

TWO NEW FLAVONES IN *CITRUS RETICULATA*

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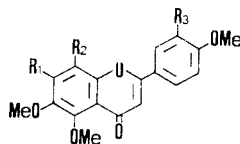
To compare the difference of constituents in the pericarp and in the leaf of *Citrus* species, polyoxygenated flavones were examined using the leaves of *Citrus reticulata* Blanco (Rutaceae). Two new flavones, which were not detected in the pericarps of the plant, were isolated, along with other polyoxygenated flavones (1). In this paper the structure elucidation and the syntheses of the flavones are described.

Besides eight known polyoxygenated flavones (5-hydroxy-3',4',6,7,8-pentamethoxy-, 3',4',5,6,7,8-hexamethoxy-, 4',5,6,7,8-pentamethoxy-, 3',4',5,6,7-pentamethoxy-, 4',5,7-trimethoxy-, 3',4',5,7-tetramethoxy-, 4',5,7,8-tetramethoxy-, and 3',4',5,7,8-pentamethoxyflavone), the two new flavones were isolated from the dried leaves of *C. reticulata* by repeated Si gel column chromatography.

Compound **1**, mp 193-194° was obtained as colorless needles. The ¹H-nmr spectrum showed five aromatic protons attributable to H-3, H-8, H-2', H-5', and H-6' of a flavone skeleton at 6.59 (s), 6.89 (s), 7.37 (d), 6.97 (d), and 7.50 (dd) ppm. The ms gave two representative fragments based on A₁ (*m/z* 181) and B₁ (*m/z* 162), which means the A ring has one hydroxy and two methoxy groups and the ring B two methoxy groups. The position of the hydroxy group was deduced at C-7 by the behavior of Band II (bathochromic shift; 6 nm) with NaOAc in the uv. Thus, **1** was presumed to be 7-hydroxy-3',4',5,6-tetramethoxyflavone (2-4) and confirmed by total synthesis. 4'-Benzyloxy-2'-hydroxy-3,4,5',6'-tetramethoxychalcone

[**3**] (4) was converted to 7-benzyloxy-3',4',5,6-tetramethoxyflavone [**4**] by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). The flavone **4** was debenzylated with Pd-C/H₂ to afford 7-hydroxy-3',4',5,6-tetramethoxyflavone, which was identical with the naturally occurring flavone.

Compound **2**, mp 139-140°, was obtained as colorless needles. The ¹H-nmr spectrum showed five methoxys, a signal due to H-3 (6.60), and signals due to 3',4'-substitution (H-2', 7.50; H-5', 6.97; and H-6', 7.48 ppm). The ms spectrum showed four methoxy groups in ring A (A₁: *m/z* 225) and a methoxy and a hydroxy group in the B ring (B₁: *m/z* 148). The position of the hydroxy was suggested to be at C-3' by the bathochromic shift (48 nm) with decreasing intensity on addition of NaOMe in the uv spectrum. Thus, **2** was presumed to



- 1** R₁=OH, R₂=H, R₃=OMe
- 2** R₁=R₂=OMe, R₃=OH

be 3'-hydroxy-4',5,6,7,8-pentamethoxyflavone and confirmed by synthesis in the following way. Condensation of 2-hydroxy-3,4,5,6-pentamethoxyacetophenone with 3-benzyloxy-4-methoxybenzaldehyde gave 3-benzyloxy-2'-hydroxy-3',4,4',5',6'-pentamethoxychalcone [**5**] which was led to 3'-benzyloxy-4',5,6,7,8-pentamethoxyflavanone [**6**] by isomerization with phosphoric acid in methyl cel-

losolve (5). Treatment of **6** with DDQ gave 3'-benzyloxy-4',5,6,7,8-pentamethoxyflavone [**7**]. The resulting flavone was debenzylated to give 3'-hydroxy-4',5,6,7,8-pentamethoxyflavone. Comparison with the synthetic sample resulted in confirming the naturally occurring flavone to be structure **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—

Melting points were determined on a Büchi melting apparatus and are uncorrected. Uv spectra were taken on a Hitachi 323 spectrometer, and ms were obtained on a JEOL-D300 operating at 70 eV. ¹H-nmr spectra were taken on a Hitachi-Perkin-Elmer R-20B (60 MHz) instrument and a JEOL JNM-GX270 (270 MHz) instrument; chemical shifts are given in δ values (ppm) with TMS as an internal standard. Tlc was carried out with Kiesgel 60F-254 (Merk), developed with C₆H₆-Me₂CO (3:1) and visualized with 10% H₂SO₄. Fujigel BW820-MH was used for column chromatography.

