

C-Methyl Flavones from the Leaf Wax of *Leptospermum laevigatum* (Myrtaceae)

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Flavonoid aglycones were detected as minor constituents of the leaf wax of the Myrtaceae *Leptospermum laevigatum*. They belong to the rarely encountered C-methyl flavonoids. One of them is a new natural product, 5-hydroxy-3,7,3',4'-tetramethoxy-6-C-methyl flavone.

Introduction

Several members of the Myrtaceae have previously been found to exhibit the rare C-methylated flavones as minor components of epicuticular wax layers (Wollenweber and Kohorst, 1981). We now have studied the leaf wax of *Leptospermum laevigatum* (Gaertner) F. Müll. for the presence of such compounds. The genus *Leptospermum* encompasses some 50 species, growing in Malaysia, Australia and New Zealand (Willis, 1973). From the epicuticular material of *L. laevigatum* we have isolated four C-methyl flavonoid aglycones, one of which is a novel natural product.

Material and Methods

Leaves of *Leptospermum laevigatum* were collected from a plant cultivated for many years in the Botanical Garden of the Technische Hochschule Darmstadt. (A voucher specimen is kept in the herbarium of the Botanical Garden of the TH Darmstadt). Fresh leaves were rinsed with acetone to dissolve the waxy epicuticular material. The gummy residue obtained upon evaporation of the solvent was defatted (MeOH, -10°) and passed over Sephadex LH-20, eluted with MeOH, to separate the flavonoids from the prevailing terpenoids. Flavonoid fractions were further chromatographed over Polyamid SC-6, eluted with toluene and increasing amounts of methylethyl ketone and

methanol. TLC was performed on silica with toluene – MeCOEt 9:1 or with toluene – dioxane – HOAc 18:5:1, and on polyamide DC-11 with petrol_{100–140°} – toluene – MeCOEt – MeOH 12:6:1:1, with toluene – petrol_{100–140°} – MeCOEt – MeOH 12:6:2:1, and with toluene – MeCOEt – MeOH 12:5:3. Chromatograms were viewed under UV₃₆₆ before and after spraying with "Naturstoffreagenz A". Terpenoids were visualized by spraying silica plates with MnCl₂ reagent, followed by heating (Jork *et al.*, 1989). Flavonoid aglycones were identified by direct comparison with markers and/ or by their spectral data. NMR spectra were recorded in DMSO-d₆ at 400 MHz (¹H) and 100 MHz (¹³C) on a Bruker ARX-400 instrument.

Two flavonoids were isolated in crystalline form, although in very small amounts. However, spectra of good quality were obtained and the data are presented below. Two additional flavonoids were identified by direct comparisons with markers available in E. W.'s lab (Wollenweber and Kohorst, 1984).

Compound **1**. Mp 195–196° C (uncorrected). UV λ_{max} (EtOH) nm: 348, 274, 259; + AlCl₃ 367, 268, AlCl₃ + HCl 366, 282, 264; NaOH 410, 267; + NaOAc 413, 270; NaOAc + H₃BO₃ 354, 273, 259. MS m/z (rel. int.): 358 (100, M⁺), 357 (57), 343 (47, M – CH₃), 327 (10), 315 (34), 181 (8), 165 (20), 151 (13). ¹H-NMR δ (ppm): 12.82 (s, OH-5); 7.68 (d, *J* = 2 Hz; H-2'); 7.63 (dd, *J* = 2, 8 Hz; H-6'); 6.97 (d, *J* = 8 Hz; H-5'); 6.80, s (H-8); 3.92, 3.90, 3.83 (s, 3 x OMe); 2.00 (s, C-Me). ¹³C-NMR δ (ppm): 178.0 (C-4), 163.1 (C-7), 157.2 (C-5), 155.5 (C-2), 154.5 (C-9), 149.9 (C-4'), 147.5 (C-3'), 138.0

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(C-3), 122.3 (C-6'), 120.8 (C-1'), 115.7 (C-5'), 112.1 (C-2'), 107.2 (C-6), 104.8 (C-10), 90.1 (C-8), 59.7 (3-OMe), 56.3 (7-OMe), 55.8 (3'-OMe), 7.3 (6-Me). The MS fragmentation is in very good agreement with (Rabesa and Voirin, 1985), while the UV data differ somewhat.

