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TWO NEW FLAVONES FROM *CALLIANDRA CALIFORNICA*

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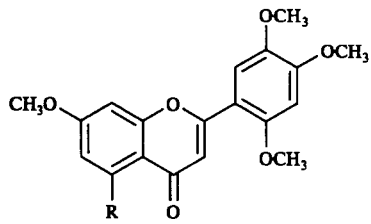
ABSTRACT.—Two new flavones were isolated from the medicinal plant *Calliandra californica*, 7,2′,4′,5′-tetramethoxyflavone [**1**] and 5-hydroxy-7,2′,4′,5′-tetramethoxyflavone [**2**]. The structures were determined from spectral evidence and both compounds were synthesized. Compound **2** exhibited antimicrobial activity for two test bacterial strains.

Decoctions of *Calliandra californica* Benth. (Leguminosae, subfamily Mimosoideae), locally known as "Tabardillo," are used in Baja California Sur to treat kidney ache, cystitis, urethritis, and gallstones (1). Preliminary examination of crude extracts of this endemic species (2), which is uninvestigated from a chemical point of view, revealed antimicrobial activity (3), and inhibition of prostaglandin synthetase activity (4). We now wish to report the structure and activity of two novel flavonoids isolated from this plant.



1 R=H
2 R=OH

The spectral data revealed that both **1** and **2** belong to the flavone group of compounds. Both possess four methoxyl groups as evidenced from their ^{1}H- and ^{13}C-nmr spectra (**1**: 3.93, s, 6H, 3.95, s, 3H, 3.98 ppm, s, 3H; 55.7, 56.0, 56.1, 56.8 ppm; **2**: 3.87, s, 3H, 3.93, s, 6H, 3.97 ppm, s, 3H; 55.8, 56.1, 56.3, 56.8 ppm). In addition **2** was assigned a hydroxyl group at position 5 as apparent from the low-field ^{1}H-nmr chemical shift (δ 12.92) of this proton due to strong hydrogen bonding with the carbonyl group. This observation, coupled with the fact that all proton signals are resolved and only two (at 6.35 and 6.45 ppm) exhibited detectable couplings (around 2 Hz), indicated **2** to be either 5-hydroxy-7,2′,4′,5′- or 5-hydroxy-7,2′,3′,5′-tetramethoxyflavone. From these data, the two possible isomers of the 2-trimethoxyphenyl substituent (viz. 2,4,5- and 2,3,5-trimethoxyphenyl) could not be unambiguously differentiated, although the 2,4,5-isomer was the more likely because of the absence of meta-coupling expected for the 2,3,5-isomer. The assignments of signals from protons at positions 3 and 6 were determined from difference nOe nmr experiments resulting in, after saturation of the hydroxyl proton signal at δ 12.92, 2% enhancements of the signals δ 7.04 and 6.35, respectively. This left only the signals from H-3′ and H-6′ unassigned. A HETCOR experiment allowed assignment of the ^{13}C-nmr signals corresponding to these proton signals. The structure of **1** was proved by synthesis from 2,4,5-trimethoxybenzoic acid and 2-hydroxy-4-methoxyacetophenone. By analogy, **2** was prepared from the same acid and phloroacetophenone (2′,4′,6′-trihydroxyacetophenone) (U. Anthoni, C. Christophersen and P.H. Nielsen, unpublished data).

The genus *Calliandra* is only fragmentarily characterized chemically

because only a few non-protein amino acids, notably monohydroxypipecolic and dihydroxypipecolic acids (5,6), have been described. The present report is thus the first account of flavonoids in this genus.

