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A 1,5-*CIS*-GUAIANOLIDE GLUCOSIDE FROM FLOWERS OF *ARNICA MOLLIS*

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Key Word Index—*Arnica mollis*; Asteraceae; sesquiterpene lactone glucoside; guaianolide; pinoresinol glucoside; benzyl glucoside.

Abstract—The novel sesquiterpene lactone glucoside 2α-*O*-β-D-glucopyranosyl-4α-hydroxy-1α,5α,11α-H-guai-10(14)-en-12,8α-olide (= 2α-hydroxy-11α,13-dihydro-1-epi-inuviscolide-2α-*O*-β-D-glucopyranoside) was isolated from flowerheads of *Arnica mollis*. Its structural elucidation was carried out by NMR spectroscopy (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HMBC, HMQC and ^{1}H–^{1}H NOESY) and mass spectrometry (DCI). Additionally, the lignan (+)-pinoresinol-4′-β-D-glucopyranoside and benzyl-β-glucoside were isolated and identified.

INTRODUCTION

Previously we reported on the isolation and identification of two xanthanolide glucosides from flowerheads of *Arnica anplexicaulis* and *A. mollis*, which have been the first sesquiterpene lactone glycosides detected within the genus *Arnica* [1]. In continuation of our chemical study of this genus [2] we have now isolated a guainolide glucoside from a methanolic extract of the flowers from *A. mollis*, which, to the best of our knowledge, is described for the first time. The aglycone is also a new compound. Additionally, the lignan, (+)-pinoresinol-4′-β-D-glucopyranoside, and benzyl-β-glucoside were found in the plant.

RESULTS AND DISCUSSION

The DCI (NH_3) mass spectrum of the isolated sesquiterpene lactone glycoside displayed a quasimolecular ion at *m/z* 446 $[M + NH_4]^+$ and fragment ions at *m/z* 428 $[M - H_2O + NH_4]^+$, 284 $[M - 162 + NH_4]^+$ and 266 $[M - H_2O - 162 + NH_4]^+$, due to the elimination of H_2O and/or a hexose, from which a molecular mass of 426 and the molecular formula $C_{21}O_9H_{32}$ could be deduced. Acid hydrolysis on a TLC plate [3] gave glucose as the sugar moiety.

Structural elucidation was achieved by ^{1}H and ^{13}C NMR spectroscopy, including ^{1}H–^{1}H COSY, HMQC and HMBC, which allowed the full assignment of all carbon and proton resonances. Additionally, the stereochemistry was determined by a ^{1}H–^{1}H NOESY spectrum. The ^{13}C and ^{1}H NMR spectra (Tables 1 and 2) show the typical signals for a β-D-glucopyranose with the doublet for H-1′ at δ 4.27 and the resonance for C-1′ at δ 102.5, being linked to a secondary hydroxyl group at the aglycone [4, 5]. The remaining ^{1}H NMR signals of the aglycone are in part similar to those of the 1,5-*cis*-guainolide, 11β,13-dihydro-1-epi-inuviscolide, from *Dittrichia graveolens* [6]. Marked difference exist only in the chemical shifts and coupling patterns of the protons H-2, H-3 and H-5, which are explainable by the assumption that the additional *O*-glucoside moiety is located at C-2. Furthermore, the signals for H-7, H-8 and H-11 are shifted downfield (0.52, 0.28 and 0.39 ppm, respectively) compared to those of the above mentioned 11β,13-dihydro-derivative, suggesting an 11β-oriented methyl group for which such downfield shifts are characteristic [1, 7, 8]. In the ^{13}C NMR spectrum (Table 2) the signal for C-12 is located at δ 182.3, which is typical for a saturated lactone ring, indicating the absence of an *exo*-methylene group at C-11 [9]. The resonance of C-6 at δ 23.5 and C-13 at δ 9.9, which show a correlation with the signal for H-11 (at δ 2.66) in the HMBC spectrum, as well as that for C-13, correspond with those for C-6, C-11 and C-13 of the guaianolide 11α,13-dihydro-2-*O*-acetyl-florilenalin (C-6: δ 22.9; C-11: δ 41.0, C-13: δ 9.9 [8]). From the HMQC spectrum the occurrence of two further quaternary carbons with δ 144.9 and 86.0 are deducible. Due to their chemical shifts and literature data [8] they have to be assigned to C-10 and C-4, respectively. Moreover, the HMBC spectrum exhibited long range couplings between the methylene protons H-14 and C-1 as well as C-9. The attachment of glucose to the hydroxyl at C-2 is confirmed by the long range coupling between H-1′ and C-2.

