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QUERCETIN 3-*O*-GALACTOSYL-(1 → 6)-GLUCOSIDE, A COMPOUND FROM NARROWLEAF VETCH WITH ANTIBACTERIAL ACTIVITY

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Key Word Index—*Vicia angustifolia*; Leguminosae; flavonol glycoside; quercetin; antibacterial.

Abstract—A new flavonol glycoside, quercetin 3-*O*-galactosyl-(1 → 6)-glucoside, has been isolated from above-ground parts of narrowleaf vetch, *Vicia angustifolia*. Its antibacterial activity against *Pseudomonas maltophilia* and *Enterobacter cloacae* is compared with that of several other flavonol glycosides.

INTRODUCTION

Vicia angustifolia Reichard, commonly called narrowleaf vetch, is one of the most widespread weeds in the southern United States [1]. In a preliminary search (unpublished data) to identify plants having biological activity against insects, extracts of narrowleaf vetch were found to inhibit the growth of two bacteria isolated from the gut of two *Heliothis* insects, *H. zea* Boddie and *H. virescens* Fabricius. Although there have been no previous reports of flavonoids from *V. angustifolia*, several other *Vicia* species (Table 1) have been investigated for their flavonoid content.

RESULTS AND DISCUSSION

A methanol–water extract of narrowleaf vetch, which showed antibacterial activity, yielded **1**, quercetin 3-*O*-galactosyl-(1 → 6)-glucoside. Compound **1** showed slight antibacterial activity when tested alone.

Comparison of the UV–visible spectra and polyamide and cellulose TLC of **1** with standard compounds indicated that it was a flavonol diglycoside with substitution at the 3-position [10–12]. Upon acid hydrolysis of the glycoside, the isolated aglycone gave UV–visible spectral data and TLC identical to those of quercetin. Cellulose TLC of the acid hydrolysate gave glucose and galactose.

When the compound was methylated, then hydrolysed, the CI mass spectrum of the product gave an $[M+1]^+$ ion at *m/z* 359, which corresponds to quercetin tetramethyl ether ($C_{19}H_{18}O_7$). The UV–visible spectra of the methylated compound in methanol and with the shift reagents NaOMe, $AlCl_3$, $AlCl_3$–HCl, NaOAc, and NaOAc–H_3BO_3 were identical to those of quercetin 5,7,3′,4′-tetramethyl ether, indicating that the disaccharide is attached at the 3-position. The ^1H NMR spectrum of the trimethylsilyl ether of **1** showed the glucosyl H-1 signal at δ5.85, confirming substitution at position 3 [10].

Oxidative cleavage of the glycoside with hydrogen peroxide gave the free disaccharide [13]. Cellulose TLC of the hydrolysis solution gave melibiose (6-*O*-α-D-galactopyranosyl-D-glucose). Enzymatic assay confirmed the α-

Table 1. Flavonoids found in *Vicia* species

Flavonoid	Source	Ref.
Cosmosiin Apiin Luteolin 7-*O*-glucoside Luteolin	*V. balansae* foliage, flowers	2
Afzelin Isoquercitrin	*V. varia* flowers	3
Diosmetin 7-*O*-glucoside 2 Diosmetin 7-*O*-biosides	*V. pannonica* leaves	3
Quercetin 3-*O*-rhamnoside	*V. atropurpurea* flowers	4
Quercetin 3-*O*-glucosido-7-*O*-rhamnoside	*V. hybrida* flowers	4
Diosmetin 7-*O*-rutinoside Kaempferol 3-*O*-α-L-rhamnoside	*V. variabilis* flowers	5
Kaempferol 3-*O*-glucosido-7-*O*-rhamnoside	*V. faba* leaves	6
Cosmosiin Luteolin 7-*O*-glucoside	*V. unijuga* leaves	6
Quercitrin	*V. sylvatica* flowers	7
Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside Kaempferol 3,7-*O*-di-glucoside Quercetin 3-*O*-glucoside-7-*O*-rhamnoside	*V. sepium* leaves	7
Quercitrin	*V. hirsuta* leaves	8
Apiin	*V. hirsuta* leaves	9

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linkage between galactose and glucose and showed that the glucose unit was in the β-configuration. Therefore, the structure of **1** is quercetin 3-*O*-galactosyl-(1 → 6)-glucoside.