The crude EtOH extract of the whole plant exhibited antimicrobial activity against *Staphylococcus aureus* (14 mm zone of inhibition), *Bacillus subtilis* (9 mm), and the pathogenic yeast *Candida albicans* (14 mm) at a concentration of 2.8 mg/disc (3). The petroleum ether and EtOAc extracts from the root exhibited activity against *S. aureus* (8 mm and 7.5 mm zones of inhibition, respectively, at 2.8 mg/disc). Pure **2** was active against *S. aureus* (1 mg/disc gave 8 mm inhibition) and *Bacillus subtilis* (1 mg/disc gave 11 mm inhibition), while pure **1** was inactive in these assays.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr experiments were performed on a Varian XL-400 spectrometer operating at 400 MHz for proton and 100.6 MHz for carbon using solutions in $CDCl_3$ with TMS as internal standard. Uv spectra were recorded on a Perkin-Elmer lambda 17 spectrophotometer, ir on a Perkin-Elmer 580 spectrometer, and ms on a VG 20-250 Quadrupole mass spectrometer and a JEOL JMS-HX/HX/10A spectrometer using the direct inlet system. Mps were determined on a Büchi 535 melting point apparatus and are uncorrected. 2′-Hydroxy-4′-methoxyacetophenone, 2′,4′,6′-trihydroxyacetophenone, and 2,4,5-trimethoxybenzoic acid were purchased from Aldrich Chemicals.

EXTRACTION AND ISOLATION.—The plant material was collected in February 1989 at Ejido Alvaro Obregon, Baja California Sur, Mexico. The identification was performed by Ing. Jorge Agúndez Espinoza from the Agronomy Department of Universidad Autónoma de Baja California Sur (UABCS). Voucher specimens are deposited in the Pharmacognosy Laboratory of the Department of Agronomy, UABCS and at the Universidad Nacional Autónoma de Mexico. The root (260 g) was extracted in a Soxhlet apparatus with petroleum ether (48 h), EtOAc (48 h), and EtOH (48 h), successively. The EtOAc extract (7.8 g) after partition between EtOAc (150 ml) and H_2O (5×30 ml) gave an EtOAc extract (7.35 g), that was active against *Staphylococcus aureus* (13 mm zone of inhibition), *B. subtilis* (11 mm), and *Streptococcus faecalis* (10 mm) when applied at 2.8 mg/disc, as well as a H_2O phase that was inactive in this regard. Separation on several Si gel columns gave two fractions, one of which after further purification by prep. tlc on Si gel with toluene-$CHCl_3$-MeOH (5.5:3.0:1.5) and recrystallization ($CHCl_3$) afforded pure **2** (5 mg, 0.002% dry wt) that was active when evaluated against *S. aureus* and *B. subtilis*. The other fraction, after further purification on Si gel columns, gave pure **1** (15 mg, 0.006% dry wt), which was inactive.

7,2′,4′,5′-Tetramethoxyflavone [**1**].—Faint yellow solid; mp 182–183°; uv ($CHCl_3$) λ max (log ε) 257 (4.26), 300 (4.14), 351 (4.22) nm; ir (KBr) ν max 3107w, 2999m, 2988w, 2933m, 2847w, 1632vs, 1587s, 1523s, 1441s, 1393s, 1366s, 1278vs, 1229s, 1208vs, 1166s, 1155s, 1090m, 1050m, 1026vs, 989w, 937w, 846m, 836m, 826m, 813m cm^{-1}; ^1H nmr δ 3.93 (6H, s, 2×MeO), 3.95 (3H, s, MeO), 3.98 (3H, s, MeO), 6.61 (1H, s, H-6′), 6.92 (1H, d, J=2.4 Hz, H-8), 6.97 (1H, dd, J=9.0 and 2.4 Hz, H-6), 7.09 (1H, s, H-3′), 7.43 (1H, s, H-3), 8.13 (1H, d, J=9.0 Hz, H-5); ^{13}C nmr δ 55.8 (MeO), 56.1 (MeO), 56.3 (MeO), 56.8 (MeO), 97.3 (C-3), 100.3 (C-6), 111.5 (C-3′), 111.9 (C-10), 112.1 (C-6′), 113.9 (C-8), 117.7 (C-1′), 127.0 (C-5), 143.1 (C-4′), 152.3 (C-2′), 153.7 (C-5′), 158.0 (C-9), 160.2 (C-2), 163.9 (C-7), 178.2 (C-4); eims *m/z* 342 $[M]^+$ (100), 327 ($[M]^+-CH_3$) (20), 311 ($[M]^+-OMe$) (8), 299 ($[M]^+-CH_3-CO$) (15), 151 (29).

