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ARTEGALLIN, A SESQUITERPENE LACTONE FROM *ARTEMISIA CAERULESCENS* SUBSP. *GALLICA*

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Key Word Index—*Artemisia caerulescens* subsp. *gallica*; Compositae; eudesmanolides; artegallin; β-santonin; artesin; arsubin.

Abstract—Four eudesmanolides were isolated from *Artemisia caerulescens* subsp. *gallica*. The constitution and stereochemistry of the new eudesmanolide artegallin were determined on the basis of its spectroscopic properties and by transformation into β-santonin.

INTRODUCTION

In our research into the chemical composition of medicinal plants growing in Castilla and Leon (Spain) we have studied the sesquiterpene lactones of *Artemisia caerulescens* subsp. *gallica* (Compositae, tribe Anthemideae; section seriphidium). The plant displays central nervous system depressive actions on motor and respiratory functions; it also decreases tone and produces hypothermia and hyperglucemic effects at high doses in rats [San Román, L. and Morán, A., personal communication].

Only one study concerning the essential oil of *A. caerulescens* subsp. *gallica* has appeared in the literature [1] and camphor and α-thujone were described as the main components, while from *A. caerulescens* three sesquiterpene lactones α- and β-santonin and artemin have been isolated [2–5]. In the present work we isolated four sesquiterpene lactones, β-santonin, artesin, arsubin and a new natural eudesmanolide that has been called artegallin.

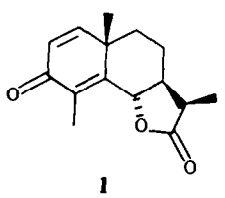
RESULTS AND DISCUSSION

The hexane extract (4% of the whole plant) was cooled to −20° and the insoluble part defatted with methanol and extracted with aq. Na_2CO_3 to give the neutral fraction (15.8% over the hexane extract) containing the sesquiterpene lactones.

Direct crystallization from ethyl acetate gave β-santonin (**1**) as a white crystalline product [6]. From the mother liquors, by repeated chromatographic separations over silica gel and/or crystallizations artesin (**2**) [7], β-santonin (**1**), artegallin (**3**) and arsubin (**4**) [8, 9] were isolated. Compounds **2** and **4** were identified by their physical and spectroscopic properties and through the conversion of **2** into **4** by sensitized singlet oxygen oxidation followed by Me_2S reduction.

Artegallin (**3**) was isolated as an oil which was slightly more polar than β-santonin. Its EIMS showed relevant peaks at *m/z* 264 $[M-CH_2CO]^+$, the highest peak observed in the spectrum, and 246 $[M-AcOH]^+$, and its UV spectrum displayed an absorption maximum at 239 nm (*sh* at 260 nm). The IR spectrum showed γ-lactone, conjugated ketone, acetate and double bond absorptions, while the ^{13}C NMR spectrum (Table 1) had peaks corresponding to three carbonyls (conjugated ketone, lactone and acetate), a tetrasubstituted double bond, two methines bearing an oxygenated function, one tetrasubstituted carbon atom, two methines, three methylenes and four methyls (one from the acetate).

The ^1H NMR spectrum (Table 1) showed signals of a *trans*-fused-α-methyl-γ-lactone [10] with the *O*-geminal hydrogen homoallylically coupled with the methyl on a



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