CUDRAFLAVONES C AND D, TWO NEW PRENYLFLAVONES FROM THE ROOT BARK OF CUDRANIA TRICUSPIDATA (CARR.) BUR.[1,2]

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Abstract — Two new prenylflavones, cudraflavones C (**1**) and D (**2**), along with six known compounds were isolated from the root bark of Cudrania tricuspidata (Carr.) Bur. (Moraceae), collected in China. The structures of cudraflavones C and D were shown to be **1** and **2**, respectively, on the basis of spectroscopic data.

In the course of our studies on the constituents of moraceous plants, we dealt with the phenolic constituents of the root bark of Cudrania tricuspidata (Carr.) Bur. (Moraceae, Japanese name "Hariguwa") collected in Japan and China, and reported the new fifteen isoprenylated xanthones, and three isoprenylated flavonoids.[3-10] This paper describes the characterization of two new prenylflavones isolated from the root bark of C. tricuspidata (Carr.) Bur.

The dried root bark of C. tricuspidata, collected in China, was extracted with ethanol. Cudraflavones C (**1**) and D (**2**) were isolated along with six known compounds, cudraflavanone A[7] (**3**), cudraflavone B[5] (**4**), umbelliferone[11] (**5**), 2,3-dihydrokaempferol[12] (**6**), erythrinin C[13] (**7**), and cycloartocarpesin[14] (**8**), from the extract as described in "EXPERIMENTAL".

Cudraflavone C (**1**), an amorphous powder, exhibited positive reaction to ferric chloride, magnesium-hydrochloric acid, and Gibbs test. The molecular formula of **1** was determined by HR-ms to be $C_{25}H_{26}O_6$. The uv spectrum exhibited maxima at 210, 234 (sh), 262, and 301 nm, and was similar to the spectrum of kuwanon C[15] (**9**) and other 3-prenylflavones.[16] The uv spectrum of **1** showed no bathochromic shift upon addition of aluminum chloride.[17] The ^1H nmr spectrum showed the signals of the following

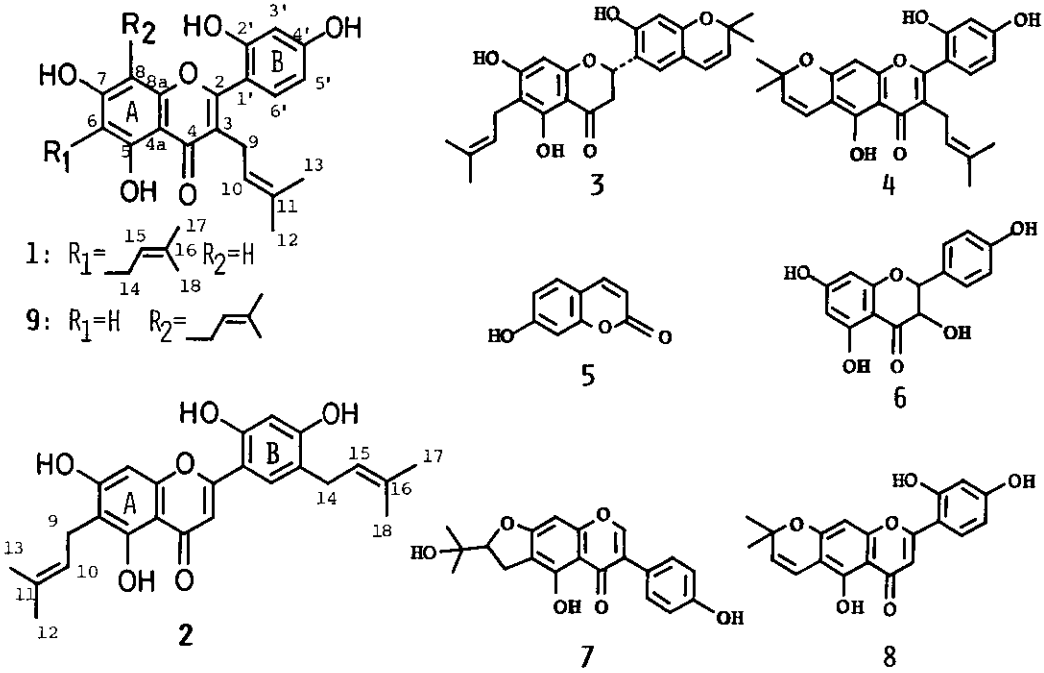

Figure 1

Table 1 ^{13}C Nmr chemical shifts (ppm)

