TWO NEW C-BENZYLATED FLAVANONES FROM *UVARIA CHAMAE* AND ^{13}C NMR ANALYSIS OF FLAVANONE METHYL ETHERS

HALA N. EL-SOHLY, WILLIAM L. LASSWELL, JR.,[1] and CHARLES D. HUFFORD[2]

Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, Mississippi 38677

ABSTRACT.—Two new racemic C-benzylated flavanones (**1a**, **2a**) were isolated from the root bark of *Uvaria chamae* (*Annonaceae*). The structures were proposed as the 5-methyl ether derivatives of dichamanetin (**1b**) and chamanetin (**2b**) based on spectral data and conversion to known compounds. The ^{13}C nmr data for a number of methyl ether derivatives of chamanetin (**2b**) and dichamanetin (**1b**) are discussed.

Previous studies carried out on the root and stem barks of *Uvaria chamae* P. Beauv. resulted in the isolation of several antimicrobial and cytotoxic flavonoids and aromatic constituents (1–6). Chamanetin (**2b**), isochamanetin (**3a**) and dichamanetin (**1b**) were identified as novel C-benzylated flavanones (2). In a continuation of attempts to isolate cytotoxic and antitumor constituents from *U. chamae* an investigation of a cytotoxic polar column fraction (ether/methanol wash) of the root bark was undertaken and has resulted in the isolation of the 5-methyl ether derivatives of chamanetin (**2b**) and dichamanetin (**1b**).

EXPERIMENTAL[3]

PLANT MATERIAL.—The plant material[4] was collected in Ghana in May, 1975.

ISOLATION OF (±)-CHAMANETIN 5-METHYL ETHER (**2a**) AND (±)-DICHAMANETIN 5-METHYL ETHER (**1a**).—The ether and methanol washes of the silicic acid column [ED_{50} 2.2 μg/ml (KB); % T/C 127, 137 (PS *in vivo*)][5] of the root bark (2) (9.9 g) were chromatographed over silica gel (MN, 70–270 mesh, 700 g) using chloroform and chloroform-methanol mixtures. The activity was concentrated in two fractions (both eluted with 8% methanol in chloroform), A and B: ED_{50}(PS) 6.4×10^{-1} and 2.4×10^{-2} μg/ml, respectively.

Fraction A (700 mg) was further column chromatographed over a silica gel G column (80 g). Elution was carried out using 5% ethanol in chloroform. A total of 100 fractions (12 ml each) were collected. Compound I was present in fractions 23–26 (510 mg). This material was further purified by preparative layer chromatography (5% MeOH-$CHCl_3$; silica gel G precoated plates 1 mm). The required zone was located under uv, scraped, extracted with chloroform-acetone, and evaporated to dryness. The residue (179 mg), after crystallization from absolute ethanol, afforded **1a** as short rods (89 mg) mp 203.5–204.5°; $[\alpha]^{25}D = 0$ (c 0.56 DMSO); cd (0.005% MeOH) $[\theta]_{400-210} = 0$; uv λ max (MeOH) 332 nm (ε 1.05×10^4), 280 (ε 5.80×10^3), 258 (ε 6.16×10^3); ir (KBr) ν max: 3160, 1660, 1600 and 1468 cm^{-1}; mass spectrum *m/e* (relative abundance) M+ 482 (13%), 405 (7%), 378 (9%), 376 (64%), 375 (26%), 299 (54%), 272 (64%), 193 (34%), 179 (23%), 167 (18%), 166 (26%), 152 (21%), and 107 (100%); ^1H nmr (acetone-d_6) δ 6.56–7.64 (m, 16H), 5.6 (X of ABX, dd, 1H, $J_{cis} = 5$ Hz, $J_{trans} = 11$ Hz), 3.96 (s, 4H), 3.76 (s, 3H) and 2.57–3.7 (AB of ABX, m, 2H); ^{13}C nmr (DMSO-d_6) δ 188.3 (s, C-4), 160.3, 160.0, 158.3 (s, C-5, 7, 9), 154.4, 154.3 (s; C-2″, 2‴), 139.3 (s; C-1′), 128.7, 128.4, 128.1, 127.1, 126.6, 126.4, 126.0 (C-2′, 3′,

[1]Present address: University of Rhode Island, Kingston, Rhode Island.

[2]To whom inquires should be addressed.

[3]Melting points were determined using a Thomas-Hoover unimelt capillary melting point apparatus and are uncorrected. The ir spectra were obtained on a Beckman IR-33 or a Perkin-Elmer 257 recording spectrophotometer. Proton nmr spectra were obtained in deuterated solvents on a JEOL FX60 spectrometer with tetramethylsilane as internal standard. Optical rotation values were measured in a 1 dm cell using a Perkin-Elmer model 141 polarimeter. Mass spectra were recorded on a Varian MAT 731 mass spectrometer at 70 ev.

