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DNA Topoisomerases I and II Inhibitory Activity and Cytotoxicity of Compounds from the Stems of *Parthenocissus tricuspidata*

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Activity-directed isolation of the methylene chloride fraction from the stems of *Parthenocissus tricuspidata* have led to the identification of two new compounds (**1**–**2**): 1-(2',3',5'-trihydroxyphenyl)-2-(4"-hydroxyphenyl)-ethane-1,2-(*E*)-epoxide (**1**, tricuspidatin A) and *erythro*-1-(3',5'-dihydroxyphenyl)-2-(4"-hydroxyphenyl)-ethane-1,2-diol (**2**, tricuspidatin B), together with four known compounds (**3**–**6**): *β*-sitosterol (**3**), nonacosan-1-ol (**4**), 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid hexacosyl ester (**5**) and betulinic acid (**6**). Their chemical structures were elucidated based on spectroscopic (IR, UV, MS, 1D and 2D NMR) and physicochemical analyses. Compounds **1** and **2** showed strong DNA topoisomerase II inhibitory activity at both concentrations of 20 and 100 μM. In addition, **3** exhibited strong cytotoxic activity against the HT-29 and HepG2 cancer cell lines, and **6** showed strong cytotoxicity against the HT-29 and MCF-7 ones.

Key Words : *Parthenocissus tricuspidata*, Vitaceae, DNA topoisomerases I and II, Cytotoxicity

Introduction

DNA topoisomerases are enzymes involved in the relaxation of DNA during a number of critical cellular processes, including replication, recombination, and transcription by transiently breaking one or two strands of DNA, passing a single- or double-stranded DNA through the break, and resealing the break.[1-3] There are five types of topoisomerases: IA, IB, IC, IIA and IIB. Human topoisomerase I is a type IB enzyme that cleaves only one strand of the DNA duplex, whereas human topoisomerase II is a type IIA enzyme that cleaves both strands. Type IB topoisomerase is thought to have evolved from a common ancestor as tyrosine recombinases and is structurally distinct from other types of topoisomerases. Type IIA topoisomerase from bacterial DNA gyrase and human topoisomerase II from topoisomerase IV are highly conserved. Only the camptothecin (CPT) family of compounds as DNA topoisomerase I-directed drugs has been introduced into the clinic to date,[4,5] although many topoisomerase II-directed drugs have been in clinical use for many years.[6] DNA topoisomerases have been established as important molecular targets for anticancer drugs.[7]

During our search for new DNA topoisomerase inhibitors from natural products using activity-guided fractionation, the organic solvent extracts of 40 medicinal plants, which are used in traditional medicine as anticancer agents, were screened for their DNA topoisomerases inhibitory activities. Among them, the methanol extract of the stems of *Parthenocissus tricuspidata* (Sieb. et Zucc.) Planch showed potent DNA inhibitory activity. *P. tricuspidata* (Vitaceae) is a woody vine that typically grows 30–50 feet or more. The leaves have been used as folk medicine in South Asia for treating arthritis, jaundice, insect bites, and neuralgia.[8] Previous phytochemical studies on this plant had revealed that it is a rich source of phenolic compounds.[9-12] Several caffeic acid derivatives have been isolated and evaluated for antioxidant activities in DPPH free radical scavenging and superoxide anion scavenging assays.[12] Stilbene dimers as parthenostilbenins were isolated from the ethyl acetate fraction of an aqueous alcohol extract of *P. tricuspidata* stems and assessed for antioxidant activity against lipid peroxidation in a rat liver homogenate.[9] A new stilbene glycoside has been isolated from the MeOH extract of *P. tricuspidata* leaves together with piceid, resveratrol, and longistylins A and C. The antiplasmodial activity of these compounds was determined *in vitro* against a chloroquine-sensitive strain of *Plasmodium falciparum*.[13] The present study describes the isolation and characterization of two new phenolic compounds (**1**–**2**) and four known ones (**3**–**6**) from the methylene chloride fraction of this plant.

