (IC_{50}).

Animals. Experimental animals used were Kunming species mice (20 ± 2 g) from the Experimental Animal Center of Liaoning University of Chinese Traditional Medicine (Shenyang, Liaoning, P. R. China). The animals were housed in climate-controlled quarters (temperature, 25 ± 1 °C; relative humidity, $50 \pm 10\%$) with a 14 h light/10 h dark cycle. Food and water were available ad libitum. All animal use procedures were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, 1988.

***In Vitro* Cytotoxicity Assay.** All of the four cell lines were seeded in 96-well plates at a density of $(1.0–1.5) \times 10^5$ cells/well in 100 μL of RMPI 1640 or DMEM containing 10% FBS for 24 h. Then cells were treated with various concentrations of the drugs. After 24 h of drug exposure, 10 μL of MTT solution (2.5 mg/mL) was added to each well for another 4 h at 37 °C. The formazine was solved in 100 μL/well dimethyl sulfoxide (DMSO), and the absorbance at 490 nm was measured using an enzyme microplate reader (TECAN).



Compound	R	MF
C-K	O-Glc	$C_{36}H_{62}O_8$
C-Mx	O-Glc$^{6\text{-}1}$Xyl	$C_{41}H_{70}O_{12}$
G-Mc	O-Glc$^{6\text{-}1}$Araf	$C_{41}H_{70}O_{12}$

Figure 4. Chemical structures and molecular formulas of C-K, C-Mx, and G-Mc.

The percentage of cytotoxicity was calculated as

$$\text{viability (\%)} = A_e/A_c$$

where A_e is the absorbance at 490 nm of the experimental well and A_c is the absorbance at 490 nm of the control well.

The median inhibitory concentration (IC_{50}) was assessed from the dose–response curves.

***In Vivo* Antitumor Activity of Transformed Products.** Ginsenoside Rg_3, Paclitaxel, and transformed products with *F. sacchari* were dissolved in a mixture of DMSO–PEG 400–DDW (1:4:5 v/v/v), which was tested in control experiments at the same dose (20 mg/kg).

Mice were divided into four groups including the untreated control group and three test groups to which 20 mg/kg ginsenoside Rg_3, Paclitaxel, and the transformed products were respectively administered *op* 3 days after inoculating S180 sarcomata in the axilla. They were killed after 12 days, and then the tumors were removed and weighed. The rate of inhibition of tumor growth (RITG) was calculated according to the equation:

$$\text{RITG (\%)} = [(X - Y)/X] \times 100$$

where X is the average tumor weight of the control group and Y is the average tumor weight of test groups.

The livability (L), thymus index (Ti), and milt index (Mi) are defined respectively as

$$L\ (\%) = \text{Nt/N0} \times 100$$

$$\text{Ti} = 10 \times \text{Tw/Bw}$$

$$\text{Mi} = 10 \times \text{Mw/Bw}$$

where Nt is the number of mice before being put to death in each group, N0 is the number of mice before the experiment in each group, Tw is thymus weight (rag), and Bw is the body weight of the mice after the experiment (grams).

RESULTS

Optimization of Transformation Medium. *PNLS* was transformed by *F. sacchari* in four different media: martin, PDA, wort, and zpek. The amounts of C-K, C-Mx, and G-Mc transformed by *F. sacchari* in these media are given in **Table 2**. It can be noted that the levels of C-K, C-Mx, and G-Mc transformed in the PDA medium were the highest among four media, followed by those in martin and wort media, while zpek

Table 4. Inhibition of Growth of Human Cancer Cells by C-K, C-Mx, and G-Mc[a]

Comp.	HL-60 (CV%)				HGC-27 (CV%)				Colon205 (CV%)				Du145 (CV%)			
	10μM	25μM	50μM	100μM	10μM	25μM	50μM	100μM	10μM	25μM	50μM	10μM	10μM	25μM	5μM	100μM
C-K																
C-Mx																
G-Mc																

[a] CV = cell viability; inhibition <20%, in light gray; inhibition 20–90%, in dark gray; inhibition >90%, in black.

Table 5. Growth Inhibitory Activity of C-K, C-Mx, and G-Mc in Human Cancer Cells

		IC_{50} (μM)		
cancer type	cell line	C-K	C-Mx	G-Mc
leukemia	HL-60	11.71	56.81	59.01
gastric	HGC-27	30.86	111.39	157.22
colon	Colon205	50.18	140.28	185.68
prostate	Du145	58.65	154.57	198.64

Table 6. Antitumor Activity *in Vivo* of the Transformed Product

compd	dose ($mg \cdot kg^{-1}$)	no. of mice (pre/post)	livability (%)	av wt of tumor (g)	RITG (%)
control[a]	20	10/8	80	2.84 ± 0.44	
transformed product	20	10/10	100	0.69 ± 0.08^b	75.7
ginsenoside Rg_3	20	10/10	100	1.18 ± 0.23^b	58.5
Paclitaxel	20	10/9	90	1.27 ± 0.31^b	55.3

[a] A mixture solvent of DMSO–PEG 400–DDW (1:4:5 v/v/v). [b] $p < 0.01$ compared with control.

is the worst medium for *F. sacchari* to transform these compounds. In fact, using zpek medium, the spores presented white, rather than purple, mature ones.

