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SESQUITERPENE GLYCOSIDES BASED ON THE ALLOAROMADENDRANE SKELETON FROM *CALENDULA ARVENSIS**

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Key Word Index—*Calendula arvensis*; Compositae; alloaromadendrole glycosides.

Abstract—Four new sesquiterpene glycosides were isolated from the aerial parts of *Calendula arvensis*. The structure of one named arvoside B, was determined as 4-*O*-(β-D-fucopyranosyl)-4-alloaromadendrole. The other three compounds were derivatives of arvoside B with different substituents in the position (-2′ of the fucopyranosyl-residue. The structure elucidation of the compounds was based upon spectral and chemical methods.

INTRODUCTION

Calendula arvensis L. (Compositae) is a herbaceous plant used in Italian folk medicine as an anti-inflammatory and antipyretic remedy. In a recent pharmacological study the extracts of aerial parts of *C. arvensis.* showed anti-inflammatory activity [1].

In previous studies we reported the isolation and structure determination of four triterpenoid saponins from the methanol extract [2] as well as an epicubebol glycoside from the chloroform extract [3].

In the present paper we describe the isolation from the aerial parts of *C. arvensis* of four new sesquiterpene glycosides **1**–**4**. The aglycone moiety of these compounds is the rare alloaromadendrole [4–6].

RESULTS AND DISCUSSION

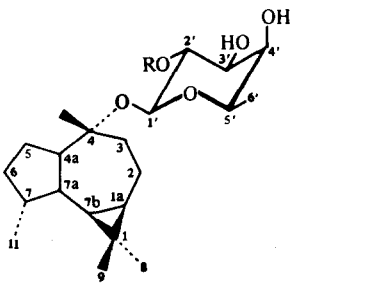
Compound **1** has $[\alpha]_D = -35.6°$. An analysis of its spectral data (^{1}H and ^{13}C NMR; see Tables 1 and 2) indicated that **1** contained one β-fucopyranosyl sugar unit. This was deduced from the following ^{1}H NMR data (CD_3OD): a large $J_{1'-2'}$ coupling (7.6 Hz) of the anomeric proton centered at δ4.45, small $J_{3'-4'}$ (3.4 Hz) and $J_{4'-5'}$ (0.9 Hz) couplings due to the equatorial nature of H-4′ and the presence of a 3H doublet at δ 1.28 (J = 6.4 Hz, Me-6′). The ^{13}C NMR signals for the sugar moiety were easily assigned for a β-fucopyranosyl residue [8]. The sugar was confirmed by acid methanolysis which yielded methyl fucoside, analysed by GLC. The molecular formula $C_{21}H_{36}O_5$ for **1** was determined by DEPT ^{13}C NMR and fast atom bombardment (FAB) mass spectral analysis in the positive ion mode, which showed molecular ion species at m/z 407 (M + K) and 391 (M + Na) and FABMS in the negative ion mode gave m/z 367 ($[M-H]^-$). The negative FAB mass spectrum also showed peaks at m/z 221 ($[(M-H)-146]^-$) and m/z 205 ($[M-H)-162]^-$), corresponding to loss of the β-fucopyranosyl sugar unit from the aglycone with cleavage on both sides of the glycosidic linkage. The molecular formula of the aglycone moiety must therefore be $C_{15}H_{25}O$, indicating its sesquiterpene nature.

The ^{13}C NMR and DEPT ^{13}C NMR spectra (after substraction of signals attributed to the sugar moiety) indicated that compound **1** contained four methyl groups, four methylene and five methine groups, and two quaternary carbons one of which was oxygenated. In the absence of any sp^2 carbon signals, the sesquiterpene **1** was tricyclic.

The ^{1}H NMR spectrum at 500 MHz showed, in addition to the sugar signals, a methyl doublet at δ 0.96, three methyl singlets at δ 1.03, 1.04, 1.195 and 13 hydrogen signals between 2.2 and 0.156 (Table 2).

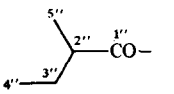
The signals at high field δ 0.156 (0.09 in $CDCl_3$) and 0.62 (0.583 in $CDCl_3$) suggested the presence of a cyclopropane ring in structure **1**. Spin decoupling experiments delineated the correlation and the sequence of all the protons in compound **1** (Table 2).

