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

Eckhard Wollenweber, Karin Mann and James N. Roitman

A Institut für Botanik der Technischen Hochschule, Schnittspahnstrasse 3, D-64287 Darmstadt, Bundesrepublik Deutschland
b Western Regional Research Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710, U.S.A.

Z. Naturforsch. 51c, 8–10 (1996); received November 10/December 11, 1995

Leptospermum laevigatum, Myrtaceae, Leaf Wax, Flavonoid Aglycones, C-Methyl Flavones

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°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.

The residue obtained upon evaporation of the solvent was defatted (MeOH, -10°C) 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 acetone to dissolve the waxy epicuticular material.
E. Wollenweber et al. • C-Methyl Flavones from Leptospermum 9

(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 \( \lambda_{\text{max}} \) (EtOH) nm: 343, 271, 253; + AlCl\(_3\) 357, 262; AlCl\(_3\) + 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-). 

In an earlier paper (Wollenweber and Kohorst, 1984) we explained how parallels in \( R_f \)-differences and in colour reactions with Naturstoffreagenz 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).

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).

Acknowledgement

Thanks are due to Lyn Craven at the Australian National Herbarium (CSIRO Division of Plant Industry) in Canberra, Australia, for determination of the plant studied. E.W. also wishes to thank Dr. K. Siems, Berlin, for NMR analysis of the terpenoid fraction.

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.
Howard G. and Mabry T. J. (1970), Myricetin 3-O-methyl ether 3'-O-b-D-glucoside, the major flavonoid of Oenothera speciosa (Onagraceae). Phytochemistry 9, 2413–2414.


