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Chemical Study on the Stem of *Cudrania tricuspidata*

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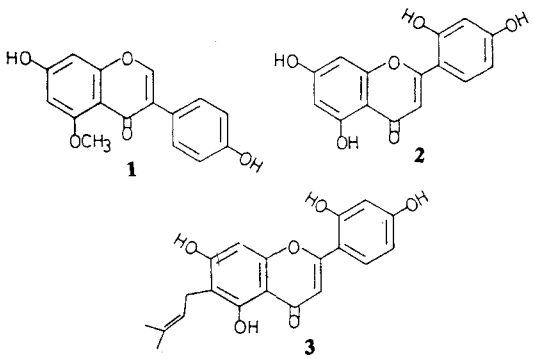
Abstract□From the stem of *Cudrania tricuspidata,* β-sitosterol, β-sitosterol glucoside, arthocarpesin, norarthocarpetin, and 5-O-methyl genistein were isolated and characterized by spectral data.

Keywords□*Cudrania tricuspidata,* Moraceae, β-sitosterol, β-sitosterol glucoside, arthocarpesin, norarthocarpetin, 5-0-methyl genistein, ^{13}C-NMR

Cudrania tricuspidata(Moraceae) is a deciduous tree which is distributed over China, Japan and Korea, and the cortex and the root bark have been used as a Chinese crude drug to treatment for neuritis and antiinflammatory.[1] Previous workers[2-4] reported that this root bark contains various xanthones and flavonoids.

On the other hand, the stem of this plant have been used as a folkloric medicine to treatment for gasteritis and liver damage in Korea. Since its chemistry has not yet been investigated, we have examined the stem and here report the isolation of sterol and flavonoid components of this plant.

Silica gel column chromatography of the chloroform soluble portion of the methanol extract yielded two compounds whch were identified as β-sitosterol and β-sitosterol glucoside by direct comparison with authentic samples. Another column chromatography of the ethylacetate soluble portion of the methanol extract yielded three compounds **1,2** and **3** in the order of increasing polarity. These compounds showed positive Mg + HCl and Zn + HCl.



Compound **1,** mp 287° was provisionally identified as an isoflavone from its UV spectrum in MeOH which possessed a principal maximum at 258 nm. A bathochromic shift in the spectrum produced upon addition of NaOAc indicated a C-7 hydroxyl. Furthermore, Unchangebility was observed in the $AlCl_3$ and $AlCl_3$ + HCl spectrum suggested the absence of free hydroxyl group at C-5.[5]

Confirmation of the isoflavone skeleton was provided by the ^{1}H-NMR spectrum which contained the characteristic singlet at δ8.05 due to the C-2 proton. A 3H singlet at δ3.79 indicated a methoxyl group and signals between δ6.35 and δ7.30 integrated for six aromatic protons. Two of these protons resonate at δ 6.37 and δ 6.39 and show *meta* coupling (J = 2.2 Hz) whilst the four remaining aromatic signals at δ6.78 and δ7.30 represent a typical *ortho* coupled doublet (J = 8.8 Hz). Mass spectrum revealed a plausible$[M]^+$ at *m/z* 284 which is the base peak of the spectrum. *Retro-Diels Alder* (RDA) fragment ions at *m/z* 166 (15.6%) and *m/z* 118 (16.4%) are attributable respectively to an A-ring fragment bearing both hydroxyl and a methoxyl substituent and B-ring fragment possessing a hydroxyl group.

The location of the methoxyl at C-5 and a hydroxyl at C-7 which is implied by the UV characteristics was deduced by absence of an intramolecular hydrogen bonded signal and ^{13}C-NMR spectrum (Table I). Thus, Compound **1** was identified as

Table I. ^{13}C-NMR spectral data of compounds 1,2 and 3 in DMSO-d_6

Carbon No.	1	3	2
2	150.6	161.6	161.7
3	124.9	103.3	103.2
4	174.2	181.9	181.8
5	161.4	158.3	161.7
6	96.6	110.6	98.5
7	162.6	161.6	163.9
8	95.0	92.9	93.7
9	157.1	155.1	157.3
10	108.0	110.6	103.5
1′	123.0	108.7	108.6
2′	130.4	161.5	161.3
3′	115.0	106.8	106.8
4′	159.4	158.7	158.7
5′	115.0	108.0	108.0
6′	130.4	129.7	129.8
1″	56.1(OMe)	20.9	
2″		122.3	
3″		130.5	
4″		25.5	
5″		17.7	

4′,7-dihydroxy 5-methoxy isoflavone (5-O-methyl genistein).