[4]The plant material was supplied through the auspices of the Drug Research and Development Program of the National Cancer Institute by the Medicinal Plant Resources Laboratory, Agricultural Research Service, U.S.D.A., Beltsville, MD. A herbarium specimen documenting this collection is deposited in the Herbarium of the National Arboretum, Agricultural Research Service, U.S.D.A., Washington, D.C.

[5]Cytotoxicity and *in vivo* activity were assayed by the procedures described in reference 7.

4', 5', 6', 1", 4", 6", 1"', and 4"', 6"'), 119.2, 119.0 (d; C-5", 5"'), 114.9, 114.6 (d; C-3", 3"'), 114.6, 110.5, 108.4 (s; C-6, 8, 10), 77.8 (d; C-2), 61.0 (q; OCH_3), 44.7 (t; C-3), 22.8, 22.5 (t; C-11, 12); ED_{50}(PS) $>1 \times 10^2$ μg/ml.

Anal. Calcd for $C_{30}H_{26}O_6$: C, 74.68; H, 5.39. Found C, 74.61; H, 5.48.

Fraction B (1.82 g) was further chromatographed on a silicic acid column (180 g) using 40% ether in benzene as eluent. A total of 80 fractions (13 ml each) were collected and fractions 28–43 were combined and evaporated to dryness (254 mg). Repeated recrystallizations from absolute ethanol gave **2a** as white needles (100 mg), mp 226–227°C; $[\alpha]^{25}D=0$ (c 0.44 DMSO); cd (0.006% MeOH) $[\theta]_{400-210}=0$; uv λ max (MeOH) 324 nm (ε 9.71 x 10^3), 286 (ε 1.02 x 10^4), 255 (ε 4.29 x 10^3), 223 (ε 1.65 x 10^4) uv λ max (MeOH+NaOAC) 324 nm (ε 2.26 x 10^4), 284 (ε 8.13 x 10^3), 253 (ε 1.27 x 10^4), 228 (ε 2.24 x 10^4); ir (KBr) ν max 3345, 1645, 1615, 1572 cm^{-1}; mass spectrum *m/e* (relative abundance) M+ 376 (100%), 299 (33%), 272 (46%), 270 (24%), 269 (23%), 193 (32%), 179 (17%), 167 (73%), and 166 (15%); ^1H nmr (acetone-d_6) δ 7.21–7.55 (m, 5H), 6.5–7.1 (4H, m), 6.28 (1H, s), 5.52 (1H, dd, X of ABX system; $J_{cis}=4.5$ Hz, $J_{trans}=11$ Hz), 3.92 (2H, s), 3.78 (3H, s), 2.8–3.3 (2H, m, AB of ABX); ^{13}C nmr (DMSO-d_6) δ 187.6 (s; C-4), 162.2, 161.7, 160.1 (s; C-5, 7, 9), 154.8 (s; C-2"), 139.4 (s; C-1'), 128.3, 128.0, 127.9, 126.2, 125.8 (d; C-2', 3', 4', 5', 6', 4", 6"), 126.8, (s; C-1"), 118.6 (d; C-5"), 114.5 (d; C-3"), 106.4, 104.9 (s; C-8, 10), 93.2 (d; C-6), 77.6 (d; C-2), 55.5 (q; OCH_3), 44.7 (t; C-3), 22.0 (t; C-11). ED_{50}(PS) $=6.7 \times 10^1$ μg/ml.

Anal. Calcd for $C_{23}H_{20}O_5$: C, 73.40; H, 5.32. Found: C, 73.40; H, 5.43.

METHYLATION OF **1a** TO **1c**.—An ethanolic solution of **1a** (30 mg) was treated with excess ethereal diazomethane for 2 days at room temperature. After evaporation to dryness, the resulting crude residue (35 mg) was separated on precoated preparative layer plates (silica gel G, 0.5 mm) using chloroform as the developing solvent. The required zone (R_f 0.7) was located under uv and eluted with benzene-acetone mixture and upon evaporation yielded a residue of **1c** (15 mg). Crystallization from absolute ethanol gave colorless shining plates of **1c** (8 mg) mp 130–131.5°C. It had a superimposable ir spectrum ($CHCl_3$) with reference sample of the tetramethyl ether of (−)-dichamanetin (**1c**) (2) as well as the same mobility on tlc (silica gel, $CHCl_3$).

METHYLATION OF **2a** TO **2c** AND **2d**.—Excess ethereal diazomethane was added to 5 ml of an ethanolic solution of **2a** (25 mg) and the reaction mixture was left for 24 hours at room temperature. After the solution was evaporated to dryness, the residue (28 mg) was shown by tlc to contain two major spots (R_f 0.44 and 0.50, system 2% methanol-chloroform). These 2 spots were separated on precoated tlc plates (silica gel G, 0.25 mm); the solvent system was the same as described above. Band I (R_f 0.44) and band II (R_f 0.50) were located under uv, scraped off, and eluted with chloroform-acetone (1:1) and after evaporation to dryness and crystallization from absolute ethanol, gave **2c** (3.5 mg, mp 188–189°C) and **2d** (5 mg, mp 159.5–160°C). Compounds **2c** and **2d** had superimposable ir spectra (chloroform) and the same mobility on tlc as samples (−)-5,7-dimethyl- and (−)-5,7,2"-trimethyl ethers of chamanetin, respectively (2).