Experimental Section

General Procedures. Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. Infrared (IR) spectra were measured on a JASCO FT/IR 300E spectrophotometer. Ultraviolet (UV) spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. ^{1}H, ^{13}C, correlation spectroscopy (COSY), heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC) nuclear magnetic resonance (NMR) spectra were recorded on Varian Unity INOVA-500 and Bruker DMX 250 spectrometers with TMS as the internal standard. Low-resolution (FAB-MS) and high-resolution fast atom bombardment mass spectroscopy (HR-FAB-MS) data were collected on a Quattro II spectrometer. Silica gel 60 F-254 (EM 5717) glass plates (0.25 mm) were used for thin-layer chromatography (TLC) and visualized by spraying with 10% sulfuric acid and heating.

Plant Material. The stems of *P. tricuspidata* were collected in August 2002 from the Palgong mountain of Kyung-Buk, Korea, and dried at room temperature for 2 weeks. The plant was verified by Professor Byung Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. A voucher specimen (CUDP 2002-02) was deposited at the College of Pharmacy, Catholic University of Daegu, Korea.

Extraction and Isolation. Stems of *Parthenocissus tricuspidata* (10 kg) were dried at room temperature, cut into small pieces, and extracted with methanol (MeOH) at refluxing temperature to yield about 1.2 kg of MeOH extract. This extract was suspended in distilled H_2O and successively partitioned with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAC), and *n*-butanol (*n*-BuOH) to yield each three fraction. Among those fractions, we chose CH_2Cl_2 fraction for activity-guided isolation which had the best DNA Topoisomerase I and II inhibitory activity. The CH_2Cl_2 soluble fraction (127.6 g) was subjected to flash column chromatography (CC) over silica gel (2.0 kg) eluted with *n*-hexane-EtOAC, EtOAC-CH_2Cl_2, and a CH_2Cl_2-MeOH gradient to increase polarity to yield 36 fractions (PT-MC-1–PT-MC-36). The PT-MC-30 fraction (2.0 g) was subjected to reverse-phase column chromatography (C_{18}) and eluted with MeOH-H_2O (1:4) to give compounds **1** (14.5 mg) and **2** (31.2 mg), respectively. The PT-MC-7 fraction (3.8 g) by silica gel CC using *n*-hexane-CH_2Cl_2 (1:1 to 0:1) and CH_2Cl_2-MeOH (1:0 to 0:1) gradient yielded 15 subfractions (PT-MC-7-1–15). The PT-MC-7-14 fraction (1.2 g) was chromatographed on a silica gel column using a mixed solvent system of *n*-hexane-CH_2Cl_2 (3:2 to 0:1) to obtain compound **3** (905.6 mg). The PT-MC-7-9 fraction (712.5 mg) was isolated on silica gel column chromatography (CC) using *n*-hexane-CH_2Cl_2 (9:1 to 0:1) to obtain compounds **4** (8.3 mg) and **5** (302.3 mg), respectively. The PT-MC-12 fraction (1.7 g) was isolated on a silica gel column, eluted with *n*-hexane-CH_2Cl_2 (1:9 to 0:1) and a CH_2Cl_2-MeOH (1:0 to 0:1) gradient to yield 21 subfractions (PT-MC-12-1–21). The PT-MC-12-12 fraction (375.3 mg) was isolated on silica gel CC using a CH_2Cl_2-MeOH (149:1–0:1) gradient to obtain compound **6** (217.8 mg). The purity of all isolated compounds was assessed by TLC, NMR and MS, and was > 99%.

Compound 1: Orange powder. $[\alpha]_D^{23}$: –42.0° (*c* 0.1, MeOH). IR (KBr) cm^{-1}: 3380 (OH), 1616, 1511 (C=C). UV (MeOH) λ_{max} (log ε): 277 (2.35), 223 (3.71), 203 (2.48) nm. FAB-MS: *m/z* 261 $[M+H]^+$. HR-FAB-MS: *m/z* 261.0768 $[M+H]^+$ (calcd. 261.0763 for $C_{14}H_{13}O_5$). ^1H NMR (CD_3OD, 250 MHz) δ 3.71 (1H, s, H-1), 4.46 (1H, s, H-2), 6.10 (1H, d, *J* = 1.9 Hz, H-4), 6.52 (1H, d, *J* = 1.9 Hz, H-6), 6.93 (2H, d, *J* = 8.5 Hz, H-2, 6), 6.66 (2H, d, *J* = 8.5 Hz, H-3, 5). ^{13}C NMR (CD_3OD, 62.5 MHz) δ 60.9 (C-1), 54.7 (C-2), 113.8 (C-1), 150.8 (C-2), 155.5 (C-3), 102.5 (C-4), 159.3 (C-5), 103.3 (C-6), 138.4 (C-1), 129.2 (C-2, 6), 116.0 (C-3, 5), 156.3 (C-4).