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Table 1. ^{1}H NMR data for compound **1** (500 MHz, CD_3OD, TMS as internal standard)*

Proton	δ (ppm)		J (Hz)	Correlated with H	NOE with H†
1	2.99	*dd*	11.8, 6.6	2, 5	5, 7, 9α, 1′
2	4.63‡	*ddd*	11.8, 9.3, 7.1	1, 3α, 3β	3β
3α	1.99	*dd*	9.3, 12.1	2, 3β	3β, 5
3β	2.16	*dd*	7.1, 12.1	2, 3α	2β, 15-H_3, 3α
5	2.43‡	*m*	6.6, 4.8, 13.6	1, 6α, 6β	1, 6α, 7
6α	1.78	*dd(br)*	4.8, 13.6	5, 6β	5, 6β, 7
6β	1.07	*ddd*	13.6, 13.6, 10.6	5, 6α, 7	6α, 8
7	2.19–2.27	*m*	10.6, 10.8	6β, 8	1, 5, 6α, 11
8	4.07	*ddd*	10.8, 10.8, 4.9	7, 9α, 9β	6β, 9β, 13-H_3
9α	2.19–2.27	*m*	10.8, 11.5	8, 9β	9β
9β	3.06	*dd*	4.9, 11.5	8, 9α	8, 9α, 14b
11	2.66	*dq*§	7.7, 7.7	7, 13	7, 13-H_3
13	1.15	*d*	7.7	11	8, 11
14a	5.28	*s*			
14b	5.18	*s*			
15	1.08	*s*			
1′	4.27	*d*	7.7	2′	
2′	3.11–3.15	*m*	8.8	3′	
3′	3.28	*t*	8.8, 8.8	2′, 4′	
4′	3.23	*t*	8.8	3′	
5′	3.11–3.15	*m*	2.3, 6.0	6a′, 6b′	
6a′	3.85	*dd*	2.3, 11.9	5, 6b′	
6b′	3.64	*dd*	6.0, 11.9	5, 6a′	

*Assignments are based on ^{1}H–^{1}H COSY.
†From ^{1}H–^{1}H NOESY.
‡Not first-order signal.
§Pseudo-quintet.

The α-configuration of H-1 and thus the evidence for a 1_α,5_α-*cis*-guaianolide is given from $^3J_{1\alpha,5\alpha} = 6.6$ Hz [7, 10], whereas in the case of a 1β,5α-*trans*-guaianolide a value of 10 Hz or greater should be anticipated [11, 12]. This stereochemistry is confirmed by strong nOe effects between H-1 and H-5.

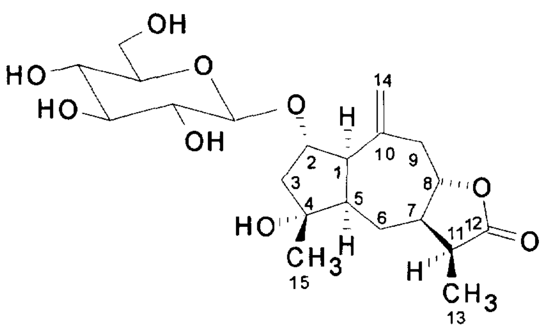
The configuration at C-2 (2β-H) follows from $^3J_{1\alpha,2\beta} = 11.8$ Hz and the lacking of a nOe correlation between H-1 and H-2. Clear nOe correlations are observed between H-7 and H-11 as well as H-8 and 13-H_3, confirming the 11β-position of the methyl group at C-13. The absence of nOe correlation between H-7 and H-8 is in accordance with the 7β(12),8α-*trans*-fusion of the lactone ring ($^3J_{7,8} = 10.8$ Hz). The methyl group at C-4 is β-orientated, because no nOe correlation exists with H-5. Hence, the structure of the isolated sesquiterpene lactone is 2α-*O*-β-D-glucopyranosyl-4α-hydroxy-1α.5α,11α-*H*-guai-10(14)-en-12,8α-olide (= 2α-hydroxy-11α,13-dihydro-1-epi-inuvisculide-2α-*O*-β-D-glucopyranoside (**1**).

Table 2. ^{13}C NMR data for compound **1** (125 MHz, CD_3OD, TMS as int. standard)*

Aglycone		Sugar	
Carbon	δ (ppm)	Carbon	δ (ppm)
1	53.5	1′	102.5
2	78.5	2′	75.3
3	*ca* 48.5†	3′	78.7
4	86.0	4′	72.0
5	54.6	5′	78.5
6	23.5	6′	62.8
7	47.0		
8	78.5		
9	44.9		
10	144.9		
11	41.8		
12	182.3		
13	9.9		
14	116.6		
15	24.4		

*Assignments are based on HMBC and HMQC.
†Hidden under solvent signals.

