Acylated Flavonoids from *Pseudognaphalium* Species

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Two new acylated flavonoids have been isolated from the resinous exudates of *Pseudognaphalium robustum* and *Pseudognaphalium cheirantifolium*. Their structures were elucidated by high-resolution spectroscopic methods as 5,7,8-trihydroxy-3-methoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (**1**) and 5,7,8-trihydroxy-3,6-dimethoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (**2**).

In the course of a phytochemical study of the resinous exudates of *Pseudognaphalium* species,[1,2] two new acylated flavonoids **1** and **2**, have been isolated from *P. robustum* (Phil.) and *P. cheiranthifolium* (Lam.) Hill & Burlt. (Asteraceae), along with the known 5,7-dihydroxy-3,8-dimethoxyflavone.[3] Both plants are widely used in Chilean folk medicine.[4]

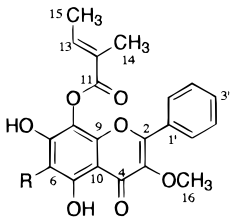
The HRMS of **1** supported the molecular formula $C_{21}H_{18}O_7$, and IR showed absorptions at 1710 (C═O α,β-unsaturated ester) and 1630 (C═O) cm^{-1}. The ^{13}C and DEPT NMR spectra exhibited signals for seven CH carbons, three CH_3 groups, and 11 quaternary carbons. The 400 MHz ($CDCl_3$) ^1H NMR spectrum showed multiplets at δ 7.97 and 7.49, singlets at δ 6.48 and 3.85, and several signals assigned to the side chain. The structure of the side chain was established through 2D NMR studies ($^1H-^1H$ COSY, HMQC, HMBC, NOESY, and $^1H-^1H$ COSYLr), which indicated *E*-stereochemistry and allowed all NMR assignments. Thus, in the HMBC spectrum, C-13 showed long-range correlation with H_3-14 and H_3-15. The cross peaks observed in the NOESY spectrum between H-13 and H_3-15 and between the two olefinic methyls revealed *E*-stereochemistry. The base peak at *m/z* 83 in the EIMS corresponding to $[C_5H_7O]^+$ supported the presence of a tigloyl group. The structure of flavonoid **1** was confirmed by comparison of its spectral data with those of its *Z*-isomer reported previously from *P. robustum*.[1]

The molecular formula $C_{22}H_{20}O_8$ for **2** was deduced from its exact mass $[M]^+$ at *m/z* 412.1176 and from the ^{13}C NMR spectrum. All spectral data (IR, UV, MS, and NMR) suggested a structure similar to that of **1** with one additional methoxyl group located on C-6. Extensive 2D NMR experiments ($^1H-^1H$ COSY, HMQC, HMBC, NOESY, and $^1H-^1H$ COSYLr) supported the structure and permitted complete assignment of all ^1H and ^{13}C NMR resonances.

Acetylation of **1** and **2** afforded 5,7-diacetoxy-8-hydroxy-3-methoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] and 5,7-diacetoxy-8-hydroxy-3,6-dimethoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate], respectively, identified by spectral data (HRMS and NMR).

Experimental Section

General Experimental Procedures. Melting points are reported uncorrected. IR spectra were recorded in KBr disks on a Matson Instrument Galaxi 2020 spectrometer. MS data were recorded on a Fisons VG Autospec mass spectrometer;



1: R= H
2: R= OCH_3

EIMS were obtained with direct inlet at 70 eV. Both ^1H and ^{13}C NMR experiments were recorded in $CDCl_3$ on a Bruker ARX-400 spectrometer with TMS as internal standard; 2D spectra were obtained using standard Bruker software. Aldrich Si gel (200−400 mesh, 60 Å) was used for column chromatography and Si gel GF_{254} for TLC.

