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CYTOTOXIC BENZYL DIHYDROFLAVONOLS FROM *CUDRANIA TRICUSPIDATA*

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Key Word Index—*Cudrania tricuspidata*; Moraceae; stem bark; dihydroflavonols; *p*-hydroxybenzyldihydroflavonols; 6,8-di-*p*-hydroxybenzyltaxifolin; 8-*p*-hydroxybenzyltaxifolin; 6-*p*-hydroxybenzyltaxifolin; gericudranins; cytotoxicity.

Abstract—Three new dihydroflavonols, gericudranins A–C were isolated from the stem bark of *Cudrania tricuspidata*. They were identified as 6,8-di-*p*-hydroxybenzyltaxifolin, 8-*p*-hydroxybenzyltaxifolin and 6-*p*-hydroxybenzyltaxifolin, respectively, by means of spectral studies. These compounds were cytotoxic to human tumor cell lines, such as CRL 1579 (skin), LOX-IMVI (skin), MOLT-4F (leukemia), KM12 (colon) and UO-31 (renal) in culture, with ED_{50} values of 2.7–31.3 $\mu g\,ml^{-1}$.

INTRODUCTION

Cudrania tricuspidata is a deciduous tree distributed over Korea, China and Japan. The cortex and root bark of this species have been used as a traditional medicine for curing neuritis and inflammation in the Orient [1] and as a folk remedy for gastritis or liver damage in Korea. From the stem or root bark of *C. tricuspidata*, xanthones [2–6], flavonoids [7–11] and benzenoids [12] have been isolated. In the course of our screening for antitumor agents from traditional medicines, we found that the 80% aqueous methanolic extract of *C. tricuspidata* exhibited relatively strong cytotoxicity against certain human cell lines in culture. The isolation and structural elucidation of the active principles are described, along with their cytotoxic activities, in the present paper.

RESULTS AND DISCUSSION

The 80% methanolic extract of *C. tricuspidata* was partitioned with *n*-hexane, benzene, $CHCl_3$, EtOAc and *n*-BuOH, consecutively. Among them, only EtOAc extracts showed notable cytotoxicity against human tumor cell lines. Silica gel column chromatography, followed by HPLC purification using Lichroprep RP-18 column of the active fraction afforded three cytotoxic compounds, named gericudranin A (**1**), gericudranin B (**2**) and gericudranin C (**3**); they gave positive colourations with $FeCl_3$ reagent on TLC.

The molecular formula of gericudranin A (**1**) was determined to be $C_{29}H_{24}O_9$ from the $[M + H]^+$ at *m/z* 517.1495 $[M + H]^+$ in the high resolution FAB-mass spectrum. The IR spectrum of **1** suggested the presence of hydroxyl (3410 and 1110 cm^{-1}) and carbonyl (1630 cm^{-1}) groups. The UV spectrum in MeOH showed maximum absorption at 299 and 304 nm, suggesting that it had a dihydroflavonol skeleton. In the ^1H spectrum, two resonances at δ4.86 (1H, *d*, J = 11.7 Hz, H-2) and 4.48 (1H, *d*, J = 11.7 Hz, H-3) were characteristic of a dihydroflavonol skeleton with *trans*-stereochemistry. ^{13}C NMR data also supported this skeleton (Table 1). The proton resonances at δ6.95 (1H, *brs*, H-2′) and 6.80 (2H, *brs*, H-5′ and H-6′) in the ^1H NMR suggested the presence of a 3,4-dihydroxyphenyl moiety. The fragmentation ion at *m/z* $[M + H - 152]^+$ in the SI-mass spectrum suggested that this moiety belonged to the B ring of dihydroflavonol skeleton. Two sets of proton resonances at δ7.04 (2H, *d*, J = 8.3 Hz, H-3″, H-7″) and 6.63 (2H, *d*, J = 8.3 Hz, H-4″, H-6″) and δ6.96 (2H, *d*, J = 8.3 Hz, H-3‴, H-7‴) and 6.59 (2H, *d*, J = 8.3 Hz, H-4‴, H-6‴) displayed typical resonances of *p*-substituted phenyl groups. In the PFG (pulse field gradient)-HMBC spectrum, a methylene carbon at δ28.12 (δ3.74 and 3.78 in ^1H NMR) correlated to H-3‴ and H-7‴. Similarly, a methylene carbon at δ27.63 (corresponding

