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ACYLATED FLAVONOLS FROM *DELPHINIUM CAROLINIANUM*

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Key Word Index—*Delphinium carolinianum*; Ranunculaceae; larkspur; systematics; flavonoids; quercetin (3‴-benzoyl-2″-glucosyl)-3-glucoside-7-rhamnoside; acylated flavonol glycosides.

Abstract—Three new benzoylated quercetin glycosides were isolated from *Delphinium carolinianum*. Quercetin (3‴-benzoyl-2″-glucosyl)-3-glucoside-7-rhamnoside was identified and two related glycosides were partially characterized.

INTRODUCTION

The flavonoids in *Delphinium carolinianum* Walter were analysed as part of a systematic study of the genus. In addition to several known compounds (galactosides, glucosides, rhamnosides and xylosides of quercetin and kaempferol), three new flavonol glycosides (**1**–**3**) were isolated and characterized; all three were found to be acylated with benzoic acid.

RESULTS AND DISCUSSION

Compound **1** was identified as quercetin (3‴-benzoyl-2″-glucosyl)-3-glucoside-7-rhamnoside. The unusual nature of **1** was first suspected when the high mobility of the compound on 2D-PC was noted. R_f values for the compound were 0.83 in 15% acetic acid and 0.82 in TBA. The former value is indicative of a di- or tri-glycosylated flavonoid, while the latter is more typical of an aglycone or highly methoxylated monoglycoside. UV spectral data (Table 1) indicated that **1** was a 3,7-disubstituted quercetin. However, ^{1}H NMR spectroscopy of the TMSi derivative showed the presence of a quercetin skeleton plus five additional aromatic protons as well as three glycosyl moieties. Unfortunately, resolution of the ^{1}H NMR spectrum was not sufficient to characterize the additional aromatic protons, although the sugars appeared to be 7-rhamnosyl and 3-glucosyl with a second glucosyl unit attached to one of the other sugars. Acid hydrolysis confirmed the presence of glucose ($R_{rha} = 0.52$) and rhamnose ($R_{rha} = 1.00$) by TLC in BBPW [1]. However, an additional carbohydrate was also detected with an R_{rha} value of 1.69 (a value not typical of any of the common saccharides). Mild alkaline hydrolysis yielded quercetin 3-diglucoside-7-rhamnoside and benzoic acid. The latter was identified by mass spectrometry and ^{1}H NMR. The ^{13}C NMR spectrum of **1** provided evidence for locating the position of attachment of the benzoyl unit as well as the second glucosyl moiety. Assignments of signals were made using known benzoylated glycosides of xanthones [2]. The signal at δ 82.1 is diagnostic for C-2 of a glucose with a glucosyl substituent at position 2. Moreover, the signal at δ 98.4 is in accord with the shift for C-1″ of quercetin 3-glucoside with a 2″-glucosyl substituent. The downfield shift of the C-3‴ signal and the concommitant upfield shifts of the C-2‴ and C-4‴ signals are indicative of 3‴-benzoylation. Based on these data the structure proposed for **1** is as shown in Fig. 1.

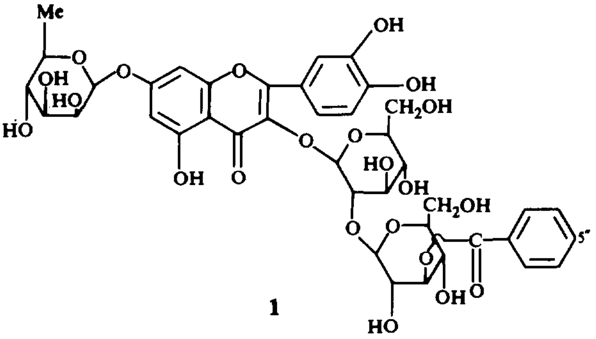

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Fig. 1.

