A Myricetin Tetramethyl Ether from the Leaf and Stem Surfaces of *Tillandsia usneoides*

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Myricetin-3,7,3′,4′-tetramethyl ether has been isolated as an aglycone from the acetone wash of *Tillandsia usneoides*. It is thus shown to be deposited externally, on leaf and stem surfaces. We suggest that the previously reported lipophilic flavonoids from this and other species are also exudate constituents.

Introduction

A tetramethyl derivative of 6-hydroxymyricetin, (5,7,4′-trihydroxy-3,6,3′,5′-tetramethoxy flavone) was found as an aglycone in the dichloromethane "extract" of *Tillandsia usneoides* (L.) L. [1]. We wished to isolate this product as a marker from some Spanish moss cultivated in a Botanical Garden, but instead we isolated from this material a different tetramethoxy flavonol.

Materials and Methods

Tillandsia usneoides (248 g, air dried), cultivated in a greenhouse at the University of Indiana, Bloomington, Indiana, was rinsed in turn with acetone then petrol 40–60 °C containing dichloromethane. After removal of some "wax" which separated from the concentrated solvent rinses, the residual material was eluted from Sephadex LH-20 with methanol. Preparative TLC of combined flavonoid fractions on silica afforded a minute amount of **1** and two additional flavonoids which did not match any of our available markers and cannot presently be identified due to scarcity of material. Thin layer chromatography was performed on polyamide DC-11 with solvents A (petrol$_{100-140°}$-toluene-methylethyl ketone-methanol 12:6:1:1) or B (toluene-petrol$_{100-140°}$-methylethyl ketone-methanol 12:6:2:1) or on silica with solvent C (toluene-methylethyl ketone 9:1). Chromatograms were viewed in UV_{366} before and after spraying with Naturstoffreagenz A (NA). MS were run at 70 eV. ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 at 200 MHz and 50 MHz, respectively.

^1H NMR of compd. **1** (δ ppm): 13.03 (s, 1H; 5-OH); 10.07 (s, 1H; 3′-OH); 7.73* (d, $J = 2.2$ Hz; H-2′); 7.65* (d, $J = 2.2$ Hz; H-6′); 7.20 (d, $J = 2.2$ Hz; H-8); 6.84 (d, $J = 2.2$ Hz; H-6); 4.31, 4.30, 4.27, 4.21 (s, 3H each; 4 × OMe). (* = values may be interchanged.)

^{13}C NMR of compd. **1** (δ ppm): 155.3 (C-2); 138.7* (C-3); 178.6 (C-4); 160.9 (C-5); 97.9 (C-6); 165.3 (C-7); 92.4 (C-8); 156.3 (C-9); 105.3 (C-10); 124.9 (C-1′); 110.0 (C-2′); 150.6 (C-3′); 138.9* (C-4′); 153.0 (C-5′); 103.7 (C-6′); 59.9# (3-OMe); 60.0# (4′-OMe); 55.9§ (5′-OMe); 56.1§ (7-OMe). (*, #, §: values may be interchanged.)

Result and Discussion

The mass spectrum of the isolated flavonoid **1** exhibited M^+ at m/z 374, corresponding to $C_{19}H_{18}O_8$, consistent with a flavone/flavonol with two hydroxy and four methoxy groups and ruled out the previously reported tetramethyl ether of 6-hydroxymyricetin (M^+ 390). ^1H and ^{13}C NMR spectra revealed that the A ring of **1** is like that of rhamnetin (quercetin-7-Me) and the B and C rings are identical to those of the previously studied 5,7,3′-trihydroxy-3,6,8,4′,5′-pentamethoxy flavone from *Gutierrezia microcephala* ([2], compd. 9). The substance is therefore 5,3′-dihydroxy-3,7,4′,5′-tetramethoxy flavone (myricetin-3,7,3′,4′-tetramethyl ether). The UV spectral data agree with those reported in the literature for the flavonol isolated from *Doliocarpus amazonicus* [3]. It was also shown, by direct comparison, to be identical with an authentic sample isolated from the leaf resin of *Cistus monspeliensis* [4].

Myricetin-3,7,3′,4′-tetramethyl ether remains a rare natural flavonol. Apart from the two sources already mentioned, it has been reported as a "farina" constituent from the leaves of the fern *Notholaena candida* var. *candida* [5] and in the glandular excretion of *Geranium macrorrhizum* [6].

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It is noteworthy that this flavonol was not present in greenhouse-grown plant material collected at the Botanical Garden in Darmstadt. Instead, a UV absorbing spot at almost the same R_f as **1**, which remains dark on spraying with Naturstoff-reagenz A (**1** turns yellow) was observed. This could well be the tetramethoxy flavonol that Lewis and Mabry have reported [1]. The existence of chemotypes (or chemically different cultivars) in *Tillandsia usneoides* should be considered.

In a survey of leaf flavonoids from Bromeliaceae, C. A. Williams [7] reported the presence of two highly methylated flavonols in *Tillandsia:* 5,7,4′-trihydroxy-3,6,3′,5′-tetramethoxy flavone in *T. usneoides* and 3,5,7,4′-tetrahydroxy-6,3′,5′-trimethoxy flavone in both *T. usneoides* (L.) L and *T. fasciculata* Swartz. The latter was present as the 3-glucoside, localized in the leaf tissue, whereas the former occurred as the aglycone [1]. The occurrence of flavonoid aglycones has also been reported for two further species of *Tillandsia.* In *T. utriculata,* the 6,3′-dimethyl and 6,7,3′-trimethyl ethers of 6-hydroxyluetolin (jacosidin and cirsilineol) and the 3,6,7,4′-tetramethyl ether of 6-hydroxykaempferol were found [8]. The latter has also been reported in *T. purpurea,* where it occurred together with quercetin-3,7,3′,4′-tetramethyl ether (retusin) and quercetagetin-3,6,7,3′,4′-pentamethyl ether (artemetin) [9].

As mentioned above, the 3,6,3′,4′-tetramethyl ether of 6-hydroxymyricetin was isolated from a dichloromethane extract [1], and jaceosidin, cirsilineol and 6-hydroxykaempferol-3,6,7,4′-tetramethylether from a chloroform extract [8]. In both cases the "extract" may be more aptly considered a leaf wash; it is evident that the flavonoid aglycones found occur on the leaf and stem surfaces of these plants. Our present finding of myricetin-3,7,3′,4′-tetramethyl ether in the acetone/petrol wash of *T. usneoides* clearly sustains this assumption. *Tillandsia* is one of the very few genera so far known within the Monocotyledonae shown to exhibit exudate flavonoids [10].

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