The signals of the ^{13}C NMR spectrum were assigned

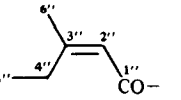


1 R = H
2 R = Ac

3 R =



4 R =



*Part 12 in the series 'Plant metabolites'. For part 11, see Pizza, C., Zong-Liang Z. and De Tommasi, N. (1987) *J. Nat. Prod.* **50**, 927.

Table 1. ^{13}C NMR spectral data of compounds **1–4** (62.9 MHz, CD_3OD, TMS int. standard)

Carbon		DEPT	**1**	**1** ($CDCl_3$)	**2**	**2** ($CDCl_3$)	**3**	**4**	Methyl-β-D-fucopyranoside [8]
Aglycone	1	C	19.7	18.8	19.7	18.8	19.7	19.6	
	1a	CH	30.1	28.6	30.1	28.8	30.0	30.0	
	2	CH_2	20.0	18.8	19.5	18.4	19.4	19.9	
	3	CH_2	38.0	37.3	38.8	37.9	39.3	38.3	
	4	C	82.9	82.3	82.8	82.1	82.6	82.9	
	4a	CH	56.1	54.8	56.2	54.6	56.0	57.2	
	5	CH_2	26.7	25.7	26.4	25.5	26.5	26.5	
	6	CH_2	30.0	28.6	30.0	28.7	30.2	30.6	
	7	CH	39.3	38.3	39.6	38.4	39.5	39.6	
	7a	CH	40.9	39.9	41.1	39.8	41.0	41.0	
	7b	CH	23.8	22.4	23.6	22.4	23.7	23.8	
	8	Me	16.8	16.2	16.6	16.2	16.8	16.9	
	9	Me	29.1	29.1	29.1	29.0	29.1	29.1	
	10	Me	27.5	27.0	27.0	26.6	26.8	26.6	
	11	Me	16.5	16.1	16.5	16.1	16.8	16.7	
Sugar	1′	CH	98.7	96.6	96.1	94.6	96.4	96.4	105.6
unit	2′	CH	72.8	71.8	74.3	73.9	74.2	74.2	72.1
	3′	CH	75.6	74.3	74.0	73.8	73.4	74.0	75.2
	4′	CH	73.1	72.3	73.3	72.2	73.4	73.6	72.6
	5′	CH	71.4	70.3	71.4	69.9	71.4	71.4	71.4
	6′	Me	16.8	16.4	16.7	16.4	16.8	16.7	16.8
Acetyl		Me			21.4	20.9			
group		C			172.1	179.2			
R	1″	C						167.6	
	2″	C						163.2	
	3″	CH						115.9	
	4″	CH_2						34.7	
	5″	Me						19.0	
	6″	Me						12.2	
R	1″	C					177.6		
	2″	CH					42.5		
	3″	CH_2					27.3		
	4″	Me					11.9		
	5″	Me					16.9		

on the basis of literature data [7], DEPT-^{13}C NMR (90 and 135°) and heteronuclear selective decoupling experiments; with the latter technique, C-1a and C-7b were discriminated (Table 1). These data indicated that the aglycone moiety of **1** must possess an aromadendrane-like skeleton.

Compounds **2–4** were derivatives of **1** at the C-2′ position of the sugar unit. Compound **2** was the C-2′ acethyl derivative, compound **3** the C-2′ (2″-methyl-butanoyl) derivative and compound **4** the C-2′(3″-methyl-2″-pentenoyl) derivative. Substitution at C-2′ of **2–4** was deduced from the ^1H NMR spectra (Table 2) (H-2′, δ 4.98–5.01, *dd*, $J = 7.6$, 9.8 Hz, was shifted downfield about 1.6 ppm if compared with **1**) and ^{13}C NMR spectra (Table 1). Comparison of the chemical shift of the ^{13}C NMR signals assigned to C-1′, C-2′, C-3′, in compounds **2–4** with the corresponding signals in **1**, indicated that C-2′ was deshielded by 1.5 ppm while C-1′ and C-3′ were shielded (γ-effect) by 2.6–2.3 ppm and 2.2–1.6 ppm, respectively, as expected for an ester bond [9].