When tested for antimicrobial activity with *Pseudomonas maltophilia* Hugh & Ryschenkow and *Enterobacter cloacae* (Jordan) Hormaeche & Edwards, **1** and several similar flavonoid glycosides showed slight growth inhibition of the two bacteria. Quercetin monosides that were tested included quercetin 3-*O*-glucoside (isoquercitrin), quercetin 3-*O*-galactoside (hyperoside), quercetin 7-*O*-glucoside (quercimeritrin), and quercetin 3′-*O*-glucoside. Also tested was a quercetin bioside, quercetin 3-*O*-rhamnoglucoside (rutin). The test results are given in Table 2.

The following observations can be made to relate chemical structure to biological activity for *P. maltophilia*: (1) all of the glycosides with substitution at the 3-position had slight activity; (2) when the sugar at the 3-position was rhamnose, higher activity was observed; (3) there was no measurable difference in activity between a glucoside and a galactoside; (4) there was no apparent difference between glycosidation at the 3- and 7-positions; and (5) when glycosidation was at the 3′-position in the B-ring, there was no activity.

Structure–activity relationships for *E. cloacae* were different from those for *Pseudomonas maltophilia*, and the following observations can be made: (1) quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside and quercetin 3-*O*-rhamnoglucoside were not active; (2) quercetin 3′-*O*-glucoside, quercetin 3-*O*-galactoglucoside and quercetin 7-*O*-glucoside were active; and (3) the highest activity was from quercetin 3-*O*-rhamnoside.

EXPERIMENTAL

The ^{1}H NMR spectrum was run on an 80 MHz instrument in $CDCl_3$ with TMS as internal standard. The mass spectra were recorded on an HP-5985B GC/MS/DS in the CI mode. The source was operated at 230 eV and 200°. Methane was used as the ionizing gas at a source pressure of 0.4–0.8 Torr.

Extraction of plant material. Vicia angustifolia Reichard, collected in Starkville, MS, was freeze-dried, then ground to give 200 g of a powdery, green solid. This solid was extracted overnight in boiling reflux with cyclohexane–EtOAc (1:1, 0.1% HOAc). After filtering, the marc was extracted overnight in boiling reflux with MeOH–H_2O (7:3). The MeOH–H_2O extract was partitioned with an equal vol. of EtOAc.

Fractionation of the MeOH–H_2O *extract.* The MeOH–H_2O extract, which showed the highest activity, was chromatographed on a polyamide column (l = 30 cm, i.d. = 2.5 cm). The column was eluted with MeOH–H_2O (7:3). Five fractions were collected.

Fraction 5 was rechromatographed on polyamide to give three subfractions: 5A, 5B and 5C. Subfraction 5B yielded a pure flavonoid glycoside, compound **1** (180 mg; 0.09% estimated yield).

Identification of compound **1**. TLC was performed on polyamide in the solvent system 3E2W [EtOH–H_2O (3:2)] and on cellulose in 15% HOAc and in TBA [*t*-BuOH–HOAc–H_2O (3:1:1)]. The chromatograms were viewed under UV light alone and after being exposed to NH_3 fumes. The R_fs of the glycoside were 0.65 on polyamide in 3E2W, 0.58 on cellulose in 15% HOAc, and 0.47 on cellulose in TBA. The spot appeared dark purple under UV light and yellow under UV light after exposure to NH_3. Compound **1** had the following spectral data: λ_{max} nm: MeOH 256, 263 sh, 297 sh, 358; NaOMe 271, 326, 398; $AlCl_3$ 272, 303 sh, 355, 430; $AlCl_3$–HCl 271, 295 sh, 342 sh, 401; NaOAc 273, 301 sh, 396; NaOAc–H_3BO_3 262, 289 sh, 388.

Acid hydrolysis. Approximately 0.5 mg of **1** was hydrolysed in 5 ml 1 M HCl. The soln was heated for 2 hr at 95°. The cooled soln was extracted with 5 ml EtOAc to separate the aglycone, the H_2O layer was allowed to evaporate to dryness, and the residue was dissolved in 0.5 ml pyridine.

