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PLANTS METABOLITES. A NEW SESQUITERPENE GLYCOSIDE FROM *CALENDULA ARVENSIS*

COSIMO PIZZA* and NUNZIATINA DE TOMMASI

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, via L. Rodinò 22, I-80138 Napoli, Italy

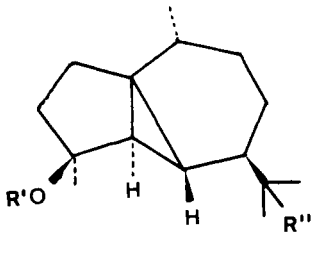
ABSTRACT.—A new sesquiterpene glycoside, named arvoside A [**3**], has been isolated from the aerial parts of *Calendula arvensis*. The structure of **3** has been determined as 4-*O*-(β-D-fucopyranosyl)-4-*epi*-cubebol by spectral studies, fabms, nmr, and chemical methods. 2D-nmr spectroscopy (proton-proton and proton-carbon chemical shift correlations direct and via 2 or 3 bonds) was also applied in the structural determination.

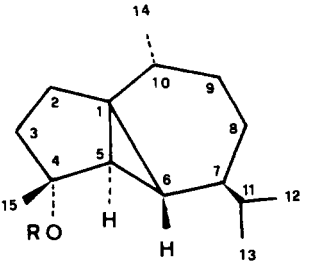
In the present paper we describe the isolation from the aerial parts of *Calendula arvensis* L. (Compositae) of a new sesquiterpene glycoside, 4-*O*-(β-D-fucopyranosyl)-4-*epi*-cubebol [**3**], designated arvoside A. The aglycone moiety of this compound is the very rare 4-*epi*-cubebol [**2**], as yet unknown in terrestrial plants but isolated from the marine brown alga *Dictyopteris divaricata* by Suzuki *et al.* (1). Compound **2** is the epimer at C-4 of cubebol [**1**], isolated from the essential oil of the fruits of *Piper cubeba* L. (2).

Recently (3) the optical antipode of cubebol [**1**] has been isolated from an Australian soft coral of *Cespitularia* species. A cubebol glycoside derivative **4** from roots of *Osteospermum auriculatum* (Compositae) (4) and an 11-hydroxy-cubebol [**5**] from the aerial parts of *Hemizonia congesta* DC. (Compositae) (5) have also been described. We note that Bohlmann (4,5) has represented the stereochemistry at C-4 of **4** and **5** opposite to that generally accepted for cubebol [**1**] in the literature (1-3). We report here a detailed analysis for **3**, including high resolution and 2D-nmr spectra.

EXPERIMENTAL

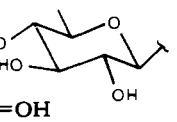
INSTRUMENTAL.—Nmr spectra were determined on a Bruker WM-250 or WM-500 spectrometer. Chemical shifts are relative to TMS. The DEPT (distortionless enhancement by polarization transfer) experiments were performed using polarization transfer pulses of 90° or 135°, obtaining in the first case only signals for the CH groups, in the second case positive signals for CH and CH_3 and negative signals for CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. These delays were also applied for two dimensional carbon-proton shift correlations on a 256×1024 data matrix (Bruker WM-250). An 8-step pulse cycle was used for P-type selection. In the case of proton-carbon shift correlation by long range coupling (COLOC), delays were adjusted to an average CH coupling 5 Hz. COSY experiments were performed on a 512×2048 data set (Bruker WM-500) with 90°-45° pulses separated by an incremental evolution time T_1. Quadrature detection was applied in both dimensions using a 16-step phase





1 R′=R″=H

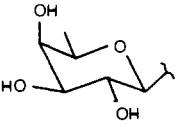
4 R′= Ang O, R″=H



5 R′=H, R″=OH

2 R=H

3 R=



cycling for N-type peak selection. Data were multiplied with a sine-bell shaping function, zero filled, Fourier transformed, and symmetrized. DEPT and 2D experiments were carried out using Bruker commercial microprograms in CD_3OD as solvent (for COSY experiments also in $CDCl_3$). Monodimensional nmr spectra were measured both in CD_3OD and $CDCl_3$. The nOe experiments were performed using the spectral subtraction technique (nOeDS). The sample for nOe measurements was previously degassed by bubbling argon through the solution for 40 min.