	1*	2**	9**
C-2	162.0	162.5	158.9
C-3	121.5	106.6	119.4
C-4	183.0	181.9	181.8
C-4a	105.0	103.3	103.4
C-5	160.0	158.4	155.0
C-6	111.8	108.3	97.9
C-7	162.3	161.7	161.7
C-8	93.5	93.1	105.5
C-8a	157.0	157.2	160.3
C-1'	113.1	110.8	111.3
C-2'	157.1	155.2	156.5
C-3'	103.9	103.2	102.7
C-4'	161.3	159.5	161.2
C-5'	108.1	119.8	106.7
C-6'	132.2	128.6	131.7
C-9	24.6	21.2	23.5
C-10	122.7a	123.1a	121.7
C-11	132.0b	131.6b	131.2
C-12	25.8c	25.7c	25.4
C-13	17.9d	17.8d	17.3
C-14	22.0	27.4	21.1
C-15	123.3a	122.7a	121.7
C-16	131.4b	130.5b	131.2
C-17	25.9c	25.7c	25.4
C-18	17.6d	17.8d	17.3

Solvent: *; acetone-d_6 **; DMSO-d_6

a-d: Assignments may be reversed in each column.

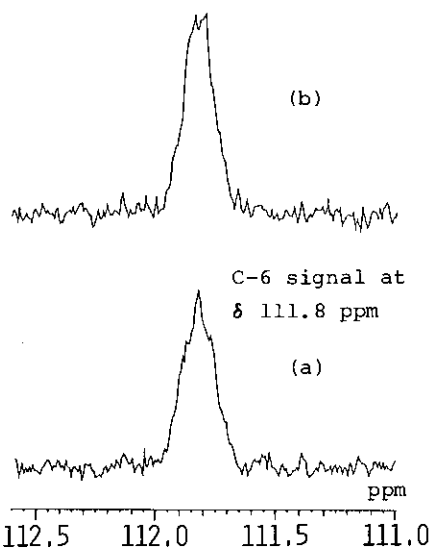

Figure 2 LSPD measurement of 1
(a) Gated decoupling with NOE.
(b) Irradiated at δ 13.43 ppm.

protons; 1) protons in two 3,3-dimethylallyl (prenyl) groups, δ 1.43, 1.57, 1.65, 1.78 (each 3H, br s), 3.12, 3.41 (each 2H, br d, J = 7 Hz), 5.14, 5.29 (each 1H, m), 2) ABC type aromatic protons, δ 6.52 (1H, dd, J = 2 and 8 Hz), 6.57 (1H, d, J = 2 Hz), 7.19 (1H, d, J = 8 Hz), 3) an aromatic proton, δ 6.40 (1H, s), and 4) a hydrogen-bonded hydroxyl group, δ 13.43 (1H, s). In the spectrum, no singlet signal due to the proton at the C-3 position was observed.[18] The ^{13}C nmr spectrum of **1** was analysed by gated decoupling with NOE technique as well as by comparison of the spectrum with those of **9**[15] and other 3-prenyl-5,7,2',4'-tetraoxygenated flavone derivatives[16,19] (Table 1). In the spectrum of **1**, the chemical shift values of the carbon atoms of the B ring and the prenyl groups were similar to those of the relevant carbon atoms of **9**, and the chemical shift value of the methylene carbon atom (δ 24.6) in the prenyl group located at the C-3 position was in good agreement with the values (δ 23.5 – 24.9) of the relevant carbon atoms of the 3-prenylflavones.[20] From the above uv, and ^{13}C and ^{1}H nmr studies, it was supported that cudraflavone C (**1**) is a 5,7,2',4'-tetraoxygenated flavone derivative having one of the two prenyl groups at the C-3 position and another in the A ring. The location of the prenyl group in the A ring was confirmed as the C-6 position, because the C-8 signal at δ 93.5 was observed as a sharp doublet (^{1}J = 163.6 Hz) in the ^{13}C nmr spectrum (acetone-d_6, gated decoupling with NOE). The assignment of the signal was confirmed by the fact that the signal at δ 111.8 (C-6) changed as shown in Figure 2 when the proton signal at δ 13.43 (C-5-OH) was irradiated, while the signal at δ 93.5 (C-8) was unchanged by the irradiation. From the above results, formula **1** was proposed for the structure of cudraflavone C. The formula **1** was proposed for the structure of mulberrin,[21] whereas the structure of mulberrin was revised by our group to the formula **9** proposed for kuwanon C.[19,22] Recently, Mereyara *et al.* reconfirmed the structure of mulberrin to be the formula **9** (kuwanon C).[23]