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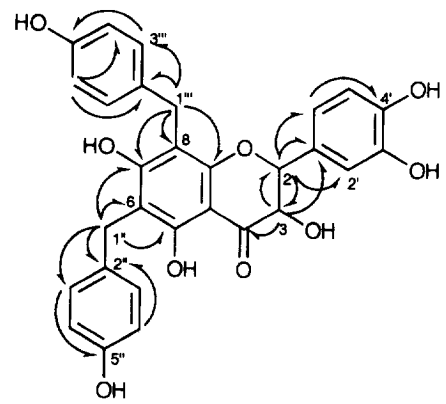

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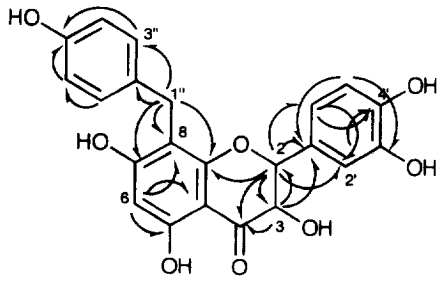

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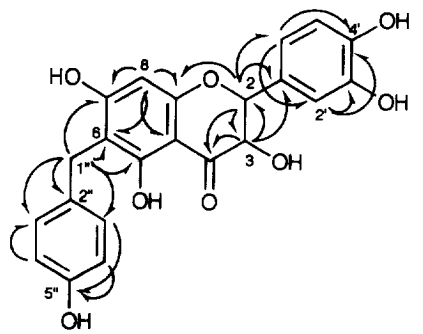

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Fig. 1. Structures and HMBC data* of gericudranins A (**1**), B (**2**) and C (**3**). Arrows indicate correlations between ^{1}H with ^{13}C.

to δ3.86 in ^{1}HNMR) correlated to H-3″ and H-7″. These correlations suggested that **1** had two *p*-substituted benzyl groups in its structure. From these observations, **1** was presumed to be a di-*p*-hydroxybenzyl substituted dihydroflavonol. The HMBC spectrum was also useful for determining the position of the 3,4-dihydroxyphenyl and the two *p*-hydroxybenzyl groups in the molecule. The proton signal for H-2 showed correlations with carbons at δ130.11 (C-1′), δ115.98 (C-2′) and δ120.87 (C-6′) of the 3,4-dihydroxyphenyl group and with carbons of the dihydroflavonol skeleton (C-3, C-4 and C-9). The H-1‴ methylene proton exhibited correlations with the three quaternary carbons at δ163.80, 109.53 and 159.64 which were assigned to C-7, C-8 and C-9, respectively. Accordingly, the C-1‴ benzylic methylene should be attached to C-8 of the taxifolin skeleton. The remaining methylene proton at H-1″ showed correlations with the δ160.58 (C-5, *s*), 110.07 (C-6, *s*) and 163.80 (C-7, *s*) carbons. All these spectral data revealed that **1** was 5,7,3′,4′-tetrahydroxy-6,8-di-*p*-hydroxybenzyldihydroflavonol, namely, 6,8-di-*p*-hydroxybenzyltaxifolin. HMBC data are summarized in Fig. 1.

Gericudranin B (**2**) showed similar patterns to those of **1** in its UV, IR and NMR spectra, suggesting that it was also a dihydroflavonol derivative. The molecular formula was determined to $C_{22}H_{18}O_8$ by high resolution FAB-mass spectrometry, and ^{1}H and ^{13}C NMR indicated a difference of one benzyl moiety from **1**. These data led us to make presumption that **2** possessed only one *p*-hydroxybenzyl group which was attached to the taxifolin skeleton. The carbon signal of C-6 of **2** was shifted upfield (13.29 ppm) from that of **1**, while the chemical shift of C-8 did not move significantly. In HMBC data (Fig. 1), H-1″ showed correlation with C-7 (δ166.37, *s*), C-8 (δ109.98, *s*) and C-9 (δ161.30, *s*). These spectral data indicated that the *p*-hydroxybenzyl group should be substituted at C-8 of taxifolin. Therefore, the structure of **2** was concluded to be 8-*p*-hydroxybenzyltaxifolin. HMBC correlations are summarized in Fig. 1.