Fabms spectra were recorded on a Kratos MS-50 mass spectrometer equipped with Kratos fab source. The 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 2-6 Kv. Optical rotation was recorded on a Perkin Elmer 241 polarimeter. Hplc separations were performed on a Waters Model 6000A pump equipped with a U6K injector and differential refractometer Model 401 detector. Glc analysis was performed with a Carlo Erba Fractovap 2900 equipped with capillary column.

TABLE 1. Nmr Data for **3** in CD_3OD[a]

Position		^{13}C[b]	DEPT	^{1}H[c]	J_2[d]	J_3[d]
Aglycone	1	36.8	C		2.20 (H-2α)	1.11 (14-H_3)
	2β			1.42 dd		
		31.6	CH_2			
	2α			2.20 ddd		
	3α			1.87 dd		1.40 (15-H_3)
		35.4	CH_2			
	3β			1.26 ddd		
	4	89.6	C		1.40 (15-H_3)	
	5	39.7	CH	1.12 d		1.40 (15-H_3)
	6	25.7	CH	0.42 dd		2.20 (H-2α)
	7	46.2	CH	1.04 dddd		
	8β			0.88 dddd		
		28.5	CH_2			
	8α			1.46 dddd		
	9α			0.65 dddd		
		33.1	CH_2			
	9β			1.62 dddd		
	10	32.4	CH	1.79 ddq	1.11 (14-H_3)	
	11	35.0	CH	1.58 dq	0.95-0.99 (12-, 13-H_3)	
	12	20.1	CH_3	0.95 d		
	13	20.5	CH_3	0.99 d		
	14	19.5	CH_3	1.11 d		
	15	24.0	CH_3	1.40 s		
Fucosyl	1′	99.2	CH	4.50 d	3.42 (H-2′)	
	2′	72.7	CH	3.42 dd	3.50 (H-3′)	3.62 (H-4′)
	3′	75.5	CH	3.50 dd	3.62 (H-4′)	
	4′	73.2	CH	3.62 dd		1.28 (6′-H3)
	5′	71.4	CH	3.66 dq	1.28 (6′-H_3)	
	6′	17.0	CH_3	1.28 d		

J_{HH}(Hz)2α-2β=11.7; 2α-3β=11.7; 2α-3α=8.3; 2β-3α=0; 2β-3β=8.3; 3α-3β=15; 5α-6β=3; 6β-7α=3; 7α-8β=13; 7α-8α=5; 7α-11=7; 8β-9α=13; 8β-9β=2.5; 8β-8α=13; 8α-9α=2.5; 8α-9β=5; 9α-9β=13; 9α-10β=13; 9β-10β=5; 10β-14-CH_3=7; 11-12, 13-CH_3=7; 1′-2′=7.6; 2′-3′=9.8; 3′-4′=3.4; 4′-5′=0.9; 5′-6′=6.4.

[a]One dimensional ^{1}H-nmr spectra were obtained at 500 MHz. One dimensional ^{13}C-nmr spectra were obtained at 62.9 MHz.

[b]Assignments confirmed by 2D-^{13}C, ^{1}H cross correlation spectroscopy.

[c]^{1}H-^{1}H 2D cross-correlation spectroscopy (COSY) at 500 MHz delineated the correlation of all the protons in **3**.

[d]In J_2 and J_3 columns are reported ^{13}C, ^{1}H cross correlation through two and three bonds, respectively, observed in 2D ^{1}H-^{13}C shift correlation by long range coupling (COLOC) spectrum.

