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QUERCETIN 3-GLUCOSYLGALACTOSIDE FROM POLLEN OF *CORYLUS AVELLANA*

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Key Word Index—*Corylus avellana*; Betulaceae; hazelnut; pollen; flavonol; quercetin glycoside; glucosylgalactose.

Abstract—The main flavonoid glycoside from the pollen of *Corylus avellana* has been characterized as quercetin 3-*O*-(2″-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside on the basis of UV, ^{1}H NMR, ^{13}C NMR and mass spectral data and GLC sugar analysis.

INTRODUCTION

The anthers and pollen of higher plants are good examples of systems showing secondary phenylpropanoid metabolism, in which accumulation of flavonoids (together with carotenoids) appears to be widespread [1]. Previous studies of pollen flavonoid pigmentation have shown that the principal flavonoids in pollen were frequently 3-di- and triglycosides [2]. As part of a comprehensive investigation of phenylpropanoids in pollen of higher plants, we report here on the main flavonoid in the pollen of *Corylus avellana*.

RESULTS AND DISCUSSION

HPLC analyses of 50% aqueous methanol extracts of freeze-dried pollen of *Corylus avellana* showed that the main flavonoid (quercetin glycoside, **1**) constituted 79% of the total flavonoid content (*ca* 50 μmol/g dry wt or *ca* 3% of the dry wt). Another quercetin glycoside (13%) and two kaempferol glycosides (5%, 3%) were minor constituents. Besides these flavonoids there were two major ferulic acid conjugates, whose structural elucidation is presently underway in these laboratories.

In the present study, the main quercetin glycoside was isolated and its structure was assigned on the basis of the data presented below. The UV spectral data (λ_{max}^{MeOH} nm: 256, 269 sh, 299 sh, 357; + NaOMe 272, 333, 405; + NaOAc 273, 325, 390; + NaOAc–H_3BO_4 262, 299, 390; + $AlCl_3$ 275, 300, 335, 432; + $AlCl_3$–HCl 275, 365 sh, 401 and colour reaction (dark purple to yellow in UV + NH_3) are consistent with those of a quercetin skeleton with the 3-hydroxyl group substituted [3]. Total acid hydrolysis gave quercetin (TLC), galactose and glucose (GLC), which were identified by direct comparison with authentic compounds. Quantitative UV spectroscopy and quantitative GLC gave a molar ratio of quercetin:galactose:glucose of 1:0.8:0.9. A negative ion fast atom bombardment mass spectrum showed a molecular ion $[M - H]^-$ at *m/z* 625 with a fragmentation pattern with sequential loss of two hexose moieties, thus identifying the 3-*O*-substituent as a disaccharide.

The ^{1}H NMR and ^{13}C NMR spectra unambiguously identified the compound as a quercetin derivative with a 3-*O*-substituent [4]. The number and characteristic shifts of the ^{13}C glycosidic signals indicated the presence of two hexose systems. Both sugars had β-glycosidic linkages from the magnitude of the vicinal proton couplings of the glycosidic protons in the ^{1}H NMR spectrum. Selective ^{1}H homonuclear decoupling, beginning with the highest frequency sugar signal H-1″, allowed identification of all the protons of the first hexose moiety. The magnitude of the vicinal couplings indicated that this sugar was galactose. Similar experiments beginning with the second glycosidic proton H-1‴ identified H-2‴ and showed that H-3‴ to H-5‴ appeared as a complex multiplet at 3.19–3.15 ppm. The axial dispositions of H-1‴, H-2‴ and H-3‴ followed from the vicinal couplings but a distinction between glucose and galactose was not possible.

The ^{13}C NMR spectrum was assigned by the use of selective proton decoupling. The correlation of H-2″ with the signal 80.68 ppm, which is characteristic of substitution at C-2″ [5], indicated that the first sugar was 1,2-disubstituted. The characteristic ^{13}C shift of C-1‴ showed that the second sugar moiety was attached via this carbon to C-2″ [5]. The nature of the second sugar moiety was established from the ^{13}C shifts [4] to be β-glucopyranose. Thus the combination of the spectroscopic data and GLC sugar analysis led us to the suggestion that the main flavonoid glycoside from the pollen of *Corylus avellana* was a quercetin 3-*O*-(2″-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside. We tentatively assume that the minor quercetin glycoside (13%) might be the quercetin 3-sophoroside reported earlier [6].

EXPERIMENTAL

Plant material. Pollen of *Corylus avellana* L. was collected from the area around Münster and was freeze-dried immediately after harvest.

Extraction and isolation. Pollen was homogenized (Ultra Turrax) and extracted with 50% aq. MeOH. The extract was

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centrifuged (3000 *g*, 15 min), concd to a small vol. under red. pres. and kept at −20°. Compound **1** was isolated by CC (Polyamide CC 6) with 40% aq. MeOH and subsequent prep. TLC on microcrystalline cellulose (20 × 40 cm) in *n*-BuOH–HOAc–H_2O (6:1:2). The band was scraped off, eluted with 50% aq. MeOH, rechromatographed on Polyamide CC and finally purified on Sephadex LH-20 CC (80 × 2 cm) with H_2O. R_f values on microcrystalline cellulose were: *n*-BuOH–HOAc–H_2O (6:1:2), 0.38; 15% HOAc, 0.64; 5% HOAc, 0.45; *i*-BuOH–HOAc–H_2O (10:7:3.5), 0.74; H_2O–EtOH–methylethylketone–acetylacetone (13:3:3:1), 0.73; H_2O–MeOH–methylethylketone (4:3:3), 0.86.