Compound 2: Orange powder. $[\alpha]_D^{23}$: 113.5° (*c* 0.1, MeOH). IR (KBr) cm^{-1}: 3385 (OH), 1612, 1516 (C=C). UV (MeOH) λ_{max} (log ε): 277 (2.41), 224 (3.76), 203 (2.52) nm. FAB-MS: *m/z* 263 $[M+H]^+$. HR-FAB-MS: *m/z* 263.0925 $[M+H]^+$ (calcd. 263.0919 for $C_{14}H_{15}O_5$). ^1H NMR (CD_3OD, 250 MHz) δ 3.42 (1H, d, *J* = 6.4 Hz, H-1), 5.19 (1H, d, *J* = 6.4 Hz, H-2), 6.08 (2H, d, *J* = 1.8 Hz, H-2, 6), 6.06 (1H, d, *J* = 1.8 Hz, H-4), 7.17 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.73 (2H, d, *J* = 8.6 Hz, H-3, 5). ^{13}C NMR (CD_3OD, 62.5 MHz) δ 89.1 (C-1), 63.9 (C-2), 141.6 (C-1), 107.7 (C-2), 159.4 (C-3), 102.2 (C-4), 159.4 (C-5), 107.7 (C-6), 133.6 (C-1), 128.7 (C-2), 113.1 (C-3), 158.1 (C-4), 113.1 (C-5), 128.7 (C-6).

DNA Topoisomerase I Inhibition Assay *in vitro*. The DNA topoisomerase I inhibition assay was carried out according to a method reported previously with minor modifications. DNA topoisomerase I activity was measured by assessing the relaxation of supercoiled pBR 322 plasmid DNA. The reaction mixture was comprised of 35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM spermidine, 0.01% bovine serum albumin (BSA), 250 ng pBR 322 plasmid DNA, and 0.3 U calf thymus DNA topoisomerase I. The reaction mixture was used to measure the inhibition of DNA relaxation by DNA topoisomerase I, in addition to a test compound solution (< 0.25% DMSO) in a final volume of 10 μL. The reaction mixtures were incubated for 30 min at 37 ºC, and terminated by adding a dye solution comprised of 2.5% SDS, 15% Ficoll-400, 0.05% bromophenol blue, 0.05% xylene cyanole, and 25 mM EDTA (pH 8.0). The reaction products were determined by electrophoresis on a 1% agarose gel in TBE (Tris-borate-EDTA) running buffer at 1.5 V/cm for 10 h. The gels were stained with ethidium bromide (0.5 μg/mL) for 30 min then distained in water for 30 min. The gels were directly scanned with an image analyzer to quantify DNA topoisomerase I activity, and the area representing supercoiled DNA was calculated.[14]

DNA Topoisomerase II Inhibition Assay *in vitro*. DNA topoisomerase II activity was measured by assessing relaxation of supercoiled pBR 322 plasmid DNA. The reaction mixtures contained 50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol, 300 ng pBR 322 plasmid DNA, 0.3 U human DNA topoisomerase II, and the indicated compound concentrations (< 0.25% DMSO), in a final volume of 20 μL. The reaction mixtures were incubated for 30 min at 37 ºC, and terminated by adding 5 μL of a mixture containing 0.77% SDS, 77 mM EDTA (pH 8.0), 30% sucrose, 0.5% bromophenol blue, and 0.5% xylene cyanole. The reaction products were determined by electrophoresis on a 1% agarose gel in TBE running buffer at 1.5 V/cm for 10 h. The gels were stained with 0.5 ìg/mL ethidium bromide for 30 min and destained in water for 30 min. The gels were directly scanned with an image analyzer to quantify DNA Topoisomerase II activity, and the area representing supercoiled DNA was calculated.[14]