Compound **2**. UV λ_{max} (EtOH) nm: 343, 271, 253; + AlCl₃ 357, 262; AlCl₃ + HCl 362, 280, 261; NaOH 344, 274, 254; no reaction with NaOAc. MS m/z (rel. int.) 372 (100, M⁺), 371 (56), 357 (41, M - CH₃), 341 (12), 329 (39). ¹H-NMR δ (ppm): 12.80 (s, OH-5); 7.74 (dd, *J* = 2, 8 Hz; H-6'); 7.67 (d, *J* = 2 Hz; H-2'); 7.17 (d, *J* = 8 Hz; H-5'); 6.87 (s, H-8); 3.93, 3.87, 3.87, 3.83 (s, 4 x OMe); 2.02 (s, C-Me). ¹³C-NMR δ (ppm): 178.0 (C-4), 163.2 (C-7), 157.2 (C-5), 155.3 (C-2), 154.6 (C-9), 151.3 (C-4'), 148.5 (C-3'), 138.4 (C-3), 122.2 (C-6'), 122.1 (C-1'), 111.6* (C-5'), 111.3* (C-2'), 107.3 (C-6), 104.9 (C-10), 90.3 (C-8), 59.8 (3-OMe), 56.4 (7-OMe), 55.72, 55.70 (3'-OMe, 4'-OMe), 7.3 (6-Me).

Compound **1** was demethylated with pyridinium hydrobromide (Howard and Mabry, 1970) to yield compound **3** (pinoquercetin, M⁺ 316): UV λ_{max} (EtOH) nm: 375, 260; + AlCl₃ 463, 275; AlCl₃ + HCl 440, 270; + NaOH 425 (dec.), 338, 246; + NaOAc 402, 340, (275); NaOAc + H₃BO₃ 385, 345, 300, 260. On polyamide TLC **3** appears as a dark spot that exhibits orange fluorescence after spraying with NA. Its R_f is about the same as that of quercetin.

Results and Discussion

The major part of the leaf wax of *Leptospermum laevigatum* consists of terpenoids, the predominant constituent being oleanolic acid. Four C-methylated flavonoid aglycones are minor constituents of this epicuticular material. 5,4'-Dihydroxy-3,7,3'-trimethoxy-6-C-methyl flavone (compound **1**) and 5-hydroxy-3,7,3',4'-tetramethoxy-6-C-methyl flavone (compound **2**) were isolated as crystalline products and identified by examining their spectroscopic data. 5,4'-Dihydroxy-7-methoxy-6-C-methyl flavone (8-desmethyl-sideroxylin) and 5,4'-dihydroxy-3,7-dimethoxy-6-C-methyl flavone (8-desmethyl-latifolin) were identified by direct comparisons with markers.

In an earlier paper (Wollenweber and Kohorst, 1984) we explained how parallels in R_f-differences and in colour reactions with Naturstoffreagens helped to find the structures of four new C-methyl flavonols. Similar considerations led us to postulate that compound **1** is a C-methyl flavonol with two OH groups, three OMe groups and one C-Me and that compound **2** is its 4'-methyl ether (corresponding to quercetin-3,7,4'-triMe and quercetin-3,7,3',4'-tetraMe). These assumptions were confirmed by the spectral data.

Compound **1** has been found once previously, namely in the bark and spines of the Didieraceae *Alluaudia humbertii* (Rabesa and Voirin, 1985). Compound **2**, its 4'-methyl ether, is a novel natural compound. The demethylation product of compound **1** has been reported as a natural product from the bark of *Pinus ponderosa* (Kurth *et al.*, 1956). 8-Desmethyl-sideroxylin was reported for the first time as a minor constituent of the leaf wax of several *Eucalyptus* species (Wollenweber and Kohorst, 1981) and found later in the leaf wax of some Ericaceae (Wollenweber and Kohorst, 1984). 8-Desmethyl-latifolin is thus far known only from *Kalmia latifolia* and from *Gaultheria procumbens* leaf wax (Wollenweber and Kohorst, 1984).

According to Wollenweber (1994) and Wollenweber and Jay (1988) some 12 flavones with C-methylation have been found thus far, 10 of which were discovered as free aglycones, mostly in Myrtaceae. Three times as many C-Methyl-flavonols have been reported and are distributed in a number of families. The reasons that plants elaborate C-methyl flavonoids are presently unknown, although one can speculate that they play a defensive role reducing attack by specific predatory insects or microorganisms (Proksch and Rodriguez, 1985).

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