METHYLATION OF DICHAMANETIN (**1b**) TO **1d**, **1e**, AND **1f**.—To 3 ml of an ethanolic solution of **1d** (367 mg) was added 8 ml of ethereal diazomethane (0.0075 gm/ml). The reaction mixture was left for 1 hour at room temperature. The solution was evaporated to dryness to yield a residue (380 mg) which by tlc was shown to contain four major spots (R_f 0.81 and 0.77 (**1e** and/or **1f**), 0.48 (**1d**), and 0.34 (**1b**), 0.5% methanol-chloroform; silica gel G (**1a** has R_f 0.14)). The different components of the reaction mixture were separated on precoated preparative layer plates (silica gel G, 1 mm); the solvent system was the same as mentioned above. The different zones were located under uv, scraped, and eluted with chloroform-acetone mixture.

Compound **1d** (R_f 0.48) (40 mg) crystallized from absolute ethanol in fine needles (25 mg) mp 169–170°C, $[\alpha]D-1.2°$ (c 0.5, $CHCl_3$), cd (0.0035% MeOH) $[\theta]_{350}+9640$, $[\theta]_{324sh}-1377$, $[\theta]_{295}-31676$, $[\theta]_{259}+757$, $[\theta]_{220}+50957$; λ max (MeOH) 357 nm (ε 4.05 x 10^3), 282 (ε 1.69 x 10^4), 225 (ε 2.73 x 10^4), λ max (MeOH+NaOAC) 360 nm (ε 4.54 x 10^3), 282 (ε 1.71 x 10^4), 228 (ε 3.06 x 10^4) ir (KBr) ν max 3420, 1642, 1600, 1500, and 1470 cm^{-1}; mass spectrum *m/e* (relative abundance) M+ 482 (27%), 450 (19%), 378 (3%), 377 (14%), 376 (57%), 375 (7%), 299 (9%), 272 (16%), 270 (53%), 269 (23%), 193 (27%), 179 (10%), 166 (8%), 152 (3%), 107 (32%); ^1H nmr ($CDCl_3$) δ 12.65 (1H, s, exchanges with D_2O), 6.46–7.43 (16H, m), 5.39 (1H, dd, X of ABX, $J_{cis}=5$ Hz, $J_{trans}=11$ Hz), 3.89 (4H, s), 3.83 (3H, s), 2.73–3.56 (2H, m, AB of ABX); ^{13}C nmr ($CDCl_3$) 197.6 (s; C-4), 163.6, 159.3, 159.1 (s; C-5, 7, 9), 154.4 (s; C-2", 2"'), 137.9 (s; C-1'), 131.2, 131.0, 129.2, 128.9, 127.0, 126.3, 125.6, 125.3 (C-2', 3', 4', 5', 6', 1", 4", 6", 1"', 4"', 6"'), 120.4, 120.2 (d; C-5", 5"'), 116.5, 116.2 (d; C-3", 3"'), 114.5, 113.2 (s; C-8, 6), 105.9 (s; C-10), 79.6 (d; C-2), 63.1 (q; C-14), 43.4 (t; C-3), 24.4, 23.8 (t; C-11, C-12).

Anal. Calcd for $C_{30}H_{26}O_6$; mol wt, 482.1783; Found: mol wt 482.1756 (MS).

Compound **1e** (R_f 0.77) (63 mg) crystallized from absolute ethanol in platelets (31 mg) mp 182–183°C, $[\alpha]D-6.4°$ (c 0.76, $CHCl_3$); cd (c 0.0055% in MeOH) $[\theta]_{355}+3333$, $[\theta]_{318}-3684$, $[\theta]_{289}-22807$, $[\theta]_{244}-526$, $[\theta]_{222(sh)}+13684$, $[\theta]_{215}+31578$; uv λ max (MeOH) 333 nm (ε 6.84 x 10^3), 297 (ε 8.41 x 10^3), 279 (ε 7.23 x 10^3), 222 (ε 1.87 x 10^4) λ max (MeOH+NaOAC) 330 (ε 1.60 x 10^4), 278 (ε 5.78 x 10^3), 228 (ε 2.31 x 10^4); ir (KBr) ν max 3200, 1650, 1610, 1505, 1460 cm^{-1}; mass spectrum *m/e* (relative abundance) M+ 482 (51%), 377 (7.5%), 376 (32%), 375 (23%), 299 (10%), 272

