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TWO BENZYLATED DIHYDROFLAVONOLS FROM *CUDRANIA TRICUSPIDATA*

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ABSTRACT.—Two new benzylated dihydroflavonols, gericudranins D [**1**] and E [**2**], were isolated from the stem bark of *Cudrania tricuspidata*, and were identified as 5,7,4′-trihydroxy-6,8-di-*p*-hydroxybenzyldihydroflavonol and 5,7,4′-trihydroxy-6-*p*-hydroxybenzyldihydroflavonol, respectively. These compounds were shown to be significantly cytotoxic to human tumor cell lines in culture such as CRL 1579 (skin), LOX-IMVI (skin), MOLT-4F (leukemia), KM12 (colon), and UO-31 (renal).

Cudrania tricuspidata Bureau (Moraceae) is a deciduous tree that grows in Korea, China, and Japan. This plant has been used as a traditional medicine for neuritis and inflammation in the Orient (1). It has also served as a folk remedy for gastritis and liver damage in Korea. Xanthones (2–6), flavonoids (7–11), and benzenoids (12) have been isolated from the stem and root bark of the plant. In the course of our search for antitumor agents from traditional medicines, three new cytotoxic benzylated dihydroflavonol derivatives were isolated from *C. tricuspidata*, namely, gericudranins A, B, and C (13). Further purification of an 80% MeOH extract of this plant afforded two more cytotoxic benzylated dihydroflavonols, gericudranins D and E. In this report, their isolation and structure elucidation are reported, along with their cytotoxic evaluation against some human tumor cell lines.

An 80% MeOH extract of *C. tricuspidata* was partitioned with *n*-hexane, C_6H_6, $CHCl_3$, EtOAc, and *n*-BuOH, consecutively. Only the EtOAc-soluble fraction showed notable cytotoxicity against human tumor cell lines. Si gel cc, followed by hplc of the active fraction afforded two cytotoxic compounds named gericudranins D [**1**] and E [**2**]. They gave a positive coloration with $FeCl_3$.

The molecular formula of **1** was determined as $C_{29}H_{24}O_8$ from the protonated molecular ion peak at *m/z* 501.1536 $[M+H]^+$ in the hrfabms. The ir spectrum of **1** suggested the presence of hydroxyl (3428 and 1172 cm^{-1}) and carbonyl (1631 cm^{-1}) groups. The uv spectrum showed maxima at 299 and 344 nm, consistent with a dihydroflavonol skeleton. Two resonances at δ 4.93 (1H, d, J=11.7 Hz, H-2) and 4.48 (1H, d, J=11.7 Hz, H-3) ppm in the ^{1}H-nmr spectrum were characteristic of the dihydroflavonol skeleton with trans stereochemistry. ^{13}C-Nmr data also supported the dihydroflavonol skeleton (Table 1). Proton resonances at δ 7.29 (2H, d, J=8.8 Hz, H-2′, H-6′) and 6.82 (2H, d, J=8.8 Hz, H-3′, H-5′) suggested the presence of a *p*-hydroxyphenyl moiety in the structure of **1**. In addition, two sets of proton resonances at δ 7.05 (2H, d, J=8.8 Hz, H-3″, H-7″) and 6.64 (2H, d, J=8.8 Hz, H-4″, H-6″), and at δ 6.95 (2H, d, J=8.8 Hz, H-3‴, H-7‴) and 6.60 (2H, d, J=8.8 Hz, H-4‴, H-6‴) were characteristic for *p*-substituted phe-

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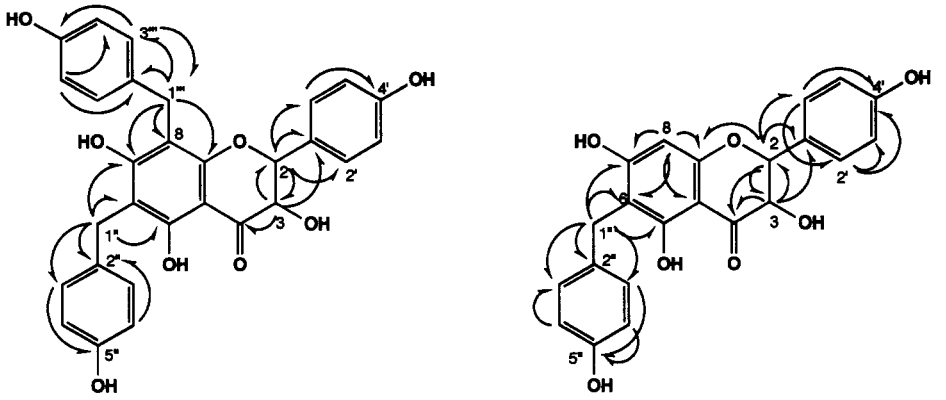