Plant Material. Specimens of *P. robustum* and *P. cheiranthifolium* were collected during the flowering season (October 1994) between Zapallar and Papudo (IV Region, Chile, 32°30′S, 71°30′W). Voucher specimens were deposited in the Herbarium of the National Museum of Natural History, Santiago, Chile (Sgo-133617 and Sgo-1333321).

Extraction and Isolation. The resinous exudates were obtained by dipping the fresh plant material in cold CH_2Cl_2 for 15−20 m. The CH_2Cl_2 extracts (10 g, 4.0% dry wt *P. robustum*; 24 g, 4.5% dry wt *P. cheiranthipholium*) were purified by column chromatography on Si gel, using hexane with increasing amounts of EtOAc. Fractions were monitored by TLC on Si gel, using hexane−EtOAc (8:1), spraying with 33% H_2SO_4, and heating to 120°. Final purification by preparative TLC (hexane−EtOAc 8:1) afforded 5,7-dihydroxy-3,8-dimethoxyflavone (20 mg) and 5,7,8-trihydroxy-3-methoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (**1**) (15 mg) from *P. robustum* and 5,7,8-trihydroxy-3,6-dimethoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (**2**) (13 mg) from *P. cheiranthyfolium*.

5,7,8-Trihydroxy-3-methoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (1): white feathers (hexane), mp 177−179 °C; IR ν_{max} 3550, 1710, 1630 cm^{-1}; UV ($CHCl_3$) ν_{max} 208, 322, 364 nm; HRMS *m/z* 382.1054 $[M]^+$, calcd for $C_{21}H_{18}O_7$ 382.1052 [M]; EIMS *m/z* (%) 382 $[M]^+$ (14), 299 (16), 83 (100); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.13 (3H, d, J = 6.7 Hz, H_3-15), 2.14 (3H, s, H_3-14), 3.85 (3H, s, H_3-16), 6.30 (1H, br s, 7-OH), 6.43 (1H, c, J = 6.7 Hz, H-13), 6.48 (1H, s, H-6), 7.49 (3H, m, H-3′, H-4′ and H-5′), 7.97 (2H, dd, J = 8.2, 1.7 Hz, H-2′ and H-6′), 12.40 (1H, br s, 5-OH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.8 (C-15), 21.2 (C-14), 60.9 (C-16), 99.9 (C-6), 106.4 (C-10), 118.6 (C-8), 126.3 (C-12), 128.7 (C-3′ and C-5′), 129.0 (C-2′ and C-6′), 130.6 (C-1′), 131.0 (C-4′), 140.1 (C-3), 143.8 (C-13), 148.1 (C-9), 154.7 (C-7), 156.3 (C-2), 159.3 (C-5), 165.6 (C-11), 179.3 (C-4).

5,7,8-Trihydroxy-3,6-dimethoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (2): yellow powder (EtOH), mp 125−127 °C; IR ν_{max} 3550, 1715, 1635 cm^{-1}; UV ($CHCl_3$) λ_{max} 208, 328, 365 nm; HRMS *m/z* $[M]^+$ 412.1176, calcd for $C_{22}H_{20}O_8$ $[M]^+$

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412.1158; EIMS *m*/*z* (%) 412 $[M]^+$ (57), 329 (34), 83 (100); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.13 (3H, d, $J = 7.0$ Hz, H_3-15), 2.15 (3H, s, H_3-14), 3.85 (3H, s, H_3-16), 4.08 (3H, s, H_3-17), 6.37 (1H, c, $J = 7.0$ Hz, H-13), 6.59 (1H, br s, 7-OH), 7.49 (3H, m, H-3′, H-4′, and H-5′), 7.99 (2H, dd, $J = 7.5$, 1.5 Hz, H-2′ and H-6′), 12.63 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.6 (C-15), 21.2 (C-14), 60.9 (C-16), 61.7 (C-17), 105.6 (C-10), 117.9 (C-8), 126.8 (C-12), 128.9 (C-3′ and C-5′), 129.0 (C-2′ and C-6′), 130.6 (C-1′), 130.7 (C-6), 131.6 (C-4′), 139.8 (C-3), 142.1 (C-13), 144.8 (C-7), 148.5 (C-9), 149.6 (C-5), 156.4 (C-2), 165.7 (C-11), 179.8 (C-4).