Compound **2**, mp 330°, showed a molecular ion peak at *m/z* 286 (100%) and other peaks at *m/z* 258 (M-CO, 6.0), 153(RDA fragment with A ring + H, 54.3) and 134(RDA fragment with B ring, 21.2) that it is a compound having flavone skeleton with two hydroxyl groups at ring A and B in the mass spectrum. The UV spectrum exhibiting band I peak at 355 nm indicated the absence of a substituent in the 3-position. A bathochromic shift of band I in the presence of $AlCl_3$ or $AlCl_3$ + HCl and of band II in the presence of NaOAc indicated the presence of free 5-hydroxyl and 7-hydroxyl groups. Addition of H_3BO_3 resulted in the decomposition of NaOAc complex, returning to original spectrum. And also a bathochromic shift with NaOMe, without a decrease in intensity, showed the presence of a free 4′-hydroxyl group.

The ^{1}H-NMR spectrum showed the two *meta*-coupled doublets of one proton at δ6.49(H-8) and δ6.17(H-6), and the signals at δ7.76(1H, d, J = 8.8 Hz), δ6.44(1H, dd, J = 8.8 and 2.2 Hz), δ6.43(1H, d, J = 2.0 Hz) assignable to the protons of a 1,2,4-trisubstituted benzene ring. The signal at δ6.99(1H, s) suggested the presence of a flavone moiety. These spectral data were in agreement with those for the structure of 5,7,2′,4′-tetrahydroxy flavone(norarthocarpetin). It was further confirmed by ^{13}C-NMR data (Table I).

Compound *3*, mp 253°, showed a similar pattern like **2** as otherwise shift reagent addition changed the spectrum in the UV spectrum (see experimental).

The ^{1}H-NMR spectrum showed a low field signal at δ 13.3 indicating the chelated hydroxyl group and the signals at δ7.74(1H, d, J = 8.8 Hz), δ6.45(1H, d, J = 2.4 Hz) and δ6.44(1H, dd, J = 8.6 and 2.2 Hz) assignable to the protons of a 1,2,4-trisubstituted benzene ring and δ6.98(1H, s) exhibiting the characteristic singlet signal of the C-3 position. As compared to **2**, Compound **3** showed more additional peaks at δ5.18(1H, t, J = 7.7 Hz), δ3.21 (2H, d, J = 7 Hz), δ1.73(3H, s, H-4″, cis) and δ1.62 (3H, s, H-5″, trans) which could be attributed a γ,γ'-dimethyl allyl group attached to an aromatic ring and did not exhibit the *meta*-coupled doublet but had a singlet at δ6.48(1H, H-8) in the ^{1}H-NMR spectrum. This means that the γ,γ'-dimethyl allyl group of **3** is located at C-6. This was further substantiated by the ^{13}C-NMR spectrum (Table I). The mass spectrum of **3** showed a molecular ion peak at *m/z* 354 and M^+-C_4H_7 at *m/z* 299 convincing the above suggestion. These spectral data were in agreement with those for the structure of 6-prenyl-5,7,2′,4′-tetrahydroxy flavone(arthocarpesin).

EXPERIMENTAL METHODS

The mps were taken on a Thomas Hoover 6406-H apparatus and are uncorrected. The UV spectra were runned with CE 599 Universal automatic scanning spectrophotometer. NMR(^{1}H and ^{13}C NMR) spectra were taken at 25° using TMS as an internal standard on a Jeol GX-270 or Jeol FX-90 Q spectrometer. MS spectra were obtained on a Hewlett-Packard 5985B GC/MS spectrometer operating at 70 eV.

Plant Material

The stem of *C. tricuspidata* used was purchased from the Chinese herb medicine shop at the Pyongwha market, Pusan. A voucher specimen is deposited in the herbarium of the Pusan National University.

Extraction and Isolation

Powdered stem of *C. tricuspidata* (2.0 kg) was refluxed with MeOH. The MeOH extract (90g) was partitioned with $CHCl_3$, EtOAc, n-BuOH and H_2O successively. The $CHCl_3$ soluble portion was chromatographed on Silica gel column with $CHCl_3$-MeOH(gradient) to give β-sitosterol and β-sitosterol glucoside. The EtOAc extract(26g) was subjected to chromatography using SiO_2(solvent; $CHCl_3$-MeOH(gradient)) column to afford 20 fractions. Compounds **3**, **2**, and **1** were obtained from fractions 2,3 and 5 as minor components. The fractionation of other fractions are now in progress.