FIGURE 1. Structures of gericudranins D [**1**] and E [**2**] and their HMBC data. (Arrows indicate ^{1}H correlations with ^{13}C).

nyl groups. In the PFG (pulse-field gradient)-HMBC nmr spectrum, a methylene carbon at δ 28.25 (δ 3.73 and 3.79 in the ^{1}H-nmr spectrum) correlated to H-3‴ and H-7‴. Similarly, a methylene carbon at δ 27.74 (corresponding to 3.87 ppm in ^{1}H nmr) 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 assigned as a di-*p*-hydroxybenzyl-substituted dihydroflavonol. The HMBC spectrum was also useful to determine the position of one *p*-hydroxyphenyl and two *p*-hydroxybenzyl groups in the molecule. The H-2 signal showed correlations with carbons at δ 129.63 (C-1′), δ 130.28 (C-2′, C-6′) of the *p*-hydroxyphenyl group and with the C-3 and C-4 carbons of the dihydroflavonol skeleton. The H-1‴ methylene proton exhibited correlations with three quaternary carbons at δ 165.50, 109.80, and 159.66, which were assigned to C-7, C-8, and C-9, respectively. Accordingly, it was inferred that C-1‴ of the benzylic methylene was attached to C-8 of an aromadendrin skeleton. The remaining methylene proton (H-1″) showed correlations with ^{13}C-nmr signals at δ 160.66 (C-5, s), 110.29 (C-6, s), and 165.50 (C-7, s). All these spectral data confirmed that **1** was 5,7,4′-trihydroxy-6,8-di-*p*-hydroxybenzyldihydroflavonol (6,8-di-*p*-hydroxybenzylaromadendrin). The term aromadendrin for 5,7,4′-trihydroxydihydroflavonol was obtained from Ref. (14). Important HMBC data are summarized in Figure 1.

Gericudranin E [**2**] showed data similar to those of **1** in its uv, ir, and nmr spectra, suggesting that **2** is also a dihydroflavonol derivative. The molecular formula, which was determined to be $C_{22}H_{19}O_7$ by hrfabms in combination with ^{1}H- and ^{13}C-nmr indicated the absence of one *p*-hydroxybenzyl moiety when compared to **1**. These data led us to presume that **2** possessed only one *p*-hydroxybenzyl group which was attached to the aromadendrin skeleton. The signal of C-8 of **2** was shifted upfield (14.04 ppm) compared with that of **1**, while the chemical shift of C-6 did not move significantly. HMBC data were analyzed to clarify the location of the *p*-hydroxybenzyl groups and to assign all nmr signals. The benzylic methylene protons at δ 3.75 ppm showed long-range correlations with the δ 162.39 (C-5), 110.60 (C-6), and 166.89 (C-7) carbons. In addition, H-8 (δ 5.93, s) correlated to the ^{13}C-nmr signals of C-6, C-7, C-9 (δ 162.47) and C-10 (δ 101.54). H-2 (δ 4.92) showed correlations to signals of C-3 (δ 73.70), C-4 (δ 198.42), C-1′ (δ 129.43), and C-2′, C-6′ (δ 130.37). From these data, **2** was identified as 6-*p*-hydroxybenzylaromadendrin.

TABLE 1. ^{13}C-Nmr Data[a] of Gericudranins D [**1**] and E [**2**].

Carbon	Compound	
	1	**2**
2	84.71 (d)	84.94 (d)
3	73.78 (d)	73.70 (d)
4	198.49 (s)	198.42 (s)
5	160.66 (s)	162.39 (s)
6	110.29 (s)	110.60 (d)
7	164.20 (s)	166.89 (s)
8	109.80 (s)	95.76 (d)
9	159.66 (s)	162.47 (s)
10	101.51 (s)	101.54 (s)
1′	129.63 (s)	129.43 (s)
2′	130.28 (d)	130.37 (d)
3′	116.02 (d)	116.12 (d)
4′	159.05 (s)	159.16 (s)
5′	116.02 (d)	116.12 (d)
6′	130.28 (d)	130.37 (d)
1″	27.74 (t)	27.54 (t)
2″	133.26 (s)	133.77 (s)
3″	130.28 (d)	130.51 (d)
4″	115.74 (d)	115.66 (d)
5″	156.13 (s)	156.04 (s)
6″	115.74 (d)	115.66 (d)
7″	130.28 (d)	130.51 (d)
1‴	28.25 (t)	
2‴	133.58 (s)	
3‴	130.43 (d)	
4‴	115.69 (d)	
5‴	156.13 (s)	
6‴	115.63 (d)	
7‴	130.43 (d)	

[a]Measured in CD_3OD and presented as δ (ppm) from TMS.