Gericudranin C (**3**) had the same molecular formula as that of **2**. The IR, UV, MS and NMR data (see experimental) were very close to those of **2**. Thus **3** was thought to be a mono-*p*-hydroxybenzyl substituted derivative taxifolin. The ^{13}C resonance of C-8 in **3** (δ95.63) was shifted upfield from that of **1** (13.90 ppm), indicating that the benzyl group of **3** was attached to the C-6 position of taxifolin. HMBC data were analysed in order to clarify the location of the *p*-hydroxybenzyl group and to assign all NMR signals. The benzylic methylene protons at δ3.75 showed long-range correlations with the δ162.39 (C-5), 110.54 (C-6) and 166.52 (C-7) carbons. In addition, H-8 was correlated to the carbons of C-6, C-7, C-9 and C-10. H-2 showed correlations with the carbons of C-3, C-4, C-1′, C-2′ and C-6′. From these data, **3** was thus identified as 6-*p*-hydroxybenzyltaxifolin.

The absolute configurations of **1**–**3** were not determined, but, the large coupling constants between H-2 and H-3 (J = 11.2–11.7 Hz) indicated that their relative configurations should be *trans*. Considering the general configurations of dihydroflavonols found in plants [13], the absolute configuration of **1**–**3** were presumed to be (2*R*, 3*R*).

Benzyl-substituted flavonoids have rarely been reported. Studies of the flavonoids of *Uvaria* have shown the presence of *C*-benzyldihydrochalcones in several species. Cole *et al.* [14] established the structure of uvaretin which showed inhibitory effect in the P-388 lymphocytic leukemia test system. Okorie also found the 3′,5′-dibenzyl derivative, chamuvaretin, in *U. chamae* [15]. However, *p*-hydroxybenzyl substituted dihydroflavonols have never been reported.

Table 1. ^{13}C NMR data of compounds **1–3**

No.	1	2	3
2	84.96 (*d*)	84.99 (*d*)	85.10 (*d*)
3	73.83 (*d*)	73.71 (*d*)	73.75 (*d*)
4	199.08 (*s*)	198.70 (*s*)	198.47 (*s*)
5	160.58 (*s*)	163.11 (*s*)	162.39 (*s*)
6	110.07 (*s*)	96.78 (*s*)	110.54 (*s*)
7	163.80 (*s*)	166.37 (*s*)	166.52 (*s*)
8	109.53 (*s*)	109.98 (*d*)	95.63 (*d*)
9	159.64 (*s*)	161.30 (*s*)	162.42 (*s*)
10	101.94 (*s*)	101.79 (*s*)	101.62 (*s*)
1′	130.11 (*s*)	130.05 (*s*)	129.99 (*s*)
2′	115.98 (*d*)	115.95 (*d*)	115.87 (*d*)
3′	146.18 (*s*)	146.24 (*s*)	146.28 (*s*)
4′	146.98 (*s*)	147.03 (*s*)	147.08 (*s*)
5′	116.07 (*d*)	116.05 (*d*)	116.05 (*d*)
6′	120.87 (*d*)	120.91 (*d*)	120.91 (*d*)
1″	27.63 (*t*)	27.92 (*t*)	27.54 (*t*)
2″	132.96 (*s*)	133.84 (*s*)	133.74 (*s*)
3″	130.22 (*d*)	130.65 (*d*)	130.50 (*d*)
4″	115.79 (*d*)	115.66 (*d*)	115.66 (*d*)
5″	156.18 (*s*)	156.00 (*s*)	156.04 (*s*)
6″	115.79 (*d*)	115.66 (*d*)	115.66 (*d*)
7″	130.22 (*d*)	130.65 (*d*)	130.50 (*d*)
1‴	28.12 (*t*)		
2‴	133.26 (*s*)		
3‴	130.40 (*d*)		
4‴	115.79 (*d*)		
5‴	156.12 (*s*)		
6‴	115.79 (*d*)		
7‴	130.40 (*d*)		

Measured in CD_3OD.