SYNTHESIS OF 2,4,5-TRIMETHOXYBENZOYL CHLORIDE.—A mixture of 2,4,5-trimethoxybenzoic acid (1.06 g, 5 mmol), C_6H_6 (5 ml) and thionyl chloride (7.5 mmol, 0.54 ml) was heated with stirring for 0.5 h at 60°. After cooling to room temperature the crystalline product was isolated by filtration and dried *in vacuo* over KOH: white solid, mp 114–115° [lit. (7) 115–6°]. Yield 90% (1.03 g); *anal.*, calcd for $C_{10}H_{11}O_4Cl$, C 52.06, H 4.78, Cl 15.40%; found C 52.35, H 5.00, Cl 15.05%. ^1H nmr δ 3.98 (3H, s, MeO), 3.93 (3H, s, MeO), 3.89 (3H, s, MeO), 6.50 (1H, s), 7.62 (1H, s).

2,4,5-Trimethoxybenzoic acid 2′-acetyl-5′-methoxyphenyl ester.—The synthesis was carried out following Ref. (8). Thus, 2-hydroxy-4-methoxyacetophenone (1.66 g, 10 mmol) in 15 ml dry pyridine with 2,4,5-trimethoxybenzoyl chloride (3.46 g, 15 mmol) was heated with stirring at 60° for 1 h. The reaction mixture was poured onto ice, neutralized with concentrated HCl, isolated, and recrystallized from EtOH. Yield 80% (2.9 g). Mp 117–8°; *anal.*, calcd for $C_{19}H_{20}O_7$, C 63.33, H 5.59%, found C 63.03, H 5.56%; ^1H nmr δ 2.47 (3H, s, CH_3), 3.82 (3H, s, MeO), 3.88 (3H, s, MeO), 3.89 (3H, s, MeO), 3.94 (3H, s, MeO), 6.55 (1H, s), 6.69 (1H, s), 6.82 (1H, dd, J=9.0 and 2.4 Hz), 7.63 (1H, s), 7.83 (1H, d, J=9.0 Hz); ^{13}C nmr δ 29.4 (CH_3), 55.5, 55.9, 56.3, 56.8 (4×MeO),

97.5, 109.0, 111.7, 114.8, 123.8, 131.7, 142.6, 151.6, 154.5, 156.7, 163.2, 163.4, (12 aromatic C), 172.0 (OC=O), 195.5 (C=O); eims *m/z* 360 $[M]^+$ (11), 195 $[C_{10}H_{11}O_4]^+$ (100).

REARRANGEMENT OF 2,4,5-TRIMETHOXYBENZOIC ACID 2′-ACETYL-5′-METHOXYPHENYL ESTER.—The product, 1-[2,4,5-trimethoxyphenyl]-3-[2-hydroxy-4-methoxyphenyl]propan-1,3-dione, was obtained following Gaydou and Bianchini (8). 2,4,5-Trimethoxybenzoic acid 2′-acetyl-5′-methoxyphenyl ester (2.85 g, 7.9 mmol) was dissolved in dry pyridine (30 ml). Solid, powdered KOH (4.2 g, 79 mmol) was added, and the suspension was stirred for 1 h at 50°. The reaction mixture was poured onto ice and neutralized with concentrated HCl. Recrystallization was effected from EtOH, in 72% yield (2.05 g). The product was used without further purification for the next step, but a sample was chromatographed on Si gel with EtOAc-heptane (80:20) as eluent; mp 117–118°; *anal.*, calcd for $C_{19}H_{20}O_7$, C 63.33, H 5.59%, found C 63.16, H 5.55%; ^{1}H nmr δ 3.64, 3.82, 3.83, 3.87, 3.89, 3.92, 3.95, 3.96 (24H, s, 2×4 MeO), 4.50 (1H, s, CH), 6.42–6.48 (1H, m, CH), 6.55 (1H, s, CH), 7.21 (1H, s, CH), 7.53 (1H, d, J=1.2 Hz), 7.60 (1H, d, J=2 Hz), 7.62 (1H, d, J=2 Hz), 12.42 (1H, s, OH), 12.66 (1H, s, OH), 15.70 (1H, s, OH); eims *m/z* 360 $[M]^+$ (60), 329 $[M-CH_3O]^+$ (45), 195 $[C_{10}H_{11}O_4]^+$ (100).