Additionally, benzyl-β-glucoside (**2**) [13, 14] and the lignan glycoside (+)-pinoresinol-4′-β-D-glucopyranoside (**3**) [15, 16] were isolated and the structures confirmed by their DCI mass spectral, ^{1}H NMR and



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(lignan) ^{13}C NMR and optical rotation data. Within the genus *Arnica* non-glycosylated (+)-pinoresinol has already been found in flowers of *A. angustifolia* [17] and roots of *A. chamissonis* ssp. *foliosa* [13] and benzyl-β-glucoside in the roots of the latter species [13].

EXPERIMENTAL

Plant material. See ref. [7]. A voucher specimen (No. 8.2) is deposited at the herbarium of the Institute for Pharmaceutical Biology, University of Düsseldorf.

Instrumentation. NMR: Bruker AMX 500, 500 MHz (^1H NMR) and 125 MHz (^{13}C NMR) in CD_3OD, TMS as int. standard; MS: DCI, Finnigan INCOS 50, NH_3 as reactant gas (emitter heating rate 10 mA s^{-1}, calibration with FC43); TCL: silica gel 60_{F254}, EtOAc–HOAc–HCO_2H–H_2O (100:11:11:27), detection: anisaldehyde–H_2SO_4; sugar: silica gel 60_{F254}, EtOAc–MeOH–HOAc–H_2O (12:3:3:2), detection: thymol–H_2SO_4.

Extraction and purification of compounds. Dried and powdered flowerheads (2.02 kg) were exhaustively extracted with $CHCl_3$ (162 g) and then with MeOH (395.6 g). A 30-g portion of the MeOH extract was sepd by CC on Sephadex LH-20 with MeOH yielding a fr. with **1** and **2** (200 mg) and a fr. with **3** (700 mg). Sepn of the first fr. by CC on Sephadex LH-20 with cyclohexane–CH_2Cl_2–MeOH (4:7:1) followed by (4:7:2) gave a fr. with **2** (26 mg) from which **2** was isolated by CC on silica gel with EtOAc–*n*-hexane (10:19) and a fr. with **1** (18 mg), which was chromatographed on silica gel with mixts of EtOAc–*n*-hexane of increasing polarity, followed by EtOAc and finally by EtOAc–Me_2CO (4:1). CC of the fr. containing **3** on Sephadex LH-20 with cyclohexane–CH_2Cl_2–MeOH (4:7:1) and subsequently (4:7:2) yielded **3**. Isolated amounts: **1**, 5 mg; **2**, 4 mg; **3**, 2.5 mg.

*2α-O-β-*D*-Glucopyranosyl-4α-hydroxy-1α,5α,11α-guai-10(14)-en-12,8α-olide* (**1**). Amorphous: R_f: 0.32; anisaldehyde–H_2SO_4: violet turning through blue to green; MS (DCI) *m/z* (rel. int.): 446 $[M + NH_4]^+$ (100), 428 $[M - H_2O + NH_4]^+$ (3), 284 $[M - 162 + NH_4]^+$, (11), 266 $[M - H_2O - 162 + NH_4]^+$ (47), 180 $[\text{hexose} - H_2O + NH_4]^+$ (24), 162 $[\text{hexose} - 2H_2O + NH_4]^+$ (9) ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Benzyl-β-glucoside (**2**). Amorphous: R_f: 0.60; anisaldehyde–H_2SO_4: green; MS (DCI) *m/z* (rel. int.): 288 $[M + NH_4]^+$ (100), 270 $[M]^+$ (5), 180 $[\text{hexose} - H_2O + NH_4]^+$ (14), 162 $[\text{hexose} - 2H_2O + NH_4]^+$ (5). ^1H NMR: refs [13, 14].

Compound **3**. Amorphous; R_f: 0.38: anisaldehyde–H_2SO_4: pink to violet; MS (DCI) *m/z* (rel. int.): 538 $[M + NH_4]^+$ (37), 376 $[M - \text{hexose} + NH_4]^+$ (22), 180 $[\text{hexose} - H_2O + NH_4]^+$ (100), 162 $[\text{hexose} - 2H_2O + NH_4]^+$ (35). ^1H and ^{13}C NMR: refs [15, 16]. $[\alpha]_D^{20}$ *ca* +6 (EtOH).

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