Acetylation of 1. Compound **1** (10 mg) was acetylated with Ac_2O (1 mL) in pyridine (1 mL) at room temperature for 12 h under Ar. H_2O (5 mL) was added, and the solution was then extracted with Et_2O (2 × 5 mL). The Et_2O solution was dried and evaporated to afford a residue that was purified by preparative TLC on Si gel using *n*-hexane−EtOAc (1:1) as eluent, to obtain 5,7-diacetoxy-8-hydroxy-3-dimethoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (11 mg): HRMS *m*/*z* $[M]^+$ 466.1275, calcd for $C_{25}H_{22}O_9$ 466.1264; EIMS *m*/*z* (%) 466 $[M]^+$ (13), 425 (12), 424 (44), 382 (18), 342 (17), 300 (39), 299 (49), 83 (100); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.10 (3H, m, H_3-15), 2.15 (3H, s, H-14), 2.33 (3H, s, OAc), 2.48 (3H, s, OAc), 3.80 (3H, s, H_3-16), 6.40 (1H, c, $J = 6.6$ Hz, H-13), 6.95 (1H, s, H-6), 7.51 (3H, m, H-3′, H-4′ and H-5′), 7.95 (2H, d, $J = 8.0$ Hz, H-2′ and H-6′).

Acetylation of 2. Compound 2 (10 mg) was acetylated with Ac_2O (1 mL) in pyridine (1 mL) at room temperature for 12 h under Ar. H_2O (5 mL) was added, and the solution was then extracted with Et_2O (2 × 5 mL). The Et_2O solution was dried and evaporated to afford a residue that was purified by preparative TLC on Si gel using *n*-hexane−EtOAc (1:1) as eluent, to obtain 5,7-diacetoxy-8-hydroxy-3,6-dimethoxyflavone 8-*O*-[(*E*)-2-methyl-2-butenoate] (10 mg): HRMS *m*/*z* $[M]^+$ 496.1374, calcd for $C_{26}H_{24}O_{10}$ 496.1370; EIMS *m*/*z* (%) 496 $[M]^+$ (9), 455 (21), 454 (80), 414 (17), 412 (34), 372 (39), 330 (100), 329 (77), 315 (20), 83 (66); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.13 (3H, m, H_3-15), 2.15 (3H, s, H-14), 2.37 (3H, s, OAc), 2.52 (3H, s, OAc), 3.79 (3H,s, H_3-16), 3.90 (3H, s, H_3-17), 6.39 (1H, m, H-13), 7.50 (3H, m, H-3′, H-4′ and H-5′), 7.95 (2H, m, H-2′ and H-6′).

5,7-Dihydroxy-3,8-dimethoxylflavone: yellow feathers (EtOH) mp 145−148 °C; UV ($CHCl_3$) λ_{max} 208, 332, 364 nm; EIMS *m*/*z* (%) 314 $[M]^+$ (71), 299 (100), 271 (14), 77 (10); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.88 (1H, s, H_3-5), 4.01 (1H, s, H_3-12), 6.44 (1H, s, H-6), 6.54 (1H, s, H-7), 7.56 (1H, m, H-3′, H-4′ and H-5′), 8.12 (2H, m, H-2′ and H-6′), 12.38 (1H, s, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 60.8 (C-11), 62.4 (C-12), 98.9 (C-6), 106.2 (C-10), 130.8 (C-1′), 128.7 (C-2′ and C-6′), 127.2 (C-8), 129.2 (C-3′ and C-5′), 131.5 (C-4′), 140.1 (C-3), 148.5 (C-9), 155.6 (C-2), 155.9 (C-7), 157.9 (C-5), 179.5 (C-4).

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References and Notes

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