Cudraflavone D (**2**), yellow needles, mp 237-242°C, exhibited positive reaction to ferric chloride, magnesium-hydrochloric acid, and Gibbs test. The molecular formula of **2** was determined by HR-ms to be $C_{25}H_{26}O_6$. The uv spectrum exhibited maxima at 206, 258, 272, 289, and 359 nm, and was similar to the spectra of flavone derivatives,[18] while the spectrum was dissimilar to those of the 3-prenylflavones.[16] The ^{1}H nmr spectrum showed the signals of the following protons; 1) protons in two prenyl groups, δ 1.68, 1.74 (each 3H, br s), 1.78 (6H, br s), 3.23, 3.26 (each 2H, br d, J = 7 Hz), 5.24, 5.34 (each 1H, m), 2) four olefinic or aromatic singlet signals, δ 6.51, 6.61, 7.04, 7.58 (each 1H, s), and 3) a hydrogen-bonded hydroxyl

group, δ 13.37 (1H, s). The ^{13}C nmr spectrum of **2** was analysed by gated decoupling with NOE technique as well as by comparison of the spectrum with the spectra of **1** and **9** (Table 1). The above results suggest that cudraflavone D is a 5,7,2',4'-tetraoxygenated flavone derivative having no prenyl group at the C-3 position and that the compound is a structural isomer of **1**. The four singlet signals being considered in the ^{1}H nmr spectrum of **2**, one of the two prenyl groups is indicated to be at the C-5' position, and another in the A ring. The location of the latter prenyl group was confirmed as the C-6 position from the fact that the sharp doublet signal at δ 93.1 (^{1}J = 164.3 Hz) in the ^{13}C nmr spectrum (gated decoupling with NOE) of **2** was assigned to the carbon atom at the C-8 position by comparing the spectrum of **2** with that of **1**. From the above results, formula **2** was proposed for the structure of cudraflavone D.

EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, sh = shoulder. The general procedures followed as described in our previous paper.[24]
The following instruments were used: Yazawa micro-melting points apparatus (hot-stage type), Shimadzu UV-265 spectrophotometer, Hitachi 260-30 IR spectrophotometer, JEOL JMS D-300 and DX-303 mass spectrometers, JEOL JNM GX-400 FTNMR spectrometer.

Isolation of Phenolic Compounds (**1** – **8**) from the Root Bark of Cudrania tricuspidata (Carr.) Bur.

The dried root bark (10 Kg) of C. tricuspidata, collected in Tian Mu Shan, the Zhejiang province, China, in september 1986, was extracted with ethanol at room temperature. Evaporation of the extract yielded 740 g of the residue, which was extracted with benzene. The benzene solution was concentrated to afford the residue (280 g).[8] This residue (180 g) was chromatographed on silica gel (1800 g) successively with n-hexane, n-hexane-benzene, benzene, benzene-ethyl acetate, and ethyl acetate as an eluent. The ethyl acetate eluted fractions was evaporated to leave the residue (20 g). This residue (20 g) was chromatographed on silica gel (400 g), successively, with chloroform (fractions 1-160), chloroform:ether = 4:1 (frs. 161-200) as an eluent, each fraction (eluted volume of 200 ml) being monitored by tlc. The combined fraction (frs. 32-42, 2.6 g of residue) was rechromatographed on silica gel (80 g) with chloroform:acetone = 15:1 as an eluent, followed by hplc (solvent, n-hexane:ethyl acetate = 1:1, column Senshu Pak, SSC-Silica 4251-N, 1 cm ø x 25 cm, detector: uv 280 nm) to give cudraflavanone A[7] (**3**, mp 209-213°C, 114 mg, 1.1 x 10^{-3}% yield from the root bark). The combined fraction (frs. 83-90, 0.8 g) was purified by preparative tlc (chloroform: acetone = 8:1, n-hexane:acetone = 1:1) to give cudraflavone B[5] (**4**, yellow amorphous powder, 153 mg, 1.5 x 10^{-3}% yield). The combined fraction (frs. 91-105, 0.6 g) was purified by hplc (under the same conditions described above) to give umbelliferone[11] (**5**, mp 234-237°C, 7 mg, 7 x 10^{-5}% yield). A part (0.5 g) of the combined fraction (frs. 177-187, 5.5 g) was purified by repeated recrystallization from n-hexane-ether to give 2,3-dihydrokaempferol[13] (**6**, mp 235-238°C, 18 mg, 1.8 x 10^{-4}% yield). A part (5.0 g) of the combined fraction (frs. 177-187, 5.5 g) was rechromatographed on silica gel (250 g) with chloroform:ethyl acetate = 9:1 (frs. 1'-19', 100 ml each) and chloroform: ethyl acetate = 8:1 (frs. 20'-35'). The combined fraction (frs. 20'-28', 0.3 g) was purified by hplc (under the same conditions described above) to give cudraflavone C (**1**, 176 mg). The combined