Evapn of the EtOAc gave a yellow solid with identical spectral and R_f data to quercetin. TLC of the pyridine soln was performed on cellulose in pyridine–isoamyl alcohol–H_2O (7:7:2). The chromatogram was developed by spraying with anisidine phthalate and then heating at 120° for 5 min. The sugars were identified by co-chromatographing authentic sugars alongside them for comparison and glucose and galactose were found.

Methylation. Permethylation with NaH–MeI in DMF of **1**, followed by hydrolysis, gave quercetin 5,7,3′,4′-tetramethyl ether, the CI mass spectrum of which showed the $[M+1]^+$ ion at m/z 359. The UV spectral data were: λ_{max} nm: MeOH 250, 355; NaOMe 260, 395; $AlCl_3$ 260, 420; $AlCl_3$–HCl 260, 420; NaOAc 250, 370, 416 sh; NaOAc–H_3BO_3 250, 360, 420 sh.

The ^{1}H NMR spectrum of the trimethylsilyl ether of **1** gave signals at δ8.10–8.30 (*dd*, 2H, H-2′ and H-6′), 7.10 (*d*, 1H, H-5′), 6.80 (*d*, 1H, H-8), 6.10 (*d*, 1H, H-6), 5.85 (*d*, 1H, H-1 glucosyl), 4.60 (*m*, 1H, H-1 galactosyl), 2.15–2.80 (*m*, 12H, 3-galactoglucosyl).

Oxidative cleavage of compound **1** *with hydrogen peroxide.* Compound **1** (3 mg) was dissolved in 1 ml 0.1 M NH_4OH and two drops of 30% H_2O_2 were added to the soln. After standing at room temp. for 4 hr, the soln was chromatographed on cellulose in pyridine–isoamyl alcohol–H_2O (7:7:2), *n*-BuOH–HOAc–H_2O (4:1:5, upper phase), and *n*-BuOH–C_6H_6–pyridine–H_2O (5:1:3:3), alongside authentic samples of rutinose (6-*O*-α-L-rhamnosyl-D-glucose), melibiose (6-*O*-α-D-galactopyranosyl-D-

Table 2. Antimicrobial test results for flavonoid glycosides

Test compound	Zone width(mm) *P. maltophilia*	*E. cloacae*
Quercetin 3-*O*-galactosyl-(1 → 6)-glucoside	≤7.0	≤7.0
Quercetin 3-*O*-glucoside	≤7.0	n.a.*
Quercetin 3-*O*-rhamnoside	8.5	8.5
Quercetin 3-*O*-galactoside	≤7.0	n.a.
Quercetin 3-*O*-rhamnosyl-(1 → 6)-glucoside	≤7.0	n.a.
Quercetin 7-*O*-glucoside	≤7.0	≤7.0
Quercetin 3′-*O*-glucoside	n.a.	≤7.0

*n.a. = no activity.

glucose), α-lactose (4-*O*-β-D-galactopyranosyl-α-D-glucose), and glucose. The chromatograms were sprayed with anisidine phthalate and dried at 120° for 5 min. The unknown disaccharide had R_g values identical to melibiose.

Enzymatic hydrolysis. A 1.0 mg sample of **1** was hydrolysed for 24 hr at room temp. with β-glucosidase (pH 5.0). TLC data of the hydrolysis soln indicated that the glycoside was completely hydrolysed to quercetin. Hydrolysis of **1** with β-galactosidase gave no changes in the TLC data.

Bioassay. Fractions were screened for antibacterial activity against the pathogens *P. maltophilia* and *E. cloacae*, with bacto-sensitivity discs (BBL). Each fraction to be tested was dissolved in an appropriate solvent (1 mg/20 μl). A 20 μl aliquot of the soln was applied to a blank sensi-disc, and the solvent was allowed to evaporate overnight. Three replications were employed for each sample. A suspension of each bacterium in a 0.85% saline soln was used to streak petri plates (15 mm × 100 mm diameter) containing solidified tryptic-soy agar. The sensi-discs were placed on the plates, no closer than 10–15 mm from each other (no more than 7 discs per plate), and the plates were incubated overnight at 37°. Inhibition was determined by measuring the diameter of the clear zone (if present) around the disc.

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