7,2′,4,′5′-Tetramethoxyflavone [**1**].—1-[2,4,5-Trimethoxyphenyl]-3-[2-hydroxy-4-methoxyphenyl]propan-1,3-dione (1.87 g, 5.2 mmol) was boiled for 2 h with HOAc (16.5 ml) and concentrated H_2SO_4 (250 μl). The solution was poured into H_2O, the product isolated, and recrystallized from EtOH. Yield 60% (1.1 g). Mp 184.5–185.0°; *anal.*, calcd for $C_{19}H_{18}O_6$, C 66.66, H 5.30%, found, C 66.45, H 5.26%. The synthetic product was identical in all aspects with the natural product [**1**].

5-Hydroxy-7,2′,4′,5′-tetramethoxyflavone [**2**].—Faint yellow solid; mp 182–183°; uv ($CHCl_3$) λ max (log ε) 260 (4.35), 291 (4.02), 360 (4.31) nm; ir (KBr) ν max 2920s, 2850m, 1652vs, 1614vs, 1583vs, 1563s, 1520s, 1505vs, 1468s, 1446s, 1391m, 1365s, 1271vs, 1230vs, 1210vs, 1162vs, 1119m, 1027s, 984w, 946w, 851m, 807m cm^{-1}; ^{1}H nmr δ 3.87 (3H, s, MeO), 3.93 (6H, s, 2×MeO), 3.97 (3H, s, MeO), 6.35 (1H, d, J=2.2 Hz, H-6), 6.45 (1H, d, J=2.2 Hz, H-8), 6.58 (1H, s, H-6′ or 3′), 7.04 (1H, s, H-3), 7.40 (1H, s, H-3′ or 6′), 12.92 (1H, s, OH); ^{13}C nmr δ 55.8 (MeO), 56.1 (MeO), 56.3 (MeO), 56.8 (MeO), 92.4 (C-8), 97.1 (C-3′ or 6′), 97.8 (C-6), 105.5 (C-10), 109.7 (C-3), 111.4 (C-1′), 111.9 (C-6′ or 3′), 143.1 (C-4′), 152.8 (C-2′), 154.0 (C-5′), 157.7 (C-5), 161.0 (C-2), 162.1 (C-9), 165.3 (C-7), 182.8 (C-4); eims *m/z* 358 $[M]^+$ (100), 343 $[M-CH_3]^+$ (11), 315 $[M-CH_3-CO]^+$ (11), 167 (37).

The product was identical in all respects with a synthetic sample of **2**.

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LITERATURE CITED

1. R. Encarnación D., R.F. Murillo, and M.L. Pantoja, *J. Ethnopharmacol.*, **20**, 209 (1987).
2. I.L Wiggins, "Flora of Baja California," Stanford University Press, Stanford, CA, 1980, p. 703.
3. R. Encarnación D. and S.K. Garcia, *J. Ethnopharmacol.*, **31**, 181 (1991).
4. C. Christophersen, C. Larsen, and R. Encarnación D., "Traditional Medicine—A Potential Resource. Exploitation of Natural Products," The H.C. Ørsted Institute, Copenhagen, 1991, p. 26.
5. J.T. Romeo and L.A. Swain, *J. Chem. Ecol.*, **12**, 2089 (1986).
6. J.T. Romeo, L.A. Swain, and A.B. Bleecker, *Phytochemistry*, **22**, 1615 (1983), and references cited therein.
7. J. Haraszti, *Acta Chem. Min. Phys.*, **2**, 59 (1931).
8. E. Gaydou and J.-P. Bianchini, *Bull Soc. Chim. Fr.*, **2**, 43 (1978).

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