fraction (frs. 30'-35', 0.2 g) was purified by preparative tlc (chloroform:methanol = 10:1, chloroform:ether = 2:1) to give erythrinin C[13] (**7**, mp 236-241°C, 7 mg, 7 x 10^{-5}% yield). The combined fraction (frs. 188-189, 2.1 g) eluted with chloroform:ethyl acetate = 4:1 was purified by preparative tlc (ethyl acetate:benzene = 1:1, chloroform:acetone = 1:1) to give cycloartocarpesin[14] (**8**, mp 271-274°C, 15 mg, 1.5 x 10^{-4}% yield) and cudraflavone D (**2**, 18 mg). The identification of the known compounds (**6**, **7**, and **8**) was carried out by comparing the physical and spectral data of these compounds with the relevant published data, and the compounds (**3**, **4**, and **5**) were identified by direct comparisons with the relevant authentic samples.

Cudraflavone C (**1**)

Compound **1** was obtained as yellow amorphous powder (176 mg, 1.8 x 10^{-3}% yield). $FeCl_3$ test: positive (dark blue). Mg-HCl test: positive. Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 210 (4.68), 234 (sh 4.44), 262 (4.39), 301 (4.16). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 213 (4.69), 264 (4.41), 317 (4.16), 386 (sh 3.75). Uv $\lambda_{max}^{MeOH+AcONa}$: 216 (4.79), 263 (4.43), 300 (sh 4.10), 328 (4.15). Uv $\lambda_{max}^{MeOH+MeONa}$: 217 (4.80), 269 (4.43), 330 (4.18), 374 (4.17). Ir ν_{max}^{KBr} cm^{-1} : 3400, 1655, 1630, 1570. EI-Ms: m/z 422 $[M]^+$, 407, 379, 367 (base peak). High-resolution ms (HR-ms): m/z 422.1725 ($C_{25}H_{26}O_6$ requires 422.1729). ^1H Nmr (acetone-d_6): δ 1.43, 1.57 (each 3H, br s, C-11-CH_3), 1.65, 1.78 (each 3H, br s, C-16-CH_3), 3.12 (2H, br d, J = 7 Hz, C-9-H x 2), 3.41 (2H, br d, J = 7 Hz, C-14-H x 2), 5.14 (1H, m, C-10-H), 5.29 (1H, m, C-15-H), 6.40 (1H, s, C-8-H), 6.52 (1H, dd, J = 2 and 8 Hz, C-5'-H), 6.57 (1H, d, J = 2 Hz, C-3'-H), 7.19 (1H, d, J = 8 Hz, C-6'-H), 13.43 (1H, s, C-5-OH).

Cudraflavone D (**2**)

Compound **2** was obtained as yellow needles, mp 237-242°C (18 mg, 1.8 x 10^{-4}% yield). $FeCl_3$ test: positive (dark green). Mg-HCl test: positive. Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 206 (4.54), 258 (4.06), 272 (4.08), 289 (4.05), 359 (4.18). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 205 (4.63), 215 (sh 4.57), 265 (4.08), 282 (4.13), 295 (4.14), 389 (4.20). Uv $\lambda_{max}^{MeOH+AcONa}$: 215 (4.75), 257 (sh 4.09), 271 (4.12), 289 (sh 4.01), 364 (4.11). Uv $\lambda_{max}^{MeOH+MeONa}$: 214 (4.95), 267 (4.09), 324 (4.06), 426 (4.26). Ir ν_{max}^{KBr} cm^{-1} : 3240, 1650, 1620, 1615 (sh), 1565, 1560 (sh). EI-Ms: m/z 422 $[M]^+$, 407, 379 (base peak), 367, 203, 165. HR-Ms: m/z 422.1716 ($C_{25}H_{26}O_6$ requires 422.1729). ^1H Nmr (acetone-d_6): δ 1.68 (3H, br s, C-11-CH_3), 1.74 (3H, br s, C-16-CH_3), 1.78 (6H, br s, C-11-CH_3 and C-16-CH_3), 3.23 (2H, br d, J = 7 Hz, C-9-H x 2), 3.26 (2H, br d, J = 7 Hz, C-14-H x 2), 5.24 (1H, m, C-10-H), 5.34 (1H, m, C-15-H), 6.51 (1H, s, C-8-H), 6.61 (1H, s, C-3'-H), 7.04 (1H, s, C-3-H), 7.58 (1H, s, C-6'-H), 13.37 (1H, s, C-5-OH).

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