HPLC analysis. The equipment used for HPLC has been described elsewhere [7]. The chromatographic separation was carried out on a RP-8 column (5 μm, 250 × 4 mm), using a 30-min gradient from 30 to 90% solvent B (1% *ortho*-phosphoric acid +20% HOAc+25% MeCN in H_2O) in solvent A (1% *ortho*-phosphoric acid in H_2O) at a flow rate of 1 ml/min. Detection was at 360 nm. Rutin was used as a standard for quantification.

Hydrolysis and GLC sugar analysis. For total hydrolysis, compound **1** was dissolved in 2 ml 1 M HCl–MeOH (1:1) and heated to 90° for 2 hr. MeOH (5 ml) was added to the soln, which was evapd to near dryness on a rotary evaporator (30°) and another 5 ml MeOH was added. This was done twice. Then the soln was evapd to complete dryness. For sugar analysis by GLC, the residue was silylated by the introduction of 200 μl MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) in 0.8 ml dry pyridine. Samples (1 μl) were injected onto a ARNC-OV-101 column (wall coated open tubular column, 25 m) in a Varian gas chromatograph (100° for 1 min, then programmed at 6°/min to 275°). Identification and quantification of the sugars were performed using glucose and galactose as standards.

^{1}H and ^{13}C NMR spectra were recorded at ambient temp., at 400 and 100 MHz, respectively, on a Bruker WM-400 NMR spectrometer locked to the deuterium resonance of the solvent, DMSO-d_6. Chemical shifts are reported in ppm relative to TMS. The following abbreviations are used to indicate the multiplicities of the signals in the ^{1}H NMR spectrum and the multiplicities of the ^{13}C signals in the single-frequency ^{1}H-decoupled off-resonance ^{13}C NMR spectrum: *s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet and *br* = broad. A negative ion fast atom bonbardment mass spectrum (FAB MS) was recorded on a Kratos MS 50 S mass spectrometer equipped with a Kratos FAB source. Glycerol was used as the matrix.

Quercetin 3-O-(2″-O-β-D-*glucopyranosyl*)-β-D-*galactopyranoside* (**1**). ^{1}H NMR DMSO-d_6: δ7.654 [*dd*, H-6′, *J* (6′-2′) = 2.0, *J* (6′-5′) = 8.5 Hz], 7.530 [*d*, H-2′], 6.845 [*d*, H-5′], 6.397 [*d*, H-8, *J* (8-6) = 2.0 Hz], 6.189 [*d*, H-6], 5.659 [*d*, H-1″, *J* (1″-2″) = 7.7 Hz], 4.570 [*d*, H-1‴, *J* (1‴-2‴) = 7.8 Hz], 3.767 [*dd*, H-2″, *J* (2‴-3‴) = 9.2 Hz], 3.674 [*br d*, H-4″, *J* (4″-3″) = 3.2, *J* (4″-5″) < 1 Hz], 3.609 [*dd*, H-3″], 3.570 [*dd*, H-6_A‴, *J* (6_A‴-6_B‴) = (−) 11.8, *J* (6_A‴-5‴) = 1.8 Hz], 3.483 [*dd*, H-6_B‴, *J* (6_B‴-5‴) = 4.0 Hz], 3.395 [*dd*, H-6_B″, *J* (6_A″-6_B″) = (−) 10.8, *J* (6_B″-5″) = 6.2 Hz], 3.331 [*t*, H-5″, *J* (5″-6_B″) = 6.3 Hz], 3.233 [*dd*, H-6_B″], 3.19–3.15 [*m*, H-3‴, H-4‴ and H-5‴], 3.078 [*dd*, H-2‴, *J* (2‴-3‴) ~ 8.5 Hz]. ^{13}C NMR DMSO-d_6: δ177.34 (*s*, C-4), 164.13 (*s*, C-7), 160.95 (*s*, C-5), 156.18 and 155.35 (*s* × 2, C-2, C-9), 148.34 (*s*, C-4′), 144.69 (*s*, C-3′), 133.04 (*s*, C-3), 122.08 (*d*, C-6′), 121.08 (*s*, C-1′), 115.81 (*d*, C-5′), 115.27 (*d*, C-2′), 104.24 (*d*, C-1‴), 104.11 (*s*, C-10), 98.56 (*d*, C-6), 98.40 (*d*, C-1″), 93.40 (*d*, C-8), 80.68 (*d*, C-2″), 75.75 (*d*, C-5″), 73.20 (*d*, C-3″), 67.42 (*d*, C-4″), 60.60 (*t*, C-6‴), 59.78 (*t*, C-6″), 76.67, 76.40, 74.26 and 69.50 (*d* × 4, C-2‴, C-3‴, C-4‴ and C-5‴). FAB MS *m/z*: 625 [M − H]$^-$; 463 [M − hexose]$^-$; 301 [M − 2 × hexose]$^-$.

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