The cytotoxicity of compounds **1–3** against several human tumor cell lines was examined (Table 2). Among them, gericudranin A was the most effective against LOX-IMVI, MOLT-4F, KM-12 and UO-31. However, none of them were as effective as adriamycin. The cytotoxicity of gericudranin B was generally very low compared to those of gericudranins A or C, suggesting that the *p*-hydroxybenzyl moiety at C-6 might be essential for the activity.

EXPERIMENTAL

General. Mps: uncorrected. ^{13}C and ^1H NMR: 150 and 600 MHz, respectively, in CD_3OD with TMS as standard. Chemical shifts are given in δ (ppm) from TMS. IR: KBr disks. FABMS: glycerol matrix. TLC was performed on silica gel (Merck, Kieselgel 60 F254), RP-TLC on Merck Art. 15683 precoated plates.

Plant material. Non-cultivated *C. tricuspidata* Bureau was collected from Chiri mountain located at the far south of The Republic of Korea. Collection was made on 15 May, 1992; at this time plants had abundant thorns and few leaves. Plant material was identified by an authority at the Pharmacognosy Laboratory, The College of Pharmacy, Pusan National University, 40 Jangjeon-Dong, Pusan, Korea. Fresh stem bark was removed using a sickle and dried in a dark, well-ventilated place.

Extraction and isolation. Air-dried stem bark (1 kg) was cut into pieces with a fodder-chopper and extracted with 80% MeOH at room temp. for several days. The MeOH extract was evapd and partitioned with *n*-hexane, benzene, $CHCl_3$, EtOAc and *n*-BuOH, consecutively. The EtOAc sol. fr. (18.3 g) was applied to silica gel [Merck, Art. 7734, 5 cm × 40 cm] and eluted with $CHCl_3$–MeOH (starting with 20:1, then 10:1 and, finally, 5:1). This chromatographic purification gave 60 frs and frs 27–43 (2.91 g) were re-chromatographed on a Lichroprep RP-18 (Merck, 3.5 cm × 28 cm) with a gradient elution of MeOH–H_2O (3:7 to 9:1). Active frs were collected and comb. (1.1 g). Final purification using HPLC (Senshu Pak ODS (20 mm × 250 mm), mobile phase 30% aq. MeCN, flow rate, 9.9 ml min^{-1}, detection; UV 290 nm) afforded gericudranin A (320 mg), gericudranin B (570 mg) and gericudranin C (165 mg).

Gericudranin A **1**. Yellow powder, mp 135°. R_fs: 0.17 on TLC [$CHCl_3$–MeOH (5:1), solvent A] and 0.38 on RP-18 TLC [MeOH–H_2O (3:2), solvent B]. UV λ_{max}^{MeOH} nm (log ε): 299 (4.14), 344 (3.67, *sh*). IR ν_{max}: 3413, 1631, 1511, 1450, 1365, 1284, 1241, 1211, 1172, 1110. ^1H NMR : δ7.04 (2H, *d*, J = 8.3 Hz, H-3″, H-7″), 6.96 (2H, *d*, J = 8.3 Hz, H-3‴, H-7‴), 6.95 (1H, *s*, H-2′), 6.80 (2H, *s*, H-5′, H-6′), 6.63 (2H, *d*, J = 8.3 Hz, H-4‴, H-6″), 6.63 (2H, *d*, J = 8.3 Hz, H-3‴, H-6‴), 4.86 (1H, *d*, J = 11.7 Hz, H-2), 4.48 (1H, *d*, J = 11.7 Hz, H-3), 3.86 (1H, *s*, H-1″), 3.74 (1H, *d*, J = 14.9 Hz, H-1‴a), 3.78 (1H, *d*, J = 14.9 Hz, H-1‴b). HR FAB-MS *m/z*: 517.1495, $C_{29}H_{24}O_9$ requires 517.1498; SIMS *m/z* (rel. int.): 555 $[M + H]^+$, 539 $[M + Na]^+$, 517 (1.5) $[M + H]^+$, 423 (0.9) $[M + H - 94]^+$, 185 (37.8) $[M + H - 332]^+$. $[\alpha]_D^{25}$ + 1.0° (MeOH; *c* 2.0).