Cytotoxicity Bioassays. The MTT assay was used as the *in vitro* cytotoxicity assay against HT-29 (Human colon carcinoma), HepG2 (Human liver carcinoma) and MCF-7 (Human breast carcinoma) cell lines.[15]

Statistical Analysis. All data in the present study were obtained as average of experiments that were performed in triplicate and are expressed as mean ± S.E. Statistical signifi-

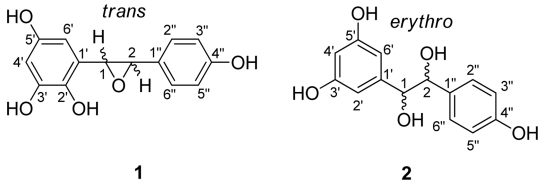

Figure 1. Chemical structures of two new compounds (**1** and **2**).

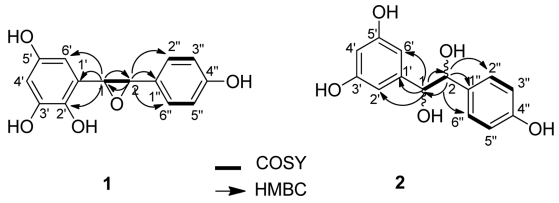

Figure 2. COSY and HMBC correlations of **1** and **2**.

cance was determined using the software SPSS 19.0.

Results and Discussion

From the methylene chloride fraction, we isolated two new compounds, tricuspidatins A and B (**1**–**2**) (Fig. 1), and four known ones (**3**–**6**), *β*-sitosterol (**3**),[16] nonacosan-1-ol (**4**),[17] 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid hexacosyl ester (**5**)[18] and betulinic acid (**6**),[19] and investigated their DNA topoisomerases I and II inhibitions[20] together with cytotoxicities against HT-29, MCF-7 and HepG2 cell lines.[21] Compounds **4**, **5** and **6** were first isolated from *P. tricuspidata.* Known compounds were identified by direct comparison with authentic samples, or by comparing their physical and spectral data with those in the literature.

Compound **1** was obtained as an amorphous powder. The FAB-MS values of **1** gave a $[M+H]^+$ peak at *m/z* 261. The HR-FAB-MS gave *m/z* 261.0768 for the $[M+H]^+$, which corresponded to the molecular formula of $C_{14}H_{13}O_5$ (calcd. 261.0763). The ^{1}H NMR spectrum of **1** showed aromatic proton signals at 6.10 (1H, d, J = 1.9 Hz, H-4') , and δ 6.52 (1H, d, J = 1.9 Hz, H-6'), characteristic of a 1',2',3',5'-tetrasubstituted aromatic ring, along with one set of *ortho*-couple at δ 6.93 (2H, d, J = 8.5 Hz, H-2", 6") and 6.66 (2H, d, J = 8.5 Hz, H-3", 5") assignable to one *para*-hydroxy phenyl group in an AA'XX'-type arrangement. The two singlet proton signals at δ 3.71 (1H, s, H-1) and δ 4.46 (1H, s, H-2) were indicated for an epoxide group with a *trans* form.[22] The ^{13}C NMR spectrum of **1** exhibited 14 carbon signals that supported the presence of two benzene ring systems, a 1',2',3',5'-tetrasubstituted aromatic ring at δ 113.8 (C-1'), 150.8 (C-2'), 155.5 (C-3'), 102.5 (C-4'), 159.3 (C-5') and 103.3 (C-6')], and a 1",4"-disubstituted aromatic ring at δ 138.4 (C-1"), 129.2 (C-2", 6"), 116.0 (C-3", 5"), and 156.3 (C-4"), and one epoxide ring at δ 60.9 (C-1) and 54.7 (C-2). The above spectroscopic data were confirmed by correlation signals from the HMQC and HMBC spectra (Fig. 2). The H-1 (δ 3.71) proton of the epoxide group showed long-range HMBC correlations with C-1', C-2', C-6', and C-2, whereas the other proton, H-2, showed long-range HMBC correlations with C-1", C-2", C-6", and C-1. The ^{1}H-^{1}H COSY spectrum exhibited three correlation pairs as H-1 and H-2, H-2" and H-3", and H-5" and H-6". Hence, the structure of **1** was elucidated as 1-(2',3',5'-trihydroxyphenyl)-2-(4"-hydroxyphenyl)-ethane-1,2-(*E*)-epoxide, and named tricuspidatin A. This is the first time this compound has been isolated from a natural source and has never been synthesized before.