EXTRACTION AND ISOLATION.—Plants of *C. arvensis* were collected near Naples, Italy, in the spring of 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 light petroleum (40°-70° bp) (8.6 g) and $CHCl_3$ (7.5 g). The $CHCl_3$ extract (3 g) was, chromatographed on a Si gel column (120 g) using $CHCl_3$ with increasing amounts of MeOH as elution solvent and collecting fractions of 15 ml. Fractions 72-80 (640 mg), eluted with $CHCl_3$-MeOH (80:20), were combined according to tlc SiO_2, $CHCl_3$-MeOH-H_2O (80:18:2) and then submitted to hplc on a C_{18} μ-bondapack column (30 cm×7.8 mm i.d.) with MeOH-H_2O (75:25) (flow rate: 5 ml/min) to give four main fractions. The fraction collected after 44 min from injection contained arvoside A [**3**] (31 mg); $[\alpha]_D = -27.6°$; fabms, positive ion mode: *m/z* 407 (M+K) and *m/z* 391 (M+Na); fabms, negative ion mode: *m/z* 367 $(M-H)^-$; ^1H- and ^{13}C-nmr data for **3** see Tables 1 and 2.

METHANOLYSIS OF ARVOSIDE A: SUGAR ANALYSIS.—A solution of **3** (ca. 0.5 mg) in anhydrous methanolic 2M HCl (0.1 ml) was heated at 80° in a stoppered reaction vial for 8 h. After being cooled, the reaction mixture was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under N_2. The residue was dissolved in TRISIL-Z [40 μl; *N*-(trimethylsilyl) imidazole in pyridine, Pierce Chemical Co.], left at room temperature for 15 min, and analysed by glc (25 m capillary column SE-30, 140°, hydrogen as carrier gas; flow 10 ml/min). Glc gave peaks which co-eluted with those of silylated methyl fucoside.

TABLE 2. Nmr Data for **2** and **3** in $CDCl_3$

Position	**2**[a]		**3**		
	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$J_{H,H}$/Hz
1	34.9		35.4		
2β				1.41 dd	11.7,8.4
	29.8		30.5		
2α				2.02 ddd	11.7,11.7,8.4
3α				1.81 dd	15,8.4
	36.6		33.4		
3β				1.18 ddd	11.7,15,8.4
4	80.8		88.8		
5	39.9	0.77	38.6	1.02 d	3
6	25.3	0.35	24.5	0.32 dd	3,3
7	44.6		44.6	0.97 m[b]	
8β				0.79 dddd	13,13,13,2.5
	27.0		27.2		
8α				1.39 m[b]	
9α				0.54 dddd	13,13,13,2.5
	31.8		31.8		
9β				1.57 m[b]	
10	30.2		30.8	1.73 ddq	13,5,7
11	33.6		33.4	1.51 dq	7,7
12	20.0	0.90	20.0	0.88 d	7
13	19.8	0.93	19.8	0.91 d	7
14	19.1	0.99	19	1.005 d	7
15	25.0	1.25	23.7	1.35 s	
1′			97.5	4.42 d	4.5
2′			71.8	3.58[c]	
3′			74.1	3.58[c]	
4′			71.8	3.71 d	1.3
5′			70.4	3.58[c]	
6′			16.5	1.26 d	6.6

[a]Literature data in Suzuki *et al.* (1). ^{13}C-nmr signals for **2** were reported in the experimental part of Suzuki *et al.* (1) but not assigned. We have assigned these data.

[b]Partially obscured from adjacent signals.

[c]Overlapped signals.