(6%), 270 (4%), 269 (12%), 361 (12%), 255 (11%), 253 (20%), 193 (7%), 121 (88%), and 107 (28%). ^{1}H nmr ($CDCl_3$) δ 13.1 (1H, s, exchangeable with D_2O), 6.6-7.63 (16H, m), 5.33 (1H, dd, X of ABX, J_{cis}=5 Hz, J_{trans}=11 Hz), 3.93 (3H, s), 3.83 (2H, s), 3.78 (2H, s), 2.5-3.3 (2H, m, AB of ABX); ^{13}C nmr ($CDCl_3$) δ 196.3 (s; C-4), 161.1, 158.8, 158.6 (s; C-5, 7, 9), 155.2, 154.4 (s; C-2", 2"'), 138.6 (s; C-1'), 131.9, 131.5, 128.9, 128.6, 128.4, 127.7, 126.4, 126.2, 125.8 (C-2', 3', 4', 5', 6', 1", 4", 6"', 1"', 4"', 6"'), 122.0, 120.0 (d; C-5", 5"'), 116.4, 110.7 (d; C-3", 3"'), 108.6, 107.2 (s; C-6, 8), 102.9 (s; C-10), 79.5 (d; C-2), 55.8 (q; C-15, 16), 43.3 (t; C-3), 22.9, 22.8 (t; C-11, 12).

Anal. Calcd for $C_{30}H_{26}O_6$; mol wt, 482.1794; Found: mol wt 482.1762 (MS).

Compound **1f** (R_f 0.81) (41 mg) as a gum, $[\alpha]D$ −6.0° (c 0.66 in $CHCl_3$); cd (c 0.007% MeOH) $[\theta]_{362}$+1101, $[\theta]_{315}$+1377, $[\theta]_{292}$−16253, $[\theta]_{250}$−1033, $[\theta]_{225}$+14807; uv λ max (MeOH) 337 nm (ε 5.47 x 10^3), 297 (ε 5.37 x 10^3), 280 (ε 4.65 x 10^3), 223 (ε 1.34 x 10^4), λ max (MeOH+NaOAC) 335 nm (ε 1.24 x 10^4), 275 (ε 3.51 x 10^3), 225 (ε 1.57 x 10^4); ir (KBr) ν max: 3300, 1650, 1505, 1470 cm^{-1}; mass spectrum *m/e* (relative abundance) M+ 482 (55%), 376 (33%), 375 (22%), 361 (21%), 299 (9%), 269 (13%), 253 (22%), 121 (100%), 107 (31%); ^{1}H nmr ($CDCl_3$) δ 12.46 (1H, s, exchangeable with D_2O), 6.4-7.6 (16H, m), 5.33 (1H, dd, X of ABX, J_{cis}=4.75 Hz, J_{trans}=12 Hz), 3.98 (3H, s), 3.86 (2H, s), 3.76 (2H, s), 2.36-3.46 (2H, m, AB of ABX); ^{13}C nmr ($CDCl_3$): δ 196.2 (s; C-4), 160.3, 160.0, 158.3 (s, C-5, 7, 9), 155, 154.4 (s; C-2", 2"'), 138.4 (s; C-1'), 131.9, 131.8, 128.9, 128.2, 127.9, 126.4, 125.9 (C-2', 3', 4', 5', 6', 1", 4", 6", 1"', 4"', 6"'), 122.4, 120 (d; C-5", 5"'), 116.2, 110.7 (d; C-3", 3"'), 108.0, 106.9 (s; C-6, 8), 103.2 (s; C-10), 79.7 (d; C-2), 56.0 (q; C-15, 16), 43.4 (t; C-3), 23.7, 21.9 (t; C-11, 12).

Anal. Calcd for $C_{30}H_{26}O_6$; mol wt, 482.1753; Found: mol wt, 482.1741 (MS).

METHYLATION OF **1b** TO YIELD **1g**.—Treatment of 130 mg of **1b** with excess ethereal diazomethane for 2 days followed by evaporation and chromatography over 20 g of silica gel in benzene afforded **1g** (40 mg), (absolute ethanol) mp 144-147°C, $[\alpha]D$ −30.8° (c 1.20, benzene); cd (c 0.0035% MeOH) $[\theta]_{350}$+5247, $[\theta]_{290}$−15743, $[\theta]_{245}$+1311, $[\theta]_{235sh}$+16618, $[\theta]_{228}$ +20116; uv λ max (MeOH) 277 nm (ε 6.50 x 10^3); ir (KBr) ν max 3210, 1620, 1590, and 1570 cm^{-1}; mass spectrum *m/e* (relative abundance) M+ 510 (52%), 389 (55%), 253 (26%), and 121 (100%); ^{1}H nmr ($CDCl_3$) δ 12.60 (1H, exchangeable with D_2O), 6.80-7.50 (13H, m), 5.50 (1H, dd, X of ABX), 4.10 (4H, s), 3.98 (3H, s), 3.85 (3H, s), 3.58 (3H, s), and 2.84-3.16 (2H, m, AB of ABX); ^{13}C nmr ($CDCl_3$) δ 197.3 (s; C-4), 166.8, 160.6, 159.1 (s; C-5, 7, 9), 157.4 (s; C-2", 2"'), 138.8 (s; C-1'), 129.5, 129.1, 128.9, 128.6, 128.5, 128.2, 126.6, 125.9 (C-2', 3', 4', 5', 6', 1", 4", 6", 1"', 4"', 6"'), 120.4 (d; C-5", 5"'), 110.2 (d; C-3", 3"'), 114.1, 112.9 (s; C-8, 6), 105.5 (s; C-10), 78.6 (d; C-2), 61.7 (q; C-14), 55.5, 55.4 (q; C-15 or 16), 43.6 (t; C-3), 23.0, 22.6 (t; C-11 or 12).

