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3-DESOXYCALLUNIN AND 2″-ACETYLCALLUNIN, TWO MINOR 2,3-DIHYDROFLAVONOID GLUCOSIDES FROM *CALLUNA VULGARIS**

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Key Word Index—*Calluna vulgaris*; Ericaceae; heather flowers; callunin; (2*R*,3*R*)-5,7,8,4′-tetrahydroxydihydroflavonol 8-glucoside; 2″-acetylcallunin; (2*R*,3*R*)-5,7,8,4′-tetrahydroxydihydroflavonol 8-(2″-acetylglucoside); 3-desoxycallunin; (2*R*)-5,7,8,4′-tetrahydroxyflavanone 8-glucoside.

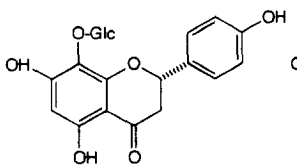
Abstract—The ethyl acetate soluble fraction obtained from the acetone extract of fresh *Calluna vulgaris* heather flowers afforded a mixture of two minor 2,3-dihydroflavonoid glucosides. Separations were achieved by column chromatography on polyamide as well as by reversed phase MPLC and HPLC. Structural elucidations were performed by UV, ^{1}H and ^{13}C NMR. The two novel compounds which are related to the major constituent, callunin were identified as 3-desoxycallunin and 2″-acetylcallunin. This is the first report of a flavanone 8-glucoside and the second report of a dihydroflavonol 8-glucoside in the plant kingdom.

INTRODUCTION

Calluna vulgaris (L.) Hull., a member of the Ericaceae, has been the subject of several previous phytochemical investigations. The major flower constituents have been identified as the flavonol aglycones kaempferol [2], quercetin [3–5] and myricetin [6, 7] and the flavonol glycosides galangin 3-methyl ether 7-glucoside [5], quercetin 3-glucoside, 3-galactoside and 3-arabinoside [5], herbacetin 8-glucoside and 8-gentiobioside [3, 5]. More recently, three acyldiglycosides, kaempferol- and quercetin 3-triacetylarabinosylglucoside and quercetin 3-triacetylarabinosylgalactoside have been described in this species [7–10]. Also two dihydroflavonols, taxifolin 3-glucoside [5] and callunin (**2**) [5, 11] have been reported. The only representatives of the flavone and the anthocyanin classes are apigenin 7-(2″-acetylglucuronic acid methyl ester) [7] and cyanidin 3-glucoside [12], respectively. We report here the findings of a further phytochemical examination of fresh heather flowers. Two dihydroflavonoid glucosides **1** and **3** were isolated and their identity established by UV, ^{1}H and ^{13}C NMR spectral analysis.

RESULTS AND DISCUSSION

The isolation of the two minor 2,3-dihydroflavonoid glucosides **1** and **3** was accomplished by a polarity gradient partitioning of the acetone extract against petrol ether and chloroform to remove non-polar constituents, followed by ethyl acetate and *n*-butanol to extract glycosides and then by repeated chromatography of the ethyl acetate-soluble portion. Final purification was achieved by reverse phase HPLC (see Experimental). UV, ^{1}H and ^{13}C NMR data suggested a 2,3-dihydroflavonoid glucoside structure for both compounds. Thus, the UV spectra showed two bands at λ 293 and 338 nm which were similar to those exhibited by the reference compound callunin (**2**). The main 293 band was shifted by alkali to λ 328 nm indicating the presence of a free 7-hydroxyl group in both compounds. Furthermore the shifts observed with aluminium trichloride (λ 318 and 398 nm) were not reversed on the addition of HCl [13, 15], indicating a free 5-hydroxyl in both **1** and **3**, which was confirmed by the sharp singlet at δ12.05 ppm for **1** and 11.73 for **3** in the ^{1}H NMR run in DMSO-d_6. This was in agreement with the singlets pointed at δ5.91 and δ5.93 for **1** and **2**, respectively (Table 1) and which reflected



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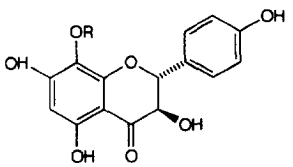

2 R = Glc

3 R = 2″-AcetylGlc

*Part 10 in the series 'Phytochemistry of the Ericaceae'. For Part 9, see ref. [1].

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Table 1. ^{1}H NMR spectra of flavonoid glucosides **1**–**3***