Compound **2** was obtained as an amorphous powder with optical rotation of $[\alpha]_D^{23}$ 113.5° (*c* 0.1, MeOH), while that of *threo* form isolated by Xiao *et al.*[23] was $[\alpha]_D^{25}$ 13.5° (*c* 0.1, MeOH). The FAB-MS value of **2** gave a $[M+H]^+$ peak at *m/z* 263. The HR-FAB-MS gave m/z 263.0925 for the $[M+H]^+$, which corresponded to the molecular formula of $C_{14}H_{15}O_5$ (calcd. 263.0919). The UV spectrum showed maxima at 277, 224, and 203 nm (MeOH). The IR spectrum showed strong bands for a hydroxyl (3385 cm^{-1}) and an aromatic ring (1612, 1516 cm^{-1}). The ^{1}H-NMR spectrum of **2** contained three sets of signals, including one set of *ortho*-coupled protons assignable to one *para*-hydroxy phenyl group in an AA'XX'-type arrangement [δ 7.17, 6.73 (2H each, d, J = 8.6 Hz)], a set of protons assignable to a 1',3',5'-trisubstituted phenyl group [δ 6.06 (H, d, J = 1.8 Hz), 6.08 (2H, d, J = 1.8 Hz)], and two aliphatic protons [δ 3.42 (1H, d, J = 6.4 Hz), 5.19 (1H, d, J = 6.4 Hz)]. The ^{13}C NMR spectrum of **2** also exhibited 14 signals for two benzene ring systems, a 1',3',5'-trisubstituted and a 1",4"-disubstituted aromatic ring and two oxygenated methine carbons at δ 89.1 (C-1) and 63.9 (C-2). It can be inferred that **2** was a resveratrol derivative; however, the double bond was saturated and oxygenated into a diol. This characteristic was very similar with 1-(3',5'-dihydroxyphenyl)-2-(4"-hydroxyphenyl)-ethane-1,2-diol, a phenolic compound isolated from *Polygonum cuspidatum*. In that study, the two aliphatic proton signals (δ 4.41 and 4.52 ppm) presented as a respective doublet form and the coupling constants were 7.6 Hz; thus, these two hydroxyls

Table 1. ^{1}H and ^{13}C NMR spectra of **1** and **2** (in CD_3OD)

Position	**1**		**2**	
	δ_H (ppm, J in Hz)	δ_C (ppm)	δ_H (ppm, J in Hz)	δ_C (ppm)
1	3.71 (1H, s)	60.9	3.42 (1H, d, 6.4)	89.1
2	4.46 (1H, s)	54.7	5.19 (1H, d, 6.4)	63.9
1'		113.8		141.6
2'		150.8	6.08 (1H, d, 1.8)	107.7
3'		155.5		159.4
4'	6.10 (1H, d, 1.9)	102.5	6.06 (1H, d, 1.8)	102.2
5'		159.3		159.4
6'	6.52 (1H, d, 1.9)	103.3	6.08 (1H, d, 1.8)	107.7
1"		138.4		133.6
2"	6.93 (1H, d, 8.5)	129.2	7.17 (1H, d, 8.6)	128.7
3"	6.66 (1H, d, 8.5)	116.0	6.73 (1H, d, 8.6)	113.1
4"		156.3		158.1
5"	6.66 (1H, d, 8.5)	116.0	6.73 (1H, d, 8.6)	113.1
6"	6.93 (1H, d, 8.5)	129.2	7.17 (1H, d, 8.6)	128.7