Optimal Transformation Conditions of *F. sacchari.* The transforming efficiency depended on several critical factors, such as transforming time, transforming temperature, rotary speed, and pH of the medium. **Figure 2** presents the effects of these factors on the amount of transformed products by *F. sacchari* in PDA medium, respectively.

From the results, the optimized transforming conditions for C-K, C-Mx, and G-Mc were as follows: transforming temperature, 30 °C; transforming time, 6 days; rotary speed, 160 rpm; pH, 5.5. Under such optimal conditions, the C-K content of transformed products by *F. sacchari* was 186.93 ± 0.57 mg/g, 215 times higher than those of the control (0.87 ± 0.36 mg/g) (23.8%). The contents of C-Mx and G-Mc from transformed products were 230.33 ± 0.89 mg/g (36.0%) and 95.61 ± 0.65 mg/g (15.0%), respectively. The HPLC chromatograms of *PNLS* and the transformed product are shown in **Figure 3**.

Structural Identification. C-K (*29–31*): white powder (EtOH–H_2O), mp 177–178 °C. ^{1}H NMR (300 MHz, pyridine-d_5): δ 0.87 (3H, s, CH_3-19), 0.91 (3H, s, CH_3-18), 0.93 (3H, s, CH_3-30), 0.99 (3H, s, CH_3-29), 1.58 (6H, s, CH_3-26, 27), 1.61 (3H, s, CH_3-21), 5.19 (1H, d, $J = 7.7$ Hz, H-1′-20-Glc). ^{13}C NMR (300 MHz, pyridine-d_5): data and its structure are shown in **Table 3** and **Figure 4**.

G-Mc ((*36*)): white powder (EtOAc), mp 181–183 °C. ^{1}H NMR (300 MHz, pyridine-d_5): δ 0.87 (3H, s, CH_3-19), 0.92 (3H, s, CH_3-18), 0.98 (3H, s, CH_3-30), 1.02 (3H, s, CH_3-29), 1.21 (3H, s, CH_3-28), 1.60 (3H, s, CH_3-26), 1.62 (3H, s, CH_3-27), 1.65 (3H, s, CH_3-21), 5.12 (1H, d, $J = 7.7$ Hz, H-1′-20-Glc), 5.65 (1H, $J = 1.7$Hz, H-1″-6′-Araf). ^{13}C NMR (300 MHz, pyridine-d_5): data and its structure are shown in **Table 3** and **Figure 4**.

C-Mx ((*37*)): white powder (EtOAc), mp 162.5–165 °C. ^{1}H NMR (300 MHz, pyridine-d_5) δ 3.58 (1H, t, $J = 10.5$, 5.1 Hz, H-3), 0.80 (1H, d, $J = 11.0$ Hz, H-5), 3.99 (1H, ddd-like, H-12), 5.30 (1H, t, $J = 7.1$ Hz, H-24), 0.94 (3H, s, H-18), 0.87 (3H, s, H-19), 1.64 (3H, s, H-21), 1.67 (3H, s, H-26), 1.67 (3H, s, H-27), 1.21 (3H, s, H-28), 1.02 (3H, s, H-29), 0.98 (3H, s, H-30), 5.11 (1H, d, $J = 7.8$ Hz, H-1′), 5.61 (1H, $J = 1.7$ Hz, H-1″). ^{13}C NMR (300 MHz, pyridine-d_5): data and its structure are shown in **Table 3** and **Figure 4**.

***In Vitro* Cytotoxicity Assay.** The *in vitro* activity of C-K, C-Mx, and G-Mc was evaluated by use of the MTT assay. Four cell lines (leukemia, HL-60; gastric cancer, HGC-27; colon cancer, Colon205; prostate cancer, Du145) were cultured and incubated with tested compounds in concentrations ranging from 0 to 250 μM for 72 h, and growth inhibitory activity of C-K, G-Mc, and C-Mx in human cancer cells was determined as shown in **Table 4**. The IC_{50} values for the three compounds were calculated, and the results are presented in **Table 5**.

***In Vivo* Antitumor Activity of the Transformed Product.** The antitumor effects of the transformed product on S180 sarcomata in mice are shown in **Table 6** and **7**. It has been shown clearly that the *in vivo* antitumor activity of the transformed product obtained under the optimized conditions was significantly higher than those of ginsenoside Rg_3 and Paclitaxel.

