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FLAVONOID AGLYCONES AND FLAVONOL GLYCOSIDES IN THE LIPOPHILIC LEAF EXUDATE OF *NOTHOFAGUS ANTARCTICA*

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Key Word Index—*Nothofagus antarctica*; Fagaceae; leaf resin; flavonoid aglycones; flavonol glycosides.

Abstract—The thin resinous layer on young leaflets of *Nothofagus antarctica* has been found to contain not only some flavonoid aglycones, but also several glycosides of quercetin and myricetin. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Nothofagus antarctica (G. Forst.) Oerst., a member of the Fagaceae native to Chile is cultivated in many European botanic gardens. The shiny surface of young leaflets, which is slightly sticky to the touch aroused our interest as such waxy layers often contain flavonoid constituents. Indeed, a preliminary examination of an acetone rinse of the leafy twigs by TLC did reveal the presence of several flavonoids.

RESULTS AND DISCUSSION

The very thin resinous coating observed on young leaflets of *N. antarctica* is easily removed by dipping briefly in acetone. After elimination of fatty and terpenoid material, five flavonoid aglycones and five flavonol glycosides were isolated from the phenolic fraction. The aglycones were unambiguously identified by direct comparison with marker compounds and by UV spectral analysis to be galangin, galangin 7-methyl ether, 8-methoxy-galangin, myricetin and pinocembrin. The identity of 8-methoxygalangin was also confirmed by its mass spectrum. This latter flavonol is a rare natural product which has been found previously only in the leaf resin of *Adenostoma sparsifolium* (Rosaceae) [1, 2] and in the bud excretion of *Platanus acerifolia* (Platanaceae) [3]. Bud exudates of members of the closely related Betulaceae exhibit a series of 6-methoxyflavonoids, but none has so far been found to produce an 8-*O*-substituted flavonoid [4, 5]. One further aglycone, probably an 8-*O*-substituted polymethoxyflavonoid, remains so far unidentified.

The polar portion of the phenolic fraction was found to contain flavonoid glycosides. Five of them were identified by spectroscopic studies to be the 3-*O*-α-L-arabinopyranosides and the 3-*O*-β-D-galactopyranosides each of quercetin and of myricetin. The fifth product was elucidated to be myricetin 3′-β-D-glucopyranoside. Their UV and NMR spectroscopic data are in agreement with literature data.

The unusual occurrence of flavonoid glycosides in lipophilic plant excretions has been reported only a few times before. We found quercetin-3-rhamnosylglucoside in the leaf and stem exudate of *Lycopersicon lycopersicum* (Solanaceae) and eriodicytol 7-glucoside in *Cotoneaster microphyllum* (Rosaceae) [6]. Earlier, quercetin glycosides and the dihydrochalcone glucoside phloridzin had been reported from the leaf surface of two species of *Kalmia* (Ericaceae) along with lipophilic *C*-methylated flavones [7]. Recently, flavonoid glycosides have been detected in the exudate of *Tragopogon pratensis* (Asteraceae, Lactucaceae), where they also co-occur with lipophilic aglycones [8].

EXPERIMENTAL

Plant material. Twigs of *N. antarctica* with young leaves were collected in April 1994 from a tree in the Botanic Garden at Darmstadt. A voucher is deposited in the herbarium of the Darmstadt Botanic Garden.

Isolation and chromatographic procedures. Freshly cut twigs with young leaves were briefly rinsed with Me_2CO to dissolve epicuticular material. After evapn of solvent, the residue was redissolved in a small amount of boiling MeOH, cooled to −18° and centrifuged to eliminate most of the fatty constituents. The supernatant was chromatographed over Sephadex LH-20, eluted with MeOH, to separate the phenolic portion from the terpenoid material. Flavonoids were

fractionated by chromatography on polyamide SC-6, eluted with toluene and increasing amounts of MeCOEt and MeOH. Frs were monitored by TLC on polyamide (DC-11, Macherey-Nagel) with petrol–toluene–MeCOEt–MeOH (12:6:1:1), toluene–petrol–MeCOEt–MeOH (12:6:2:1) and toluene–MeCOEt–MeOH (12:5:3) and on silica gel with toluene–MeCOEt (9:1). Chromatograms were viewed under UV radiation (366 nm) before and after spraying with Naturstoffreagenz A (diphenyl boric acid–β-aminoethyl ester). Identification of flavonoid aglycones was achieved by co-TLC with authentic markers available in E.W.'s laboratory and by evaluation of their UV spectra. Flavonoid glycosides were analysed by UV spectroscopy and from their NMR (400 MHz: 1D; 500 MHz: 2D) and FAB-MS (Xe, 5–6 keV, glycerol as matrix) spectra.

Hydrolysis. A few mg each of quercetin 3-arabinoside, quercetin 3-galactoside and myricetin 3′-glucoside, respectively, were hydrolysed with 1 N TFA (1 h with reflux). The sugars were sepd from the aglycones by SPE on RP-18 and analysed by TLC with EtOAc–MeOH–HOAc–H_2O (12:3:3:2) on silica gel and MeCOEt–HOAc–satd aq. H_3BO_3 (9:1:1) on silica gel impregnated with Pi buffer adjusted to pH 8 according to ref. [9] (useful for distinguishing pentoses arabinose, xylose, lyxose and ribose). Sugars were detected by spraying with naphthalene-1,3-diol–H_2SO_4 and heating to 110°.

Spectroscopic data. UV and NMR data for the glycosides agreed with lit. data; the results from FAB-MS were as expected. ^{1}H NMR data for quercetin 3-*O*-α-L-arabinopyranoside are given here, as complete sugar analysis has been conducted: ^{1}H NMR (MeOH-d_4, 500 MHz) δ (ppm): 6.19 (*d*, *J* = 1.5 Hz, H-6), 6.38 (*d*, *J* = 1.3 Hz, H-8), 6.86 (*d*, *J* = 8.3 Hz, H-5′), 7.56 (*dd*, *J* = 1.9, 8.6 Hz, H-6′), 7.73 (*d*, *J* = 1.9 Hz, H-2′), 5.15 (*d*, *J* = 6.4 Hz, H-1″), 3.89 (*dd*, *J* = 6.5, 8.4 Hz, H-2″), 3.81 (*dd*, *J* = 3.7, 13.2 Hz, H-5″a), 3.80 (*d*, *J* = 3.3 Hz, H-4″), 3.64 (*dd*, *J* = 2.9, 8.3 Hz, H-3″), 3.43 (*dd*, *J* = 3.0, 13.4 Hz, H-5″b). Assignments were accomplished by ^{1}H–^{1}H COSY and *J*-resolved COSY.

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