The relative stereochemistry of the aglycone moiety in compounds **1–4** was ascertained by ^1H NOE difference spectra (NOEDs) of compound **2** in different solvents (Fig. 1). By irradiation of the signal for H-7b we observed NOE with signals C-11 methyl, C-8 methyl and H-1a. Irradiation of the H-7a (in C_6D_6) gave NOE with signals C-9 methyl and H-4a (*dd*, $J = 13.3$ and 11.3 Hz). An intense NOE was also observed on H-1′ of fucose by irradiation of C-10 methyl; on the other hand the latter signal gave no NOE with the cyclopropyl hydrogens.

These NOE experiments led us to establish: (i) the orientation of C-11 methyl; (ii) the *cis*-junction between the two larger rings; (iii) the β-orientation of C-10 methyl. Further evidence for the *cis*-junction and relative stereochemistry at C-4 was obtained by comparison of ^1H NMR spectra of compounds **1** and **4** (Table 2). Signals of the C-11 methyl, C-8 methyl, H-1a and H-7b in **4** were shifted to highfield by 0.06, 0.12, 0.046 and 0.02 ppm, respectively. Inspection of molecular models showed that the relatively strong shielding effect observed on these signals, due to the spatial orientation of the double bond, was possible only if the aglycone was based on the alloaromadendrole skeleton with the 2′-(3″-methyl-2″-pentenoyl) fucosyl residue in **4** in a *cis*-re-

Table 2. ^{1}H NMR spectral data of compounds **1–4** (500 MHz, CD_3OD, TMS int. standard)

		1	2	2 (C_6D_6)	3*	4
Aglycone	1a	0.62 *ddd*	0.63	0.58	0.63	0.602
	2α	1.49 *ddd*	1.51	1.66	‡	1.49
	2β	1.57†	1.57	1.93	‡	1.56
	3α	1.78 *br dd*	1.76	‡	‡	1.76
	3β	1.65†	1.66	1.46	‡	†
	4a	1.62†	1.60	1.53	‡	1.62
	5α	‡	‡	‡	‡	‡
	5β	‡	‡	‡	‡	‡
	6α	1.32 *m*	1.31	‡	‡	1.32
	6β	1.88 m	1.88	1.79	‡	1.82
	7β	1.99†	2.0	1.91	‡	1.95
	7a	1.73 *ddd*	1.72	1.88	‡	1.71
	7b	0.156 *dd*	0.156	0.04	1.15	0.11
	8	1.04 *s*	1.00	1.05	1.01	0.92
	9	1.04 s	1.04	1.12	1.04	1.01
	10	1.195 *s*	1.21	1.15	1.21	1.20
	11	0.96 *d*	0.97	0.97	0.95	0.90
Fucosyl	1′	4.45 *d*	4.66	4.35	4.68	4.69
	2′	3.42 *dd*	5.01	5.15	4.98	5.05
	3′	3.50 *dd*	3.65†	3.42	3.65	3.65
	4′	3.60 *dd*	3.65†	3.30	3.65	3.65
	5′	3.66 *dq*	3.65†	2.97	3.65	3.65
	6′	1.28 *d*	1.28	1.09	1.28	1.28
OAc			2.11 *s*	1.92 *s*		
R	2″				2.38 *tq*	5.70 *q*
	3″				1.88†	
	4″				0.98 *t*	2.24 *q*
	5″				1.22 *d*	1.12 *t*
	6″					2.20 *d*

* ^{1}H NMR spectrum obtained at 250 MHz.

† Partially overlapping signals.

‡ Overlapped with other signals in the region 1–2 ppm.

J Values (Hz) compound **1**: 1a–7b = 9.5; 1a–2β = 9.5; 1a–2α = 6; 2α–2β = 13.8; 2α–3α = 7.2; 2α–3β = 0; 3α–3β = 13.8; 7b–7a = 9.5; 11-Me–7β = 7; 1′–2′ = 7.6; 2′–3′ = 9.8; 3′–4′ = 3.4; 4′–5′ = 0.9; 5′–6′ = 6.4. **3**: 2″–3″ = 2″–5″ = 3″–4″ = 7; **4**: 2″–6″ = 2; 4″–5″ = 7.