H	1†	1‡	2†	2‡	3†
2	5.42 *br d* (11.8–3.0)	5.45 *dd* (11.8–4.0)	4.47 *br d* (11.4)	4.40 *br d* (11.1)	4.63 *br d* (11.4)
3			5.03 *d* (11.4)	5.09 *d* (11.1)	5.03 *d* (11.4)
3_A	3.04 *dd* (17.2–11.8)	3.02 *dd* (17.1–11.8)			
3_B	2.75 *dd* (17.2–3.0)	2.80 *dd* (17.1–4.0)			
6	5.91 *s*	5.93 *s*	5.96 *s*	5.98 *s*	5.93 *s*
HO-5		12.01 *s*		11.73 *s*	
2′,6′	7.37 *d* (8.5)	7.39 *d* (8.4)	7.38 *d* (8.5)	7.36 *d* (8.5)	7.39 *d* (8.6)
3′,5′	6.78 *d* (8.5)	6.76 *d* (8.4)	6.79 *d* (8.5)	6.76 *d* (8.5)	6.81 *d* (8.6)
1″	4.61 *d* (6.4)	4.52 *d* (6.8)	4.60 *d* (7.2)	4.54 *d* (7.0)	under H_2O peak
2″	3.20–3.40 *m*	3.20–3.50 *m*	3.20–3.40 *m*	3.10–3.20 *m*	4.80 *m*
3″	3.20–3.40 *m*	3.20–3.50 *m*	3.20–3.40 *m*	3.20–3.20 *m*	3.45 *t* (8.3)
4″	3.20–3.40 *m*	3.20–3.50 *m*	3.20–3.40 *m*	3.10–3.20 *m*	3.30 *m*
5″	3.20–3.40 *m*	3.20–3.40 *m*	3.20–3.40 *m*	3.10–3.20 *m*	3.30 *m*
6″	3.70 *m*	3.20–3.50 *m*	3.37 *m*		3.68 *m*
$6''_A$				3.52 *d* (11.4)	
$6''_B$				3.45 *br d* (11.4)	
AcO-2″					1.59 *s*

*At 200 MHz for **1** and **3** and 400 MHz for **2**.
†In CD_3OD (δ3.27).
‡In DMSO-d_6 (δ2.49). *J* (Hz) in parentheses.

a 5,6,7- or 5,7,8-trisubstituted A-ring. It was thus demonstrated that two hydroxyl groups are located on the A-ring. Another one must be on the B-ring at position 4′ as indicated by the upfield doublet for H-3′, H-5′ at δ6.78 for **1** and δ6.81 in **2**, respectively (Table 1). For flavonoid glycoside **1**, three further protons recorded at δ5.42 (J = 11.8–3 Hz), δ3.04 (J = 17.2–11.8 Hz) and δ2.75 (J = 17.2–3 Hz) were ascribable, respectively, to H-2, H-3_A and H-3_B on the C-ring of a flavanone structure because of their multiplicity and coupling constants [17]. These ^{1}H NMR data required that the aglycone be a 5,6,7,4′- or a 5,7,8,4′-tetraoxygenated flavanone. The ^{13}C NMR of the glycoside itself confirmed these findings and the six signals at δ107.2, 78.3, 77.6, 75.2, 70.7 and 62.2 ppm defined **1** as a monoglucoside (Table 2) [18–20]. Thus, the sugar unit is linked through a phenolic group to the A-ring at either the 6- or the 8-position. Evidence for localization of the glucose moiety was given by ^{13}C NMR and particularly by the quaternary C-8 peak (δ127.5), which could not be confused with a quaternary C-6 generally appearing towards δ130 [18, 20]. This result is obviously confirmed by the upfield signals relative to the conjugated position C-5 (δ158.9), C-7 (δ162.0) and C-9 (δ155.4) owing to the electron donating 8-*O*-substituent [18, 20]. Therefore, from the foregoing evidence compound **1** is identified as 3-desoxycallunin, a new natural product.

The third dihydroflavonoid glycoside isolated from the same source, exhibited similar R_f values to **1** on silica gel and cellulose (BAW) TLC but showed more polar behaviour on cellulose (5% HOAc) TLC and reverse phase HPLC (aq. MeOH) (Table 3). Both ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) revealed a 2,3-dihydroflavonol glycoside from the doublet (J = 11.4 Hz) at δ4.63 for H-3

Table 2. ^{13}C NMR spectra of flavonoid glucosides **1**–**3***

C	1	2	3
2	80.6	85.1	85.2
3	44.0	73.8	73.0
4	197.7	198.6	198.0
5	158.9	159.2	159.5
6	97.4	97.7	98.1
7	162.0	161.7	162.2
8	127.5	127.3	127.8
9	155.4	155.2	155.0
10	103.2	101.9	101.7
1′	129.2	129.1	129.3
2′,6′	128.8	130.3	130.9
3′,5′	116.4	116.2	116.3
4′	161.5	161.2	161.3
1″	107.2	107.0	104.1
2″	75.2	75.3	75.8†
3″	78.3	78.4	75.0†
4″	70.7	70.8	70.5
5″	77.6	77.6	78.1
6″	62.2	62.2	61.7
AcO-2″			20.6
			168.3

*At 50 MHz, in CD_3OD (δ49.0).
†Assignments with the same superscript in one column may be interchanged.

and the peak at δ73.0 for the corresponding C atom [14, 16–18, 20]. The osidic moiety identified with glucose by TLC (see Experimental) was, similarly to glucosides **1** and **2**, bound through an ether linkage to the flavonoid 8-position as suggested by the ^{13}C NMR resonance at

Table 3. Chromatographic data for flavonoid glucosides **1**–**3***

Component	R_f values System 1	System 2	R_t (min) System 3	System 4
1 3-Desoxycallunin	0.62	0.65	0.40	20.0
2 Callunin (8-hydroxydihydrokaempferol 8-glucoside)	0.49	0.53	0.59	5.5
3 2″-Acetylcallunin	0.64	0.69	0.57	8.8

*System 1: silica gel F-254; EtOAc–HCOOH–HOAc–H_2O = 20:1:1:2.
System 2: Cellulose F-254; *n*-BuOH–HOAc–H_2O = 4:1:5 (upper phase).
System 3: Cellulose F-254; HOAc–H_2O = 1:19.
System 4: Radial peak C_{18} 4μ (8 × 10 mm); MeOH–H_2O–HOAc = 35:64:1 (1 ml min^{-1}).