Anal. Calcd for $C_{32}H_{30}O_6$; mol wt 510.2060; Found: mol wt 510.2056 (MS).

^{13}C NMR SPECTRAL CONDITIONS.—The ^{13}C nmr spectra were obtained on a JEOL FX60 spectrometer operating at 15.03 MHz. The samples were run in 5 mm tubes using deuteroacetone, deuterochloroform, or deuterodimethyl sulfoxide as solvent, and tetramethylsilane as reference. The sample sizes ranged from 25-50 mg per 0.4 ml. The spectra were obtained using a spectral width of 4000 Hz, a 45° pulse, a 5 second repetition rate, and 8K data points. The chemical shift assignments were made with aid of literature precedent and off-resonance experiments.

^{13}C NMR DATA FOR FLAVANONE METHYL ETHERS.—Compounds **1c**, **2c**, **2d**, **2e**, **2f**, **3b**, **3c**, and **4** have been reported (2); however, their ^{13}C nmr spectral data were not reported and are listed here:

1c ($CDCl_3$); δ 189.8(s), 164.6(s), 160.8(s), 159.1(s), 157.1(s), 157.0(s), 138.8(s), 129.6(s), 129.0, 128.4, 128.2, 126.6, 125.6, 121.5(s), 120.2(d), 118.3(s), 112.0(s), 109.9(d), 78.2(d), 16.9(q), 61.6(q), 55.3(q), 55.2(q), 45.6(t), 23.4(t), 23.0(t).

2c (DMSO-d_6); 188.0(s), 163.4(s), 160.8(s), 160.7(s), 155.0(s), 139.3(s), 128.3, 127.9, 127.6, 126.6, 126.1, 125.9, 118.6(d), 114.5(d), 1.07.6(s), 105.8(s), 90.1(d), 77.6(d), 56.0(q), 56.0 (q), 44.7(t), 22.0(t).

2d ($CDCl_3$); 189.8(s), 164.1(s), 161.7(s), 161.2(s), 157.5(s), 139.3(s), 129.4, 128.5, 128.3, 128.1, 126.5, 125.9, 120.2(d), 110.1(d), 109.1(s), 106.3(s), 88.9(d), 78.4(d), 56.1(q), 55.7(q), 55.4(q), 45.6(t), 22.2(t).

2e ($CDCl_3$); 196.1(s), 165.3(s), 162.0(s), 159.1(s), 154.7(s), 138.3(s), 131.7, 129.0, 127.9, 126.5, 126.2, 125.9, 120.1(d), 116.6(d), 109.1(s), 103.0(s), 91.9(d), 79.5(d), 56.1(q), 43.2(t), 22.7(t).

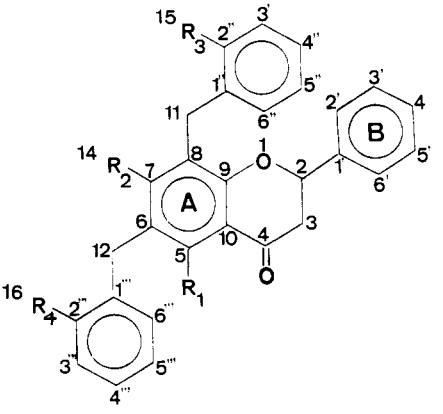
2f ($CDCl_3$); 196.3(s), 166.4(s), 163.1(s), 159.6(s), 157.5(s), 138.9(s), 129.4, 128.8, 128.6, 128.4, 126.6, 125.9, 120.3(d), 110.1(d), 107.8(s), 103.2(s), 92.7(d), 78.5(d), 55.9(q), 55.4(q), 43.3(t), 22.0(t).

3b ($CDCl_3$); 188.4(s), 163.7(s), 163.5(s), 158.1(s), 154.8(s), 138.6(s), 131.6, 128.9, 127.9, 126.2, 125.5, 119.9(d), 116.4(d), 116.4(s), 108.9(s), 96.6(d), 79.2(d), 62.7(q), 56.1(q), 45.5(t), 23.8(t).