RESULTS AND DISCUSSION

Compound **3** has $[\alpha]_D = -27.6°$. An analysis of its spectral data (^{1}H and ^{13}C-nmr; see Tables 1 and 2) indicated that **3** contains one β-fucopyranosyl sugar unit. This was deduced from the following ^{1}H-nmr data: a large $J_{1'-2'}$ coupling (7.6 Hz) of the anomeric proton centered at δ 4.51, small $J_{3'-4'}$ (3.4 Hz) and $J_{4'-5'}$ (0.9 Hz) coupling due to equatorial nature of H-4′ and the presence of a 3H doublet at δ 1.28 (J=6.4 Hz; CH_3-6). ^{13}C-nmr signals for sugar moiety are easily assigned for a β-fucopyranosyl residue. The sugar was confirmed by acid methanolysis which yielded methyl fucoside, analyzed by glc. The molecular formula $C_{21}H_{36}O_5$ for **3** was determined by DEPT ^{13}C-nmr and fabms analysis in positive ion mode, which showed a molecular ion species at *m/z* 407 (M+K) and 391 (M+Na) and fabms in negative ion mode *m/z* 367 ([M-H]$^-$). The negative fabms spectrum also showed peaks at *m/z* 221 ([(M-H)-146]$^-$) and *m/z* 205 ([(M-H)-162]$^-$), corresponding to loss of a β-fucopyranosyl sugar unit from the aglycone with cleavage on both sides of the glycosidic linkage. The molecular formula of the aglycone moiety must be, therefore, $C_{15}H_{25}O$, indicating a sesquiterpene nature.

The ^{13}C- and DEPT ^{13}C-nmr spectra (after subtraction of signals attributed to the sugar moiety) indicated that **3** 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 **3** was tricyclic.

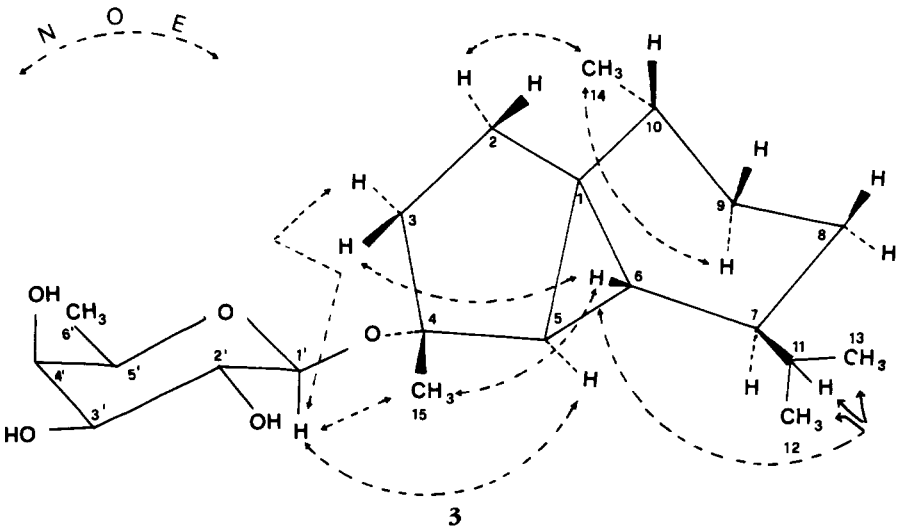
The ^{1}H-nmr spectrum at 500 MHz in CD_3OD (Table 1) showed, in addition to the sugar signals, three methyl doublets located at δ 0.95, 0.99, 1.11, a methyl singlet at δ 1.40 and 13 hydrogen signals between δ 2.2 and 0.42 which were generally well resolved. Those signals not well resolved in CD_3OD became visible at 500 MHz using $CDCl_3$ as solvent (Table 2). The signal at high field [δ 0.42 (0.32 in $CDCl_3$), dd, J=3.0, 3.0 Hz] suggested the presence of a cyclopropane ring in structure **3**.

A 2D proton-proton shift-correlation (COSY) experiment delineated the correlation of all the protons in **3**; it also allowed us to identify the further cyclopropane proton from correlation between the signals at δ 0.42 (H-6) and 1.12 (H-5), the latter signal overlapping with a methyl doublet at δ 1.11 (CH_3-14) in the CD_3OD spectrum. The proton sequence from H-5 to CH_3-14 was observed by spin decoupling experiments in different solvents (CD_3OD and $CDCl_3$), starting from the H-5 signal and continuing till CH_3-14 (Tables 1 and 2). It is to be noted that Suzuki *et al.* (1), probably because of the low resolution ^{1}H nmr, attributed the two cyclopropane protons to signals at δ 0.35 and 0.77 ($CDCl_3$); according to our results, the signal at 0.77 is assigned to H-8β.