Compound 1

White needles from MeOH, mp 287°, UV λ_{max} nm(MeOH)(log ε); 258(4.24), 285(sh, 3.90), 316 (sh, 3.63), λ_{max} (MeOH + NaOMe) 267(4.24), 300 (sh, 3.98), λ_{max}(MeOH + NaOAc) 266(4.21), 320 (3.75), λ_{max}(MeOH + NaOAc + H_3BO_3) 257(4.20), 321(sh, 3.57), λ_{max}(MeOH + $AlCl_3$) 260(4.20), 288 (sh, 3.85), 320(sh, 3.57), λ_{max}(MeOH + $AlCl_3$ + HCl) 256(4.20), 286(sh. 3.85), 320(sh. 3.57), MS (*m/z*, %); 284(M^+, 100), 283(M^+-H, 44.4), 253 (M^+-OCH_3, 14.3), 167(RDA fragment with A ring + H, 6.6), 118(RDA fragment with B ring, 16.4), ^{1}H-NMR(DMSO-d_6, 270MHz) δ; 8.05(1H, s, H-2), 7.30(2H, d, J = 8.8 Hz, H-3′,5′), 6.78(2H, d, J = 8.8 Hz, H-2′,6′), 6.39(1H, d, J = 2.2 Hz, H-8), 6.37(1H, d, J = 2.2 Hz, H-6), 3.79(3H, s, -OCH_3), ^{13}C-NMR(DMSO-d_6, 22.53MHz) δ; see Table I.

Compound 2

Yellowish needles from acetone, mp 330°, UV λ_{max}nm(MeOH)(log ε); 254(4.08), 266(4.11), 285 (3.89), 355(4.23), λ_{max}(MeOH + NaOMe) 268 (4.18), 320(3.85), 402(4.34), λ_{max}(MeOH + NaOAc) 268(4.14), 290(sh. 3.87), 320(3.90), 370(4.13), λ_{max} (MeOH + NaOAc + H_3BO_3) 259(4.05), 270(4.09), 290(3.87), 360(4.20), λ_{max}(MeOH + $AlCl_3$) 265(sh, 4.05), 275(4.08), 296(sh. 3.93), 360(4.09), 395 (4.23), λ_{max}(MeOH + $AlCl_3$ + HCl) 260(sh, 4.01), 275(4.06), 293(3.93), 360(sh, 4.11), 390(4.18), MS (*m/z*, %); 286(M^+, 100), 258(M^+-CO, 6.0), 153 (RDA fragment with A ring + H, 54.3), 134(RDA fragment with B ring, 21.2), 124(134-CO, 8.0), ^{1}H-NMR(DMSO-d_6, 270MHz) δ; 13.0(1H, brs, H-5), 7.76(1H, d, J = 8.8 Hz, H-6′), 6.99(1H, s, H-3), 6.49(1H, d, J = 2.0 Hz, H-8), 6.44(1H, dd, J = 8.8 and 2.2 Hz, H-5′), 6.43(1H, d, J = 2.0 Hz, H-3′), 6.17(1H, d, J = 2.0 Hz, H-6), ^{13}C-NMR(DMSO-d_6, 22.53MHz) δ; see Table I

Compound 3

Yellowish needles from MeOH, mp 253°, UV λ_{max}nm(MeOH)(log ε); 253(4.09), 272(4.16), 290 (sh. 4.01), 353(4.32), λ_{max}(MeOH + NaOMe) 271 (4.20), 326(3.96), 406(4.41), λ_{max}(MeOH + NaOAc) 271(4.21), 292(sh. 3.95), 375(4.22), λ_{max}(MeOH + NaOAc + H_3BO_3) 253(4.10), 271(4.18), 288(4.04), 371(4.29), λ_{max}(MeOH + $AlCl_3$) 260(4.06), 280(4.12), 295 (4.10), 373(4.29), $_{max}$(MeOH + $AlCl_3$ + HCl) 260 (4.06), 281(4.13), 294(4.11), 371(4.29), MS(*m/z*, %); 354(M^+, 20), 299(M^+-C_4H_7, 100), ^{1}H-NMR (DMSO-d_6, 270MHz) δ; 13.3(1H, brs, H-5), 7.74 (1H, d, J = 8.6 Hz, H-6′), 6.98(1H, s, H-3), 6.48 (1H, s, H-8), 6.45(1H, d, J = 2.4 Hz, H-3′), 6.44 (1H, dd, J = 8.6 and 2.2 Hz, H-5′), 5.18(1H, t, J = 7.7 Hz, -CH-), 3.21(2H, d, J = 7.0 Hz, -CH_2-), 1.73(3H,s, -CH_3, cis), 1.62(3H, s, -CH_3, trans), ^{13}C-NMR(DMSO-d_6, 22.53MHz) δ; see Table I.

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