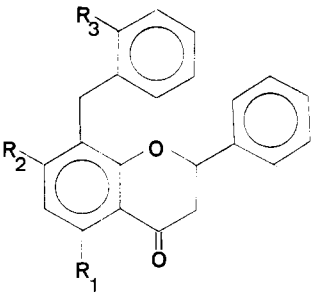
3c ($CDCl_3$); 195.8(s), 166.3(s), 161.9(s), 161.3(s), 157.6(s), 138.7(s), 128.9, 128.1, 126.6, 126.2, 120.3(d), 110.3(d), 108.5(s), 103.1(s), 91.1(d), 79.5(d), 55.9(q), 55.6(q), 43.6(t), 21.5(t).

3d ($CDCl_3$); 188.7(s), 164.7(s), 163.5(s), 160.5(s), 139.0(s), 129.6, 128.8, 128.1, 126.6, 126.2, 125.9, 120.3(d), 116.9(s), 110.1(d), 109.1(s), 95.7(d), 79.2(d), 61.9(q), 55.9(q), 55.4(q), 45.7(t), 22.3(t).

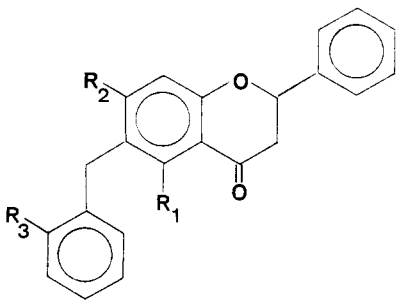
4 (Acetone-d_6); 197.1(s), 169.0(s), 165.1(s), 164.0(s), 140.0(s), 129.5(d), 129.5(s), 127.3(d), 103.9(s), 95.7(d), 94.7(d), 80.0(d), 56.3(q), 43.7(t).



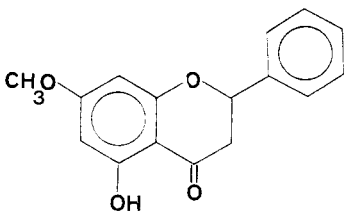
1a $R_1=OCH_3$; $R_2=R_3=R_4=OH$
1b $R_1=R_2=R_3=R_4=OH$
1c $R_1=R_2=R_3=R_4=OCH_3$
1d $R_1=R_3=R_4=OH$; $R_2=OCH_3$
1e and **1f** $R_1=R_2=R_3=OH$; $R_4=OCH_3$ or $R_1=R_2=R_4=OH$; $R_3=OCH_3$
1g $R_1=OH$; $R_2=R_3=R_4=OCH_3$



2a $R_1=OCH_3$; $R_2=R_3=OH$
2b $R_1=R_2=R_3=OH$
2c $R_1=R_2=OCH_3$; $R_3=OH$
2d $R_1=R_2=R_3=OCH_3$
2e $R_1=R_3=OH$; $R_2=OCH_3$
2f $R_1=OH$; $R_2=R_3=OCH_3$



3a $R_1=R_2=R_3=OH$
3b $R_1=R_2=OCH_3$; $R_3=OH$
3c $R_1=OH$; $R_2=R_3=OCH_3$
3d $R_1=R_2=R_3=OCH_3$



4

RESULTS AND DISCUSSION

An investigation of the active ether-methanol wash fraction of a silicic acid column of the root bark of *Uvaria chamae* (2) has resulted in concentration of the

cytotoxic activity (PS *in vitro*) into two fractions from which two optically inactive C-benzylated flavanones (PS inactive)[6] have been isolated.

The uv and ir spectra of **2a** were consistent with a flavanone nucleus (1, 2). The band at λ max 286 shifted to 324 nm with sodium acetate suggesting the presence of a phenolic hydroxyl group at C-7. The mass spectrum showed, in addition to the parent ion peak at *m/e* 376, peaks at 299 (M+ −77) and 272 (M+ −104) consistent with a flavanone nucleus with an unsubstituted B ring. The ^{1}H nmr spectrum showed the characteristic ABX pattern of flavanones (δ5.52, X, H-2; δ 2.8–3.3, AB, H-3). In addition there was a 2H singlet at δ 3.92 (Ar-CH_2-Ar), a 3H singlet at δ 3.78 (OCH_3), and 9 aromatic protons between δ 6.7–7.5. An additional 1H singlet was evident at δ 6.28 (H-6). These data together with the molecular formula, $C_{23}H_{20}O_5$, suggested that **2a** was a monomethylated ether derivative of either chamanetin (**2b**) or isochamanetin (**3a**).

Support for the placement of the methoxyl group at C-5 was deduced from ^{13}C nmr data. The chemical shift of the carbonyl carbon (C-4) of a 5-hydroxyflavanone nucleus appears near δ 197 (5). The signal for C-4 in **2a** appears at δ 187.6. This upfield shift is consistent with placement of the methoxyl group at C-5 since a similar upfield shift upon methylation was noted in xanthone glycosides (8).