Compound **2** was partially characterized as quercetin (*X*″-xylosyl-*X*″-benzoyl)-3-glucoside (R_f TBA = 0.91; R_f acetic acid = 0.66). UV spectral data (Table 1) of **2** indicated a quercetin 3-glycoside. Acid hydrolysis produced quercetin, xylose ($R_{rha} = 0.76$) and an unusual carbohydrate ($R_{rha} = 1.62$). ^{1}H NMR data of the TMSi derivative indicated a quercetin skeleton with two glycosyl groups and five additional aromatic protons. As observed for **1**, mild alkaline hydrolysis of **2** yielded benzoic acid. Insufficient material was available for ^{13}C NMR spectroscopy. However, the order of attachment may be deduced since acid hydrolysis gave free xylose and a substituted glycosyl moiety (assumed to be glucose because of ^{1}H NMR signals and color with aniline phthalate), thus

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Table 1. UV-visible spectral data for compounds **1**–**3** (λ_{max})

Reagent	1	2	3
MeOH	256, 267sh, 356	256, 267sh, 353	255, 264sh, 354
NaOMe	264, 390 ↑	270, 327, 402 ↑	271, 326, 410 ↑
$AlCl_3$	273, 333, 438	272, 332, 434	274, 330sh, 434
$AlCl_3$–HCl	268, 366sh, 400	266, 352sh, 398	268, 362sh, 398
NaOAc	259, 402	270, 326, 390	270, 326, 385
NaOAc–H_3BO_3	258, 378	259, 290sh, 372	260, 376

establishing linkage of the benzoyl to the glucosyl moiety in **2**. Finally, ^{1}H NMR data (shift of H-1 = δ 5.98) established attachment of the glucose to position 3 of the flavonol, as we have found quercetin 3-xylosides to have an H-1″ signal δ 0.5–0.7 further upfield. The R_{rha} value of 1.62 for the unusual hydrolysis product suggests that the benzoic acid may be attached to position 3″ of the glucose, as in **1**.

Compound **3** was partially characterized as quercetin (X'' or X'''-benzoyl-X''-glucosyl)-3-glucoside (R_f TBA = 0.74; R_f acetic acid = 0.70). UV spectral data indicated that **3** was a 3-substituted quercetin (Table 1) and ^{1}H NMR of its TMSi ether again showed five additional aromatic protons, typical for a benzoyl group. Acid hydrolysis yielded quercetin, glucose and a saccharide with R_{rha} = 1.42. Mild alkaline hydrolysis of **3** yielded quercetin 3-diglucoside and benzoic acid. Insufficient material was available to determine to which glucosyl moiety the benzoic acid is attached. However, the R_{rha} of 1.42 for the hydrolysis product from **3** suggests that the benzoic acid is not attached to positions 3″ or 3‴.

This constitutes the first report of the natural occurrence of benzoylated quercetin glycosides. Also, benzoic acid has not previously been reported as a flavonoid acyl group in the Ranunculaceae. However, caffeoylated and related kaempferol glycosides have been found in *Aconitum* [3], a genus closely related to *Delphinium*. Other acylated flavonoid glycosides have been reported from the Ranunculaceae by Harborne [4] and Fujiwara *et al.* [5], who found a caffeoylated flavonol glycoside in *Helleborus* and acetylated quercetin glycosides in *Coptis*, respectively. This communication also provides the first NMR spectral data for benzoylated flavonoids from the Ranunculaceae.

EXPERIMENTAL

Plant material was collected from wild populations of *D. carolinianum* Walter identified by the senior author. Vouchers are deposited in the Herbarium of the University of Illinois. Flavonol glycosides were extracted from air-dried leaves (303 g) of *D. carolinianum* in 80% MeOH. Isolation and purification was completed through combined use of solvent partitioning, PC, Sephadex LH-20, cellulose and Polyclar CC [6]. The benzoylated compounds were partitioned from the crude extract (concd and dissolved in H_2O) into EtOAc, and placed on a Polyclar column in 100% MeOH. Fractions containing **1**, **2** and **3** were the first, ninth and tenth, respectively, of the flavonoid-containing fractions eluted from the column. Compounds **1**–**3** were easily separated from other quercetin di- and tri-glycosides in their respective fractions by elution from a Sephadex LH-20 column with 100% MeOH (benzoylated compounds were always the first flavonoids eluted). Identification was facilitated by R_f values on PC, acid hydrolysis, UV, ^{1}H NMR (90 MHz) and ^{13}C NMR (22.6 MHz) spectroscopy using standard techniques [1, 7]. TLC, MS and ^{1}H NMR were employed to identify hydrolysis products.