Table 7. Immune Organ Weights and Immune Organ Indexes of the Transformed Product

compound	dose ($mg \cdot kg^{-1}$)	no. of mice (pre/post)	immune organ wt (g), thymus spleen	mice wt (post) (g)	immune organ index, thymus spleen
control[a]	20	10/8	0.072 ± 0.017 0.208 ± 0.034	35.9 ± 4.33 57.94 ± 13.65	23.16 ± 12.11
transformed product	20	10/10	0.102 ± 0.011^c 0.284 ± 0.036^c	34.2 ± 2.21 83.04 ± 18.71^c	28.93 ± 8.96
ginsenoside Rg_3	20	10/10	0.109 ± 0.014^c 0.226 ± 0.015	31.7 ± 3.18^b 71.29 ± 10.92^b	30.85 ± 13.26
Paclitaxel	20	10/9	0.115 ± 0.021^c 0.201 ± 0.027	33.0 ± 3.47 60.91 ± 15.62	31.06 ± 14.51

[a] A mixture solvent of DMSO–PEG 400–DDW (1:4:5 v/v/v). [b] $p < 0.05$. [c] $p < 0.01$ compared with control.

DISCUSSION

Owing to the potent antitumor activities *in vitro* as well as *in vivo* of C-K, C-Mx, and G-Mc, which were believed to be responsible for the main pharmacological activities of ginseng, the transformation of such bioactive compounds with high efficiency has been a subject of several authors (*38–41*). In this work, we have been successful in isolating, for the first time, one filamentous fungus, *F. sacchari*, which showed potent transforming capacity of C-K, C-Mx, and G-Mc. Furthermore, the conditions of transforming C-K, C-Mx, and G-Mc by *F. sacchari* were optimized, which permitted to obtain high levels of such bioactive compounds in the transformed product. These results may have practical importance in developing the antitumor compounds, i.e., C-K, C-Mx, and G-Mc.

It should be mentioned that the transforming efficiency of *F. sacchari* depends on its concentrations and activities of enzymes produced in metabolism. In other words, the optimized transforming conditions for C-K, C-Mx, and G-Mc by *F. sacchari* may be the optimized conditions to produce the active enzyme from *F. sacchari* in metabolism. The results should be of interest from the point of view of research and developing enzyme preparation.

C-K, G-Mc, and C-Mx are the metabolites of protopanaxadiol ginsenosides Rb_1, Rb_2, Rb_3, and Rc, which are the main components of *PNLS* produced by intestinal bacteria in humans, rats, and crude snailase and had moderate cytotoxicity (*7, 26, 42, 43*). In our present work, C-K, C-Mx, and G-Mc were evaluated for their *in vitro* activities by use of the MTT assay. Four cell lines were cultured and incubated with test compounds in concentrations ranging from 0 to 250 μM for 72 h, and viability of the cells was determined. The IC_{50} values for the three compounds were calculated (**Table 5**). Substantial variations of sensitivity to the compounds were observed among the different cell lines. For C-K, the IC_{50} values for four cell lines were in the lower micromolar range, a 3–5-fold greater cytotoxicity relative to C-Mx and G-Mc. C-K inhibited the growth of human cancer cells (HL-60, HGC-27, Colon205, and Du145) in a dose-dependent manner. The results demonstrated that the cytotoxicities of the C-K, C-Mx, and G-Mc were dependent upon their chemical structures, particularly the number of sugar moieties linked to the core structure, because C-Mx and G-Mc differ from C-K in the presence of an additional sugar moiety, which is β-D-xylopyranosyl for C-Mx but α-L-arabinofuranosyl for G-Mc (**Figure 4**).

On the other hand, the *in vivo* antitumor activities of the transformed product were also verified and compared with those of ginsenoside Rg_3, which is currently in use for cancer therapy (*44, 45*), and Paclitaxel. As expected, the transformed product obtained under the optimized conditions showed a much higher inhibition rate of tumor growth (75.7%) than those of ginsenoside Rg_3 (58.5%) and Paclitaxel (55.3%). From these results, it is a reasonable suggestion that C-K, C-Mx, and G-Mc would be the main compounds responsible for antitumor activities of the transformed product, even though the mechanisms of action for the latter product have not been elucidated.

In conclusion, this is the first report of the high transformation efficiency of bioactive C-K, C-Mx, and G-Mc by a new selected fungus, *F. sacchari*. The *in vitro* study showed that C-K possessed much more potent antitumor activity than C-Mx and G-Mc, and the transformed product presented much higher *in vivo* antitumor activity than the commonly used ginsenoside Rg_3 and Paclitaxel. Further molecular and pharmacological studies on the transformed product are underway in order to reassess its *in vivo* antitumor activities and to identify its underlying mechanisms of action. The results will be useful for preparing the bioactive products such as C-K, C-Mx, and G-Mc and developing the transformed product into a novel adjuvant for the treatment of cancer or antitumor.

ABBREVIATIONS USED

C-K, 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol; C-Mx, 20-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl-20(*S*)-protopanaxadiol; G-Mc, 20-*O*-α-L-arabinofuranosyl-β-D-glucopyranosyl-20(*S*)-protopanaxadiol; *PNLS*, saponins of *Panax notoginseng* leaves; *F. sacchari*, *Fusarium sacchari*.

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