Table 2. Inhibitory Effects of Compounds **1**–**6** for DNA Topoisomerases I and II (%, Inhibition Ratio of Relaxation) and Their IC_{50}[a] Values against HT-29, MCF-7 and HepG2 Cell Lines

Comp.	Topoisomerase I (%)		Topoisomerase II (%)		Cytotoxicity IC_{50} (μM)		
	20 (μM)	100 (μM)	20 (μM)	100 (μM)	HT-29[b]	MCF-7[c]	HepG2[d]
1	4.6 ± 0.5	22.1 ± 0.5	96.8 ± 0.4	100 ± 0.0	> 100	> 100	> 100
2	4.3 ± 0.5	9.3 ± 0.9	78.7 ± 0.3	100 ± 0.0	> 100	> 100	> 100
3	NA	NA	1.2 ± 0.2	11.1 ± 0.2	3.1 ± 0.2	> 100	13.5 ± 0.6
4	NA	NA	1.0 ± 0.1	1.8 ± 0.2	> 100	> 100	> 100
5	NA	8.5 ± 0.1	2.8 ± 0.2	100 ± 0.0	> 100	> 100	> 100
6	NA	NA	3.4 ± 0.2	90.4 ± 0.2	3.4 ± 0.1	17.2 ± 0.3	> 100
CPT[e]	65.5 ± 0.8	86.7 ± 0.6	NA		8.4 ± 0.3	7.5 ± 0.2	9.5 ± 0.5
VP-16[f]	NA[g]		44.3 ± 0.2	58.2 ± 0.2	6.2 ± 0.2	4.1 ± 0.1	3.7 ± 0.1

[a]IC_{50} was defined as the concentration that caused 50% inhibition of cell growth *in vitro*. [b]HT-29: Human colon carcinoma. [c]MCF-7: Human breast carcinoma. [d]HepG2: Human liver carcinoma. [e]Camptothecin: positive control for topoisomerase I. [f]Etoposide: positive control for topoisomerase II. [g]NA: not applicable. All the results are presented as the average of triplicate experiments with mean ± S.E.

were in the *threo* form.[23] Interestingly, in our ^{1}H NMR spectrum, the two aliphatic protons (δ 3.42 and 5.19 ppm) presented as doublets with coupling constants of 6.4 Hz (Table 1), so it was assigned as the *erythro* form. The above spectroscopic data were confirmed by the HMQC, HMBC, and COSY spectra (Fig. 2). Hence, the structure of **2** was elucidated to be *erythro*-1-(3,5-dihydroxyphenyl)-2-(4"-hydroxyphenyl)-ethane-1,2-diol, and named tricuspidatin B.

In the DNA topoisomerase I assay as shown in Table 2, compound **1** showed 22.1% inhibition at 100 μM. Whereas the positive control, camptothecin, exhibited 65.5 and 86.7% inhibitory activities at the concentrations of 20 and 100 μM, respectively. In the DNA topoisomerase II assay, compounds **1** and **2** showed 96.8 and 78.7% strong inhibitions at 20 μM, respectively, whereas the positive control, etoposide,[24] exhibited 44.3 and 58.2% inhibitions at the concentrations of 20 and 100 μM, respectively. To investigate whether these compounds (**1**–**6**) mediated DNA topoisomerase inhibitory activity to induce cell death, the compounds were tested on human cancer cell lines. The tetrazolium-based colorimetric assay (MTT assay) was used to determine cytotoxicity towards HT-29, MCF-7 and HepG2 cell lines. As shown in Table 2, compounds **3** and **6** were potently cytotoxic to the HT-29 cell lines with IC_{50} values of 3.1 and 3.4 μM, respectively. Compound **3** showed significant inhibitory activity against HepG2 (IC_{50} = 13.5 μM). Compound **6** also showed significant inhibitory activity against the MCF-7 cell line (IC_{50} = 17.2 μM). The other compounds (**1**, **2**, **4** and **5**) displayed no inhibitory activity in any of the tested cancer cell lines.