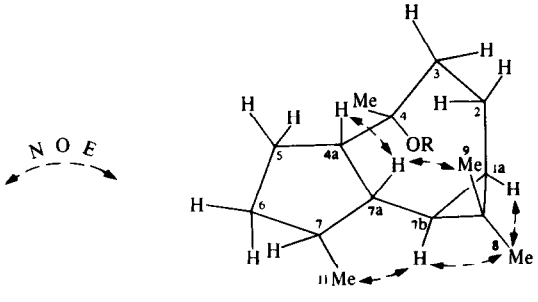


Fig. 1.

lationship with H-1a and H-7b. Thus, the structure of arvoside B (**1**) has been determined as 4-*O*-(β-D-fucopyranosyl)-4-alloaromandendrole.

EXPERIMENTAL

Chemical shifts are relative to TMS. The DEPT (Distortionless Enhancement by Polarization Transfer) experiments were performed using polarization transfer pulse of 90 or 135°, obtaining in the first case only signals for the CH groups, in the second case positive signals for CH and CH_3 and negative signals for CH_2 groups. The NOE experiments were performed using the spectral substraction technique (NOEDS). The samples for NOE measurements were previously degassed by bubbling argon through the soln for 40 min. FABMS spectra were obtained by dissolving the sample in a glycerol matrix (−ve ion) and a glycerol-thioglycerol matrix (+ve ion) and placing them on a copper probe tip prior to bombardment with Ar atoms of energy of 2–6 kV.

Extraction and isolation. Plants of *Calendula arvensis* L. were collected near Naples (Italy) in Spring 1985; a sample has been deposited in Dipartimento di Chimica delle Sostanze Naturali, University of Naples.

The aerial parts of air-dried plant material (800 g) were extracted in a Soxhlet apparatus, successively with petrol (40–70° bp) (8.6 g) and $CHCl_3$ (7 g). A portion of the $CHCl_3$ extract (3 g) was chromatographed on a silica gel column (120 g), using $CHCl_3$ with increasing amount of MeOH as elution solvent and collecting fractions of 15 ml. Fractions 73–74 and 77–79, eluted with $CHCl_3$–MeOH (8:2) were combined according to TLC- on

silica gel developed with $CHCl_3$–MeOH–H_2O (40:9:1) and separately submitted to HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.), differential refractometer detector. From the fractions 73–74 (180 mg) (MeOH–H_2O; 8:2; flow rate 5 ml/min) were collected compounds **2** (10 mg), **3** (8 mg) and **4** (12 mg), respectively, after 17 min, 27 min and 25 min. from injection. From the fractions 77–79 (510 mg) (McOH–H_2O; 3:1; flow rate 5 ml/min) was isolated **1** (15 mg) R_t 40 min. **1**: $[\alpha]_D = -35.6°$; FABMS negative ions *m/z* 367 $(M-H)^-$. **2**: $[\alpha]_D = -39.5°$; FABMS negative ions *m/z* 409 $(M-H)^-$. **3**: $[\alpha]_D = -39.9°$; FABMS negative ions *m/z* 451 $(M-H)^-$. **4**: $[\alpha]_D = -28.6°$; FABMS negative ions *m/z* 463 $(M-H)^-$. ^1H NMR and ^{13}C NMR data for **1–4** are in Tables 1 and 2.

Methanolysis of **1**: *sugar analysis.* A soln of **1** (*ca* 0.5 mg) in dry methanolic 2 M HCl (0.1 ml) was heated at 80° in stoppered reaction vials for 8 hr. After being cooled, the reaction mixture was neutralized with Ag_2CO_3 centrifuged and the supernatant evapd to dryness under N_2. The residue was then analysed as the silyl derivative by GLC (25 cm capillary column Se-30, 140°, hydrogen as carrier gas). GLC gave peaks which co-eluted with those of silylated methyl-fucoside.

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