^{1}H NMR (90 MHz, CCl_4). **1** (TMSi ether): δ 8.11 (2H, *m*, 4,6-benzoyl), 7.50 (5H, *m*, H-2′, H-6′ and 3,5,7-benzoyl), 6.82 (1H, *d*, H-5′), 6.77 (1H, *d*, H-8), 6.40 (1H, *d*, H-6), 6.00 (1H, *d*, H-1 glc-1), 5.78 (1H, *d*, H-1 glc-2), 5.22 (1H, *d*, H-1 rha), 3.3–4.2 (17 H, *m*, sugar protons), 1.28 (3H, *d*, H-6 rha). **2** (TMSi ether): δ 8.09 (2H, *m*, H-4, 6-benzoyl), 7.49 (5H, *m*, H-2′, H-6′ and 3,5,7-benzoyl), 6.83 (1H, *d*, H-5′), 6.44 (1H, *d*, H-8), 6.16 (1H, *d*, H-6), 5.98 (1H, *d*, H-1 glc), 5.20 (1H, *m*, H-1 xyl), 3.3–4.1 (11H, *m*, sugar protons). **3** (TMSi ether): δ 8.12 (2H, *m*, H-4, 6-benzoyl), 7.49 (5H, *m*, H-2′, H-6′ and 3,5,7-benzoyl), 6.88 (1H, *d*, H-5′), 6.49 (1H, *d*, H-8), 6.19 (1H, *d*, H-6), 5.98 (1H, *d*, H-1 glc-1), 5.70 (1H, *d*, H-1 glc-2), 3.3–4.2(12 H, *m*, sugar protons).

^{13}C NMR (22.6 MHz, underivatized, DMSO-d_6). **1**, rhamnose carbons are designated by ′′′′′, benzoyl by ′′′′ and proximal glucose by ″: δ 177.4 (*s*, C-4), 167.9 (*s*, C-1⁗), 161.8 (*s*, C-7), 161.2 (*s*, C-5), 157.1 (*s*, C-9), 156.3(*s*, C-2), 148.4 (*s*, C-4′), 144.7 (*s*, C-3′), 136.4 (*d*, C-5⁗), 133.7 (*s*, C-3), 129.5 (*s*, C-2⁗), 129.4 (*d*, C-3⁗, C-7⁗), 128.6 (*d*, C-4⁗, C-6⁗), 121.8 (*d*, C-6′), 121.2 (*s*, C-1′), 116.2 (*d*, C-5′), 115.2 (*d*, C-2′), 105.8 (*s*, C-10), 103.6 (*d*, C-1‴), 99.4 (*d*, C-6), 98.9 (*d*, C-1′′′′′), 98.4 (*d*, C-1″) 94.5 (*d*, C-8), 82.1 (*d*, C-2″), 78.1 (*d*, C-3‴), 77.2 (*d*, C-5″), 76.6 (*d*, C-3″, C-5‴), 71.9 (*d*, C-4′′′′′), 71.2 (*d*, C-2‴), 70.6 (*d*, C-3′′′′′), 70.0 (*d*, C-2′′′′′)*, 69.9 (*d*, C-4″)*, 69.8 (*d*, C-5′′′′′)*, 67.2 (*d*, C-4‴), 61.3 (*t*, C-6‴)†, 61.0 (*t*, C-6″)†, 17.5 (*q*, C-6′′′′′).

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*, † Assignments with the same sign may be interchanged.