PLANT MATERIAL.—The leaves of *C. reticulata* were collected at the farm of Saga University in October 1985, and a voucher specimen is kept in the Herbarium of Gifu Pharmaceutical University, Gifu-city, Japan.

EXTRACTION AND ISOLATION.—The dried leaves (840 g) were extracted with MeOH (5 liters) for 30 h on a H₂O bath. The extract was concentrated under reduced pressure, and the resultant residue was suspended in H₂O and extracted with EtOAc and *n*-BuOH, successively. The EtOAc fraction (29 g) was subjected to Si gel chromatography developed successively with C₆H₆-Me₂CO (5:1, 3:1, 1:1).

7-HYDROXY-3',4',5,6-TETRAMETHOXYFLAVONE [1].—Colorless needles (*n*-hexane/EtOAc), mp 193-194°; ms *m/z* (rel. int.) 358 (M⁺) (17), 343 (100), 181 (6), 162 (4); ¹H nmr (CDCl₃, 270 MHz) δ 3.96, 3.97, 3.99, 4.02 (12H, each s, 4×OCH₃), 6.59 (1H, s, H-3), 6.89 (1H, s, H-8), 6.97 (1H, d, *J*=8.56 Hz, H-5'), 7.37 (1H, d, *J*=2.13 Hz, H-2'), 7.50 (1H, dd, *J*=8.56, 2.13 Hz, H-6'); uv λ max (MeOH) (log ε) 265 sh (4.13), 330 (4.41) nm; (MeOH/NaOMe) 269, 314, 360; (MeOH/AlCl₃) 265, 330; (MeOH/AlCl₃/HCl) 265, 330, 405; (MeOH/NaOAc) 271, 315, 350; (MeOH/NaOAc/H₃BO₃) 265, 329.

SYNTHESIS OF 1.—4'-Benzyloxy-2'-hydroxy-3,4,5',6'-tetramethoxychalcone [**3**], mp 167-168° [lit. (4) mp 148-150°], (1.0 g, 2.2 mmol) was heated in a dry dioxane solution (30 ml) containing DDQ (1.0 g, 4.4 mmol) under reflux for

30 h. The reaction mixture was purified with column chromatography on Si gel (eluent: *n*-C₆H₁₄-EtOAc, 1:1) to give **4** (380 mg) as colorless needles, mp 176-177°. ¹H nmr (CDCl₃, 60 MHz) δ 3.94, 3.96, 3.98, 4.02 (12H, each s, 4×OCH₃), 5.25 (2H, s, CH₂Ph), 6.65 (1H, s, H-3), 6.91 (1H, s, H-8), 6.92-7.73 (8H, H-2', 5', 6' and CH₂Ph); ms *m/z* (rel. int.) 448 (M⁺) (22), 357 (36), 228 (9), 91 (100). The resulting flavone **4** (350 mg, 0.78 mmol) was debenzylated with Pd-C/H₂ (atmospheric pressure) in EtOAc for 2 h to give 7-hydroxy-3',4',5,6-tetramethoxyflavone (180 mg) as colorless needles, mp 197°. ¹H nmr (CDCl₃, 270 MHz) δ 3.96, 3.97, 3.99, 4.05 (12H, each s, 4×OCH₃), 6.60 (1H, s, H-3), 6.90 (1H, s, H-8), 6.97 (1H, d, *J*=8.56 Hz, H-5'), 7.33 (1H, d, *J*=2.13 Hz, H-2'), 7.51 (1H, dd, *J*=8.56, 2.13 Hz, H-6'); ms *m/z* (rel. int.) 358 (M⁺) (22), 343 (100), 181 (4), 171 (9); uv λ max (MeOH) 240 sh, 265 sh, 332 nm; (MeOH/NaOMe) 236, 271, 316, 361; (MeOH/AlCl₃) 240 sh, 264 sh, 332; (MeOH/AlCl₃/HCl) 264 sh, 332, 406; (MeOH/NaOAc) 271, 316, 360; (MeOH/NaOAc/H₃BO₃) 268, 332.

3'-HYDROXY-4',5,6,7,8-PENTAMETHOXYFLAVONE [2].—Colorless needles (*n*-C₆H₁₄/EtOAc), mp 139-140°; ¹H nmr (CDCl₃, 270 MHz) δ 3.95 (2), 3.98, 4.02, 4.10 (15H, each s, 5×OCH₃), 6.60 (1H, s, H-3), 6.97 (1H, d, *J*=8.13 Hz, H-5'), 7.48 (1H, dd, *J*=8.13, 2.13 Hz, H-6'), 7.50 (1H, d, *J*=2.13 Hz, H-2'); ms *m/z* (rel. int.) 388 (M⁺) (31), 373 (100), 225 (9), 197 (23), 182 (17), 148 (26); uv λ max (MeOH) (log ε) 252 (4.26), 268 (4.17), 334 (4.26) nm; (MeOH/NaOMe) 262, 348, 382; (MeOH/AlCl₃) 250, 268, 334; (MeOH/AlCl₃/HCl) 248, 268, 334; (MeOH/NaOAc) 252, 268, 334; (MeOH/NaOAc/H₃BO₃) 252, 268, 334.