The methoxyl carbon signal appears at δ 55.5 in **2a**. Other methyl ether derivatives of chamanetin (**2b**) show similar methoxyl carbon signals (**2c**, 55.4 and 55.9; **2d**, 55.4, 55.7, and 56.1; **2e**, 56.1; **2f**, 55.4 and 55.9). The methyl ether derivatives of isochamanetin (**3a**) show methoxyl carbon signals near δ 56 but also signals near δ 62 (**3b**, 56.5 and 62.2; **3c**, 55.6 and 55.9; **3d**, 55.4, 55.9, and 61.9). The assignment of the carbon signal near δ 62 to the methoxyl group at C 5 in **3b** and **3d** follows because a methoxyl group that has a substituent at *both* ortho positions is known to appear in the range of δ 60–63 (9, 10). Several reports have appeared (11–14) that use this empirical observation in the assignment of molecular structures. Thus, since the methoxyl carbon signal appears at δ 55.5, then **2a** is a 5-methyl ether of chamanetin (**2b**)[7] rather than isochamanetin (**3a**).

Compound **2a** was shown to be racemic since $[\alpha]D = 0$, and $[\theta] = 0$ between 400 and 210 nm in the cd spectrum. Optically active flavanones show intense peaks in the cd spectrum and these have been correlated with absolute stereochemistry (15). Thus, from the evidence presented, **2a** can be represented as (±)-5-0-methyl chamanetin. Methylation of **2a** with diazomethane produced two ether derivatives which were shown to be (±)-5,7-0-dimethyl chamanetin (**2c**) and (±)-chamanetin trimethyl ether (**2d**).

The uv, ir and mass spectral data for **1a** were also consistent with a flavanone nucleus with an unsubstituted B ring. The ^{1}H nmr spectrum showed signals for aromatic protons (δ 6.56–7.64 16H; 3H were exchangeable with D_2O), 1 proton at δ 5.60 (dd, H-2, X of ABX), two protons between δ 2.57–3.70 (H-3, AB of ABX), four protons at δ 3.96 (s, 2Ar-CH_2-Ar), and three protons at δ 3.76 (s, OCH_3). The absence of an upfield aromatic signal near δ 6.0 coupled with the presence of additional aromatic and benzylic protons suggested that **1a** was a dibenzylated flavanone (1, 2). The signal at δ 3.76 was attributed to a methoxyl group. The molecular formula was established as $C_{30}H_{26}O_6$ (elemental analysis) and, when taken with the other data, allows formulation of **1a** as a monomethyl ether deriva-

[6]Work is continuing toward identifying the active constituents in these fractions.

[7]Chamanetin-7-methyl ether (**2e**) has been prepared from chamanetin (2) and can be easily distinguished from **2a** by tlc.

tive of dichamanetin (**1b**). Permethylation of **1a** gave a tetramethyl ether which was shown to be (±)-dichamanetin tetramethyl ether (**1c**). The ^{13}C nmr spectrum of **1a** shows the signal for C–4 at δ 188.3 and the methoxyl carbon at δ 61.0, which indicates that the methoxyl group can be located only at C–5.[8] Thus, **1a** can be formulated as (±)-5-O-methyl dichamanetin.[9]

Three dichamanetin monomethyl ether derivatives (**1d**, **1e**, **1f**) were prepared by careful methylation of dichamanetin (**1b**) with diazomethane.[10] These three monomethyl ethers could easily be distinguished on tlc and none of them corresponded to **1a**. They had spectral data (uv, ir, and ^1H nmr) consistent with their proposed structures. Since these monomethyl ethers were prepared from dichamanetin which has been shown to have the S configuration at C–2, the methyl ethers (**1d**, **1e**, and **1f**) also have the 2S configuration (also supported from cd data). The ^{13}C nmr data were useful in assigning structures. All three derivatives showed the signal for C–4 near δ 197 (196.2, 196.3, 197.6) indicating that none of them were methylated at C–5 (5, 8). One of the monomethyl ethers showed a signal for the methoxyl carbon at δ 63.1(q) while the other two had signals at δ 55.8(q) and 55.6(q). This allows formulation of 7-*O*-methyl dichamanetin (**1d**) as the monomethyl ether derivative that has its methoxyl carbon signal δ 63.1 since this is the only methoxyl group other than C–5 that has *both* ortho positions substituted (9, 10). Additionally, **1d** shows signals (doublets in off-resonance) for C–3" and C–3"' at 116.2 and/or 116.5 and for C–5" and C–5"' at 120.2 and/or 120.4 while in dichamanetin (**1b**) these signals appear at δ 115.0, 115.9, 120.8 and 120.9, respectively (5). These data provide additional evidence that methoxylation has occurred at C–7 and not in the O-hydroxybenzyl moiety. The two monomethyl ethers (**1e** and **1f**) had nearly identical ^{13}C nmr data. Both spectra showed a signal at δ 110.7(d), which is assigned to the carbon ortho to the methoxyl group clearly indicating that methoxylation has occurred in the O-hydroxylbenzyl moiety. The other signals (for C–3" or C–3"', 5", 5"') appear at δ 116.2 (116.4), 120.0(120.1), and 122.4–122.0). The data do not allow assignment of specific structures to **1e** and **1f**.