Gericudranin B **2**. Yellow powder, mp 174°. R_fs: 0.17 on silica gel TLC (solvent A) and 0.45 on RP-18 TLC (solvent B). UV λ_{max}^{MeOH} nm (log ε): 297 (4.32), 338 (3.79, *sh*). IR ν_{max}: 3737, 3471, 1639, 1562, 1542, 1508, 1457, 1438, 1249, 1122, 1076. ^1H NMR : δ7.00 (2H, *d*, J = 8.3 Hz, H-3″, H-7″), 6.99 (2H, *brd*, J = 1.0 Hz, H-2′), 6.82 (1H, *dd*, $J_{5',6'}$ = 7.8 Hz, $J_{2',6'}$ = 1.0 Hz, H-6′), 6.82 (1H, *d*, J = 7.8 Hz, H-5′), 6.58 (2H, *d*, J = 8.3 Hz, H-4″, H-6″),

Table 2. ED_{50} values (μg ml^{-1}) of compounds **1–3** against human tumor cell lines

	CRL1579 (skin)	LOX-IMVI (skin)	MOLT-4F (leukemia)	KM12 (colon)	UO-31 (renal)
1	3.65	11.99	2.65	13.70	6.99
2	13.12	31.26	23.07	28.05	9.78
3	3.34	13.46	7.62	13.84	16.82
Adriamycin	0.16	0.13	0.02	0.11	0.30

5.99 (1H, *s*, H-6), 4.86 (1H, *d*, J = 11.2 Hz, H-2), 4.47 (1H, *d*, J = 11.2 Hz, H-3), 3.64 (1H, *d*, J = 13.9 Hz, H-1″a), 3.69 (1H, *d*, J = 11.2 Hz, H-2), 4.47 (1H, *d*, J = 11.2 Hz, H-3), 3.64 (1H, *d*, J = 13.9 Hz, H-1″a), 3.69 (1H, *d*, J = 13.9 Hz, H-1″b). HR FAB-MS *m/z*: 411.1084, $[M + H]^+$, $C_{22}H_{18}O_8$ requires 411.1079; SIMS *m/z* (rel. int.): 449 $[M + H]^+$, 433 $[M + Na]^+$, 411 (8.1) $[M + 1]^+$, 369 (5.4) $[M + H - 42]^+$, 277 (3.4) $[M + H - 134]^+$, 185 (79.7) $[M + H - 226]^+$. $[\alpha]_D^{25}$ + 1.9° (MeOH; *c* 2.0).

Gericudranin C **3**. Yellow powder, mp 197°. R_fs: 0.16 on silica gel TLC (solvent **A**) and 0.40 on RP-18 TLC (solvent **B**). UV λ_{max}^{MeOH} nm (log ε): 297 (4.12), 340 (3.49, *sh*). IR ν_{max}: 3428, 2931, 2360, 1635, 1511, 1450, 1400, 1288, 1253, 1187, 1114, 1079. ^{1}H NMR : δ7.09 (2H, *d*, J = 8.3 Hz, H-3″, H-7″), 6.95 (2H, *brd*, J = 1.0 Hz, H-2′), 6.83 (1H, *dd*, $J_{5',6'}$ = 7.8 Hz, $J_{2',6'}$ = 1.0 Hz, H-6′), 6.79 (1H, *d*, J = 7.8 Hz, H-5′), 6.62 (2H, *d*, J = 8.3 Hz, H-4″, H-6″), 5.94 (2H, *s*, H-8), 4.89 (1H, *d*, J = 11.7 Hz, H-2), 4.49 (1H, *d*, J = 11.7 Hz, H-3), 3.75 (2H, *s*, H-1″). HR FAB-MS *m/z*: $[M + 1]^+$ 411.1086, $C_{22}H_{18}O_8$ requires 411.1079; SIMS *m/z* (rel. int.): 449 $[M + K]^+$, 433 $[M + Na]^+$, 411 (17.6) $[M + 1]^+$, 394 (4.1) $[M + H - 17]^+$, 317 (12.2) $[M + H - 94]^+$, 185 (94.6) $[M + H - 226]^+$. $[\alpha]_D^{25}$ + 1.6° (MeOH; *c* 1.2)

Cytotoxicity. Cytotoxic activity against human tumor cell lines was estimated according to NCI protocols [16].

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