When the ^{1}H-nmr spectrum of **1** was measured in DMSO-d_6, a broad singlet appeared at δ 12.25 ppm which could be assigned as a hydrogen-bonded hydroxyl proton at C-5. Similarly, **2** in DMSO-d_6 showed a broad singlet at δ 12.23 ppm. In addition, compared to aromadendrin (14), the chemical shifts of C-6 and C-8 of **1** moved downfield 12.80 and 13.40 ppm and C-6 of **2** downfield 13.10 ppm. From these observations, **1** and **2** could not be 5,7-*p*-hydroxydibenzyldihydroflavonols. The absolute configurations of **1** and **2** were not determined, but the large coupling constant between H-2 and H-3 (J=11.7 Hz) indicated that their relative configurations should be trans (15).

Benzyl-substituted flavonoids have been reported rarely. Studies of flavonoids of the genus *Uvaria* have shown the presence of *C*-benzyldihydrochalcones in several species. Cole *et al.* established the structure of uvaretin, which showed significant inhibition of the P-388 lymphocytic leukemia cell line (16). Okorie also found the 3′,5′-dibenzyl derivative chamuvaretin in *U. chamae* (17). However, except for our recent contribution (13), this is the first report of *p*-hydroxybenzyl-substituted dihydroflavonols.

The cytotoxicity of **1** and **2** against several human tumor cell lines was examined. Although the data are not extensive enough to discuss structure-activity relationships of the gericudranins, the *p*-hydroxybenzyl moiety at C-6 seems to be essential for the activity. This was because gericudranin B, which has no *p*-hydroxybenzyl group at that position, showed the least potent activity among gericudranins A–E (13). Structure-activity relationships of synthetic gericudranin derivatives are being investigated in our laboratory and will be published elsewhere. The cytotoxic activities of gericudranins D and E are summarized in Table 2.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were uncorrected. ^{13}C- and ^{1}H-nmr spectra were recorded on a JEOL JNM-A600 (150 and 600 MHz) instrument in CD_3OD with TMS as internal standard. Chemical shifts are given in δ (ppm) from TMS. Ir spectra were measured on a JEOL JIR-RFX-3001 spectrometer in KBr disks. Hrfabms were measured on a JEOL HX-110 mass spectrometer (glycerol). Uv spectra were run on a Kontron Unicon-903 spectrophotometer. Hplc was carried out with a Tosoh SC-8010 system. Tlc was performed on Si gel (Merck, Art. 5554 Kieselgel 60 F_{254}). Reversed-phase tlc was performed on Merck Art. 15683 precoated plates.

PLANT MATERIAL.—As reported previously (13).

EXTRACTION AND ISOLATION.—The dried stem bark of *C. tricuspidata* (1 kg) was extracted with 80% MeOH as described previously (13).

TABLE 2. Cytotoxicity[a] of Gericudranins D [**1**] and E [**2**].

Compound	CRL1579 (skin)	LOX-IMVI (skin)	MOLT-4F (leukemia)	KM12 (colon)	UO-31 (renal)
1	9.5	16.6	8.9	5.0	5.2
2	2.9	12.5	10.7	11.9	7.6
Adriamycin ...	0.16	0.13	0.02	0.11	0.3

[a]ED_{50} (μg/ml).

The MeOH extract was partitioned with *n*-hexane, C_6H_6, $CHCl_3$, EtOAc, and *n*-BuOH, consecutively. The EtOAc-soluble fraction (18.3 g) was applied to Si gel (Merck, Art. 7734, 4 cm×20 cm) and eluted with a $CHCl_3$-MeOH gradient 30:1→5:1). This chromatographic purification gave 50 fractions and fractions 20 to 33 (1.5 g) were combined and re-chromatographed using prep. tlc (Merck, Art 5744, $CHCl_3$-MeOH, 5:1). Two bands (R_f 0.53 and 0.63) were recovered and extracted with MeOH. The final purification using hplc [Senshu Pak ODS (20 mm×250 mm), mobile phase; 33% aqueous CH_3CN, flow rate; 9.9 ml min^{-1}, detection; uv 290 nm] afforded gericudranins D (150 mg) and E (100 mg).