Dichamanetin trimethyl ether (**1g**) was also prepared; its ^{13}C nmr data showed signals for C–4 at δ 197.6, C–3" and C–3"' at 110.2, C–5" and C–5"' at 120.4, and methoxyl carbons at 61.7(C–7) and 55.5 and 55.4(C–2" and/or C–2"'). Dichamanetin tetramethyl ether (**1c**) (2) showed C–4 at δ 189.8, C–3" and C–3"' at 109.9, C–5" and C–5"' at 120.2, and methoxyl carbons at 61.6 and 61.9(C–5 and/or C–7) and 55.2 and 55.3(C–2" and/or C–2"').

The flavanones previously isolated from *U. chamae* (**1**–**2b**, , **3a**) were all optically active and were shown to have the 2S configuration (1, 2). The C–5 methoxylated flavanones, **1a** and 2**a**, reported here have been shown to be optically inactive.

When a 5-hydroxyl group is present in flavanones, the flavanone⇌chalcone isomerism strongly favors the flavanone side due to hydrogen bonding stabilization of the C–5 hydroxyl group to the C–4 carbonyl group (16). A methoxyl group at C–5 would eliminate this stabilization influence and thus might lead to facile racemization. Thus, it may be that the flavanones have been racemized, although the compounds were not subjected to extreme conditions during isolation.

---

[8]A methoxyl carbon signal at δ 61.0 would be consistent with placement at either C–5 or C–7 since both have diortho substituents (9–14) but, since the signal for C–4 appears at δ 188.4 and not δ 197 (5), the methoxyl carbon must be at C–5.

[9]**1a** is racemic since $[\alpha]D=0$ and showed no cotton effect in the cd spectrum.

[10]Only the tetramethyl ether was previously prepared (2).

ACKNOWLEDGMENTS

This investigation was supported by Grant CA-15590 awarded by the National Cancer Institute, DHEW, and also in part by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi. WLL acknowledges a fellowship from the American Foundation for Pharmaceutical Education. The authors gratefully acknowledge mass spectral data obtained from Dr. James McCloskey, University of Utah.

Received 13 November 1978.

LITERATURE CITED

1. C. D. HUFFORD and W. L. LASSWELL, JR., *J. Org. Chem.*, **41**, 1297 (1976).
2. W. L. LASSWELL, JR. and C. D. HUFFORD, *ibid*, **42**, 1295 (1977).
3. W. L. LASSWELL, JR., Ph.D. Dissertation, University of Mississippi, 1977.
4. W. L. LASSWELL, JR. and C. D. HUFFORD, *Phytochemistry*, **16**, 1439 (1977).
5. C. D. HUFFORD and W. L. LASSWELL, JR., *Lloydia*, **41**, 151 (1978).
6. C. D. HUFFORD and W. L. LASSWELL, JR., *Lloydia* **41**, 156 (1978).
7. R. I. GERAN, A. H. GREENBERG, M. M. MACDONALD, A. M. SCHUMACHER, B. J. ABBOTT, *Cancer Chemother. Rep.*, Part 3, **3**, 1(1972).
8. K. HOSTETTMANN and H. WAGNER, *Phytochemistry*, **16**, 821 (1977).
9. K. S. DHAMI and J. B. STOTHERS, *Can. J. Chem.*, **44**, 2855 (1966).
10. G. W. BUCHANAN, G. MONTAUDO and P. FINOCCHAIRO, *ibid.*, **52**, 767 (1974).
11. A. F. ERASMUSON, R. J. FERRIER, N. C. FRANCA, H. E. GOTTLIEB and E. WENKERT, *J.C.S. Perkin I*, 492 (1977).
12. J. F. CASTELAO, JR., O. R. GOTTLIEB, R. A. DELIMA, A. A. L. MESQUITA, H. E. GOTTLIEB and E. WENKERT, *Phytochemistry*, **16**, 735 (1977).
13. K. PANICHPOL and P. G. WATERMAN, *ibid.*, **17**, 1363 (1978).
14. H. DUDDECK, G. SNATZKE and S. S. YEMUL, *ibid.*, **17**, 1369 (1978).
15. W. GAFFIELD, *Tetrahedron*, **26**, 4093 (1970).
16. T. A. GEISSMAN, "The Chemistry of Flavonoid Compounds," MacMillan Company, New York, 1962, p. 289.