δ127.8 for C-8 as well as peaks corresponding to the conjugated positions at δ155.0 (C-9), 162.2 (C-7) and 159.5 (C-5) (Table 2). In comparison with dihydroflavonoid glucosides **1** and **2**, compound **3** showed an acetyl group on both NMR spectra (δ1.59 and δ20.6 and 168.3) (Tables 1 and 2). The downfield shift of H-2″ indicated that the acetyl group was attached to C-2″. Further evidence was given by ^{13}C NMR. In comparison with the corresponding carbon of callunin, the upfield shift of 2.9 ppm for C-1″ and 3.4 ppm for C-3″ confirmed acylation at C-2″ [18–21]. Surprisingly, the C-2″ which was expected to move downfield was not changed as reported for apigenin 7-(2″-acetyl)glucuronic acid methyl ester [9]. Accordingly, **3** is characterized as 2″-acetylcallunin, a previously unknown compound.

2″-Acetylcallunin along with apigenin 7-(2-acetyl)glucuronic acid methyl ester and flavonol 3-triacetyldiglycosides, was detected by TLC and HPLC in a direct acetone extract of fresh flower material confirming that it is a genuine plant product and is not formed by acyl shift during work up. *Calluna vulgaris* is a species characterized by a diverse flavonoid metabolism where 8-*O*-substitution is often present as well as acylglycosylation. However, 8-*O*-substituted flavonoids are relatively more common in the family Ericaceae than acylated glycosides which are more restricted in their occurrence [7–10, 22–24]. Compound **3** is the only known example of a flavonoid showing both 8-*O*-substitution and acylglycosylation.

EXPERIMENTAL

Plant material. See ref. [7].

General. Microcrystalline cellulose plastic sheets (Merck) and silica gel 60F-254 aluminium sheets (Merck) were used for TLC analysis. UV spectral analyses with the usual shift reagents were made according to standard procedures [13]. Glucose was identified by TLC on silica gel (EtOAc–H_2O–MeOH–HOAC, 13:3:3:4) in the presence of *p*-anisidine phthalate reagent. MPLC was achieved on polyamide SC-6 (Macherey Nagel) (460 mm × 27 mm i.d.) and silica gel C60-RP18 20–40 μm (Sorbsil Prolabo) (460 mm × 15 mm i.d.) using a Büchi 681 pump. Purification was accomplished by semi-prep. HPLC on a Waters model equipped with a 510 pump, a variable wavelength detector and a μbondapak C-18 column (10 μm, 10 × 5 cm). All NMR experiments were performed with either a Bruker AC-200 spectrometer at 200 MHz (^1H) and 50 MHz (^{13}C) or a Bruker AM-400 apparatus at 400 MHz (^1H). The solvent signal was used as ref.

Extraction and isolation of the dihydroflavonoid glucosides. The general extraction procedure has been previously reported [7]. The EtOAc extract (14.5 g) was fractionated by polyamide MPLC (250 g) using toluene–MeOH with increasing polarity. Fractions eluted with 20% MeOH (0.8 g) were combined and then subjected to RP18 MPLC (35 g) using H_2O–MeOH gradient. Fractions containing **1** and **3** were eluted with H_2O–MeOH, 80:20, which yielded 110 mg of the impure mixed compounds. Final purification was achieved by semi-prep. reverse phase C_{18} HPLC packed with 30% aq. MeOH for **1** (16 mg) and 40% aq. MeOH for **3** (9.5 mg).

(2R)-5,7,8,4′-*Tetrahydroxy flavanone* 8-O-β-D-*glucoside or* 3-*desoxycallunin* (**1**). UV λ_{max}^{MeOH} 291, 337sh; + $AlCl_3$ 314, 395; + $AlCl_3$ + HCl 313, 395; + NaOH 243, 324, 424; + NaOAc 251sh, 279sh, 287sh, 327; + NaOAc + H_3BO_3 291, 327 nm. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2. Chromatographic data: see Table 3.

(2R, 3R)-5,7,8,4′-*Tetrahydroxy dihydroflavonol* 8-O-β-D-(2″-*acetylglucoside) or* 2″-*acetylcallunin* (**3**) *powder.* UV λ_{max}^{MeOH}: 293, 338sh; + $AlCl_3$ 318, 398; + $AlCl_3$ + HCl 316, 395; + NaOH 247, 325; + NaOAc 251sh, 278sh, 284sh, 332; + NaOAc + H_3BO_3 294, 334 nm. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2. Chromatographic data: see Table 3.

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