Gericudranin D [**1**].—Yellow powder; mp 120±1°; $[\alpha]^{25}D$ +9.6° (*c*=0.3, MeOH); uv λ max (MeOH) 298 (log ε 3.67), 344 sh (3.25) nm; ir ν max 3428 (OH), 1631 (C=O) cm^{-1}; ^1H nmr (CD_3OD) δ 7.29 (2H, d, *J*=8.8 Hz, H-2′, H-6′), 7.05 (2H, d, *J*=8.8 Hz, H-3″, H-7″), 6.95 (2H, d, *J*=8.8 Hz, H-3‴, H-7‴), 6.82 (2H, d, *J*=8.8 Hz, H-5′, H-6′), 6.64 (2H, d, *J*=8.8 Hz, H-4″, H-6″), 6.60 (2H, d, *J*=8.8 Hz, H-4‴, H-6‴), 4.93 (1H, d, *J*=11.7 Hz, H-2), 4.48 (1H, d, *J*=11.7 Hz, H-3), 3.87 (1H, s, H-1″), 3.73 (1H, d, *J*=15.1 Hz, H-1‴a), 3.79 (1H, d, *J*=15.1 Hz, H-1‴b); ^{13}C-nmr data, see Table 1; hrfabms *m/z* 501.1536, $C_{29}H_{24}O_8$ requires 501.1550.

Gericudranin E [**2**].—Yellow powder; mp 125±1°; $[\alpha]^{25}D$ +11.8° (*c*=0.5, MeOH); uv λ max (MeOH) 295 (log ε 4.21), 340 (3.26) nm; ir ν max 3413 (OH), 1635 (C=O); ^1H nmr (CD_3OD) δ 7.34 (2H, d, *J*=8.3 Hz, H-2′, H-6′), 7.09 (2H, d, *J*=8.3 Hz, H-3″, H-7″), 6.82 (2H, d, *J*=8.3 Hz, H-3′, H-5′), 6.62 (2H, d, *J*=8.3 Hz, H-4″, H-6″), 5.93 (1H, s, H-8), 4.92 (1H, d, *J*=11.7 Hz, H-2), 4.53 (1H, d, *J*=11.7 Hz, H-3), 3.75 (1H, s, H-1″); ^{13}C-nmr data, see Table 1; hrfabms *m/z* 395.1132 $[M+1]^+$, $C_{22}H_{19}O_7$ requires 395.1131.

BIOLOGICAL TESTING.—Cytotoxic activity against human tumor cell lines was estimated according to the NCI protocols (18).

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LITERATURE CITED

1. Shanghai Science and Technological Publishers, "The Dictionary of Chinese Drugs," Shougakukan, Tokyo, 1985, Vol. 2, p. 2383.
2. T. Nomura, Y. Hano, and T. Fujimoto, *Heterocycles*, **20**, 213 (1983).
3. T. Fujimoto, Y. Hano, and T. Nomura, *Planta Med.*, **50**, 218 (1984).
4. Y. Hano, Y. Matsumoto, J.Y. Sun, and T. Nomura, *Planta Med.*, **56**, 399 (1990).
5. Y. Hano, Y. Matsumoto, J.Y. Sun, and T. Nomura, *Planta Med.*, **56**, 478 (1990).
6. Y. Hano, Y. Matsumoto, K. Shinora, J.Y. Sun, and T. Nomura, *Planta Med.*, **57**, 172 (1991).
7. T. Fujimoto, Y. Hano, T. Nomura, and J. Uzawa, *Planta Med.*, **50**, 161 (1984).
8. Y. Hano, Y. Matsumoto, K. Shinora, J.Y. Sun, and T. Nomura, *Heterocycles*, **31**, 1339 (1990).
9. T. Fugimoto and T. Nomura, *Heterocycles*, **22**, 997 (1990).
10. J.C. Park, H.S. Young, and J.S. Choi, *Yakhak Hoeji*, **36**, 40 (1992).
11. H.S. Young, J.H. Park, H.J. Park, and J.S. Choi, *Arch. Pharm. Res.*, **12**, 39 (1989).
12. T. Nomura, *Planta Med.*, **51**, 190 (1985).
13. I.K. Lee, C.J. Kim, K.S. Song, H.M. Kim, H. Koshino, M. Uramoto, and I.D. Yoo, *Phytochemistry*, in press.
14. Z. Shen and O. Theander, *Phytochemistry*, **24**, 155 (1985).
15. K.R. Markham and T.J. Mabry, in: "The Flavonoids." Ed. by J.B. Harborne, T.J. Mabry, and H. Mabry, Academic Press, New York, 1975, Part 1, Chapter 2, pp. 68.
16. J.R. Cole, S.J. Torrance, R.M. Wiedhopf, S.K. Arora, and R.B. Bates, *J. Org. Chem.*, **41**, 1852 (1976).
17. D.A. Okorie, *Phytochemistry*, **16**, 1591 (1977).
18. A. Monks, D. Scudiero, P. Skehan, P. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolfe, M. Gray-Goodrich, H. Campbell, and M.R. Boyd, *J. Natl. Cancer Inst.*, **83**, 757 (1991).

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