The signals of the ^{13}C-nmr spectrum were assigned on the basis of direct 2D-^{13}C-^{1}H chemical shift cross correlation spectroscopy (Table 1) with the only ambiguity being for C-2 and C-3 (see below). The location of the C-15 methyl on C-4 and connection between C-5, C-4, and C-15 methyl was deduced by a 2D-^{1}H-^{13}C shift correlation by long range coupling (COLOC) spectrum, via 2 or 3 bonds (CD_3OD, Table 1). The methyl singlet at δ 1.40 (C-15 methyl) correlates with carbon resonances at 89.6 (C-4) and 39.7 (C-5) ppm. The ^{1}H-^{13}C COLOC spectrum also indicated the connection between the quaternary carbon C-1 (36.8 ppm) and the C-14 methyl (δ 1.11), showing the sequence C-1, C-7, C-14. ^{13}C-nmr signals (unassigned) for 4-*epi*-cubebol aglycone in $CDCl_3$ were reported by Suzuki *et al.* (1), and we obtained good agreement with their data in $CDCl_3$ (Table 2).

Comparison of the chemical shifts of the signals for **3** in ^{13}C-nmr ($CDCl_3$) (Table 2), assigned to C-3, C-4, C-5, and C-15 with those of the corresponding signals for **2** indicates that C-4 (glycosidation site) is deshielded by 8 ppm (β-effect), while C-3, C-5, and C-15 are shielded, respectively, by 3.2, 1.3, and 1.6 ppm (γ-effects), as expected for a glycosidation shift (6).

The relative stereochemistry at C-4 and C-7 was ascertained by ^{1}H-nOe difference spectra (nOeDS) (Figure 1): by irradiation of the signal at δ 0.42 (H-6β), we observed nOe with signals at δ 1.40 (C-15 methyl), 1.26 (H-3β), 1.58 (H-11), 0.95 and 0.99 (CH_3-12 and 13); by irradiation of fucose H-1′ signal, nOe with C-15 methyl and signals at 1.12 (H-5α), 1.87 (H-3α) [in addition to the sugar signals at 3.50 (H-3′) and 3.66 (H-5′): 1-3 diaxial relationship]. These nOe experiments led us to establish a) the β orientation of C-15 methyl at C-4; b) the β orientation of the isopropyl group at C-7; c) the spatial orientation of the two hydrogens of C-3 and their relative position to C-4. It is possible now to make the correct assignments in 2D-^{1}H-^{13}C cross correlation for C-3 (35.4 ppm) and C-2 (31.6 ppm). The ^{1}H coupling constant analysis for protons of the six-membered ring led us to exclude a boat conformation for this ring because of the magnitude of the J values in this ring (Table 1). The value of $J_{9\text{-}10\beta} = 13$ Hz excludes a 10β-methyl possibility, while the β orientation of the isopropyl group, located at C-7 is also confirmed by $J_{7\text{-}8\beta} = 13$ Hz. The relative stereochemistry at C-10 was also established by an nOe experiment (Figure 1); irradiation of the signal at δ 1.11 (CH_3-14) gave an intense enhancement of protons at δ 2.20 (H-2), 0.65 (H-9), and 1.79 (H-10β).



3

FIGURE 1. Relative stereochemistry of **3** ascertained by nOeDS.

Application of the molecular rotation method (7) shows that the sugar is β-D-fucopyranose (M_D of **3**: −102°; M_D of **2** methylated (1)+M_D of methyl β-D-fucopyranoside: −115°).

Thus, the structure of arvoside A has been determined as 4-*O*-(β-D-fucopyranosyl)-4-*epi*-cubebol.

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