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SHORT COMMUNICATION

MYRICETIN 3-*O*-METHYL ETHER 3′-*O*-β-D-GLUCOSIDE, THE MAJOR FLAVONOID OF *OENOTHERA SPECIOSA* (ONAGRACEAE)

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Abstract—The major flavonoid of *Oenothera speciosa* has been shown to be a new natural product, myricetin 3-*O*-methyl ether 3′-*O*-β-D-glucoside.

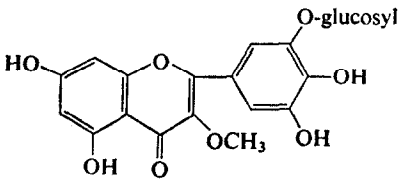
WE REPORT the isolation and structure determination of myricetin 3-*O*-methyl ether 3′-*O*-β-D-glucoside, the major flavonoid in the leaves of *Oenothera speciosa* Nutt. Aq. methanol (1:4) extracts of the dried leaves of *O. speciosa* were chromatographed two-dimensionally and the major flavonoid was isolated as an amorphous, chromatographically pure solid from several chromatograms. The NMR spectrum of the trimethylsilyl ether of the flavonoid indicated that the flavonoid was a glycosyl-methoxyl derivative of myricetin: one methoxyl group (3·8 δ); one monosaccharide (the glycosyl H-1 signal was centered at 4·9); and aromatic proton signals at 6·1 (d, $J = 2{\cdot}5$) and 6·4 (d, $J = 2{\cdot}5$) for H-6 and H-8, respectively, and a two-proton singlet at 7·3 for the H-2′ and H-6′. Demethylation (with concurrent deglycosylation) of the flavonoid by a 5-min treatment with pyridinium hydrobromide* yielded myricetin, confirming the 3,5,7,3′,4′,5′-oxygenation pattern for the compound.

Treatment of the flavonoid with β-glucosidase afforded a myricetin methyl ether which still appeared dark-purple when viewed as a chromatographic spot in u.v. light, indicating that the 3-position was still substituted. Comparison of the u.v. data (a standard set of six spectra was recorded for each compound)[1] for this substance with those obtained for myricetin confirmed that the methoxyl group was attached at C-3. At this point the only structural question remaining concerned the position of attachment of the glucosyl moiety. The u.v. data for the myricetin glycoside indicated that the compound contained free hydroxyl groups at the 4′, 5′, 5 and 7 positions;[1] and, since the methoxyl group was assigned to the 3-position, the glucosyl moiety must be attached at C-3′. This assignment was confirmed by comparing the effect of NaOMe (under spectral analysis conditions)[1] upon the aglycone and the glycoside: the latter was stable to NaOMe while the aglycone rapidly decomposed; this result is in accord with the presence of the alkali-sensitive 3′,4′,5′-hydroxylation pattern in the aglycone. Therefore, the flavonoid from *O. speciosa* must be myricetin 3-*O*-

* Dr. Heinz Rösler, who first employed pyridinium hydrobromide in our laboratory, found that it is a convenient and general reagent for the rapid demethylation (with concurrent de-*O*-glycosylation) of all types of flavonoids.

[1] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York (1970).

methyl ether 3'-*O*-β-D-glucoside (I). The only previous report of myricetin 3-methyl ether is from *Aegialitis annulata* (Plumbaginaceae).[2]



(I)

EXPERIMENTAL

The *Oenothera speciosa* plant material was provided by Dr. Peter Raven, Stanford University, from his experimental garden. All the two-dimensional chromatograms were on Whatman 3MM paper and were developed first in TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and then in 15% HOAc. The NMR spectrum was recorded in CCl_4 using tetramethylsilane as an internal standard. The enzyme hydrolysis of the flavonoid and preparation of its TMS ether were carried out by standard procedures.[1] All the u.v. spectra were recorded using standard procedures.[1]

Myricetin 3-O-*methyl ether* 3'-O-β-D-*glucoside*. The flavonoid glycoside (I) from *O. speciosa* showed the following properties: R_f (TBA) 0·40; R_f (HOAc) 0·18; u.v. λ_{max} (MeOH): 252, 268 sh, 302, 357 nm; λ_{max} (NaOMe): 266, 272 sh, 327, 413 nm; λ_{max} ($AlCl_3$): 273, 311 sh, 439 nm; λ_{max} ($AlCl_3$/HCl): 260 sh, 272, 301, 360, 402 nm; λ_{max} (NaOAc): 265, 272 sh, 326, 408 nm; λ_{max} (NaOAc/H_3BO_3): 258, 299, 376 nm.

Myricetin 3-O-*methyl ether*. The β-glucosidase-hydrolyzed product from I showed the following properties: R_f (TBA) 0·60; R_f (HOAc) 0·085; u.v. λ_{max} (MeOH); 252, 263 sh, 301 sh, 360 nm; λ_{max} (NaOMe): 266, dec.; λ_{max} ($AlCl_3$): 270, 311, 427 nm; λ_{max} ($AlCl_3$/HCl): 270, 306, 366 sh, 403 nm; λ_{max} (NaOAc): 266, 391 dec.; λ_{max} (NaOAc/H_3BO_3): 257, 381 nm.

Demethylation (with concurrent deglycosylation) of the flavonoid glycoside (I) with pyridinium hydrobromide. 1 mg of I was placed in a test-tube with 10 mg of dry pyridinium hydrobromide (prepared by bubbling HBr into benzene and then adding dry pyridine; the resultant salt was filtered, washed with benzene and dried under high vacuum). The mixture was just maintained as a melt for 5 min by carefully heating the test-tube in a flame. The cooled residue was mixed with 5 ml of H_2O and the aqueous solution was then extracted with 5 ml EtOAc This extract was spotted directly onto paper for chromatographic purification and identified as myricetin.

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[2] J. B. HARBORNE, *Phytochem.* **6**, 1415 (1967).