SYNTHESIS OF 2.—3-Benzyloxy-4-methoxybenzaldehyde (480 mg, 2.0 mmol) was condensed with 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (500 mg, 2.0 mmol) to give **5** (780 mg) as orange yellow needles, mp 68°. ¹H nmr (CDCl₃, 60 MHz) δ 3.80, 3.88 (2), 3.95, 4.10 (15H, each s, 5×OCH₃), 5.24 (2H, s, CH₂Ph), 6.92-7.45 (8H, H-2, 5, 6 and CH₂Ph), 7.80 (2H, s, H-β, α), 13.72 (1H, s, OH); ms *m/z* (rel. int.) 480 (M⁺) (32), 241 (17), 235 (39), 162 (12). A solution of **5** (600 mg, 1.3 mmol) dissolved in EtOH (50 ml) containing 85% H₃PO₄ (5 g) was boiled under reflux for 5 h. The mixture was extracted with H₂O and EtOAc. The EtOAc extract was purified by column chromatography on Si gel to obtain **6** (228 mg) as colorless needles, mp 104-105°. ¹H nmr (CDCl₃, 60 MHz) δ 2.85 (1H, d, *J*=4.8 Hz, H-3 *cis*), 2.90 (1H, *J*=11.4 Hz, H-3 *trans*), 3.79, 3.86, 3.90 (2), 4.08 (15H, each s, 5×OCH₃), 5.02 (2H, s, CH₂Ph), 5.38 (1H, dd, *J*=11.4, 4.8 Hz, H-2), 6.88-7.55 (8H, H-2', 5', 6' and CH₂Ph); ms *m/z* (rel. int.)

480 (M^+) (24), 240 (100), 235 (47), 197 (24). Compound **6** (200 mg, 0.4 mmol) was refluxed in a dry dioxane (30 ml) containing DDQ (95 mg, 0.4 mmol) for 7 h. The reaction mixture was subjected to column chromatography on Si gel (eluent: C_6H_{14} -EtOAc, 1:1) to give **7** (155 mg) as colorless needles, mp 141-142°. 1H nmr ($CDCl_3$, 60 MHz) δ 3.95 (2), 3.98, 4.02, 4.10 (15H, each s, $5 \times OCH_3$), 5.24 (2H, s, CH_2Ph), 6.61 (1H, s, H-3), 6.91-7.52 (8H, H-2', 5', 6' and CH_2Ph). Ms m/z (rel. int.) 478 (M^+) (18), 463 (33), 228 (100). Compound **7** was debenzylated with Pd- C/H_2 (atmospheric pressure) in EtOAc for 1 h to give 3'-hydroxy-4',5,6,7,8-pentamethoxyflavone (105 mg) as colorless needles, mp 143-144°. 1H nmr ($CDCl_3$, 270 MHz) δ 3.95 (2), 3.98, 4.03, 4.10 (15H, each s, $5 \times OCH_3$), 6.60 (1H, s, H-3), 6.97 (1H, d, $J=8.56$ Hz, H-5'), 7.48 (1H, dd, $J=8.56, 2.13$ Hz, H-6'),

7.50 (1H, d, $J=2.13$ Hz); ms m/z (rel. int.) 388 (M^+) (33), 373 (100), 225 (4), 197 (11), 182 (11); uv λ max (MeOH) 252, 270, 334 nm; (MeOH-NaOMe) 263, 349, 382.

LITERATURE CITED

1. M. Iinuma, S. Matsuura, K. Kuroguchi, and T. Tanaka, *Chem. Pharm. Bull.*, **28**, 717 (1980).
2. H. Wagner, R. Hoer, T. Murakami, and L. Farkas, *Chem. Ber.*, **106**, 20 (1973).
3. H. Wagner, L. Farkas, G. Florres, and J. Strelisky, *Chem. Ber.*, **107**, 1049 (1974).
4. J.B. Harborne and C.I. Stacey, *Phytochemistry*, **17**, 588 (1978).
5. M. Iinuma, S. Matsuura, and T. Tanaka, *Chem. Pharm. Bull.*, **32**, 1472 (1984).

Received 15 December 1986