Conclusion

In conclusion, six constituents including two new compounds (**1**–**2**) were isolated from *P. tricuspidata.* Considering the selectivity between the inhibitory activities of topoisomerases I and II, compounds **1** and **2** exhibited strong inhibitory activity against DNA topoisomerase II at the concentrations of 20 and 100 μM. Compounds **3** and **6**, a sterol and a triterpenoid, showed strong cytotoxicity against the HT-29 cell line.

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References

1. D'Arpa, P.; Liu, L. F. *Biochim. Biophys. Acta* **1989**, *989*, 163.
2. Pommier, Y. *Cancer Chemother. Pharmacol*. **1993**, *32*, 103.
3. Wang, J. C. *Annu. Rev. Biochem*. **1996**, *65*, 635.
4. Slichenmyer, W. J.; Rowinsky, E. K.; Donehower, R. C.; Kaufmann, S. H. *J. Natl. Cancer Inst*. **1993**, *85*, 271.
5. Potmesil, M. *Cancer Res*. **1994**, *54*, 1431.
6. Chen, A. Y.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol*. **1994**, *34*, 191.
7. Liu, L. F. *Annu. Rev. Biochem*. **1989**, *58*, 351.
8. Hwang, H. K.; Sung, H. K.; Wang, W. K.; Kim, I. H. *Yakhak Hoechi* **1995**, *39*, 289.
9. Kim, H. J.; Saleem, M.; Seo, S. H.; Jin, C.; Lee, Y. S. *Planta Med*. **1995**, *71*, 973.
10. Paulino, L. A.; Darc, J. M.; Maia, B.; Carlos, R. L. *Phytochemistry* **1991**, *30*, 3144.
11. Toshiyuki, T.; Masayoshi, O.; Kuniyasu, M.; Fujio, A.; Munekazu, I. A. *Phytochemistry* **1998**, *48*, 1241.
12. Saleem, M.; Kim, H. J.; Jin, C. B.; Lee, Y. S. *Arch. Pharm. Res*. **2004**, *27*, 300.
13. Son, I. H.; Chung, I. M.; Lee, S. J.; Moon, H. I. *Parasitol. Res*. **2007**, *101*, 237.
14. Son, J. K.; Jung, J. H.; Lee, C. S.; Moon, D. C.; Choi, S. W.; Min, B. S.; Woo, M. H. *Bull. Korean Chem. Soc.* **2006**, *27*, 1231.
15. Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Natl. Cancer* **1990**, *82*, 1113.
16. Grella, M. D.; Monako, P.; Previera, L. *J. Nat. Prod*. **1990**, *53*, 1430.
17. Kokpol, U.; Chavasiri, W.; Chittawong, V.; Bruce, M.; Cunningham, G. N.; Miles, D. H. *Phytochemistry* **1993**, *33*, 1129.
18. Delazar, A.; Shoeb, M.; Kumarasamy, Y.; Byres, M.; Naher, L.; Modarresi, M.; Sarker, S. D. *DARU*. **2004**, *12*, 49.
19. Sholichin, M.; Yamasaki, K.; Kasai, R.; Tanaka, O. *Chem. Pharm. Bull*. **1980**, *28*, 1006.
20. Fukuda, M.; Nishio, K.; Kanzawa, H.; Ogasawa, H.; Ishida, T.; Arioka, H.; Bojamowki, K.; Oka, M.; Sajio, N. *Cancer Res.* **1996**,

56, 789.
21. Rubinstein, L. V.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer inst.* **1990**, *82*, 1113.
22. Pouchert, C. J.; Behnke, J. In *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, 1st ed.; Aldrich Chemical Company, Inc.: U.S.A., 1993; Vol. 2, p 400.
23. Xiao, K.; Xuan, L.; Xu, Y.; Bai, D.; Zhong, D. *Chem. Pharm. Bull*. **2002**, *50*, 605.
24. Jeong, S. Y.; Zhao, B. T.; Lee, C. S.; Son, J. K.; Min, B. S.; Woo, M. H. *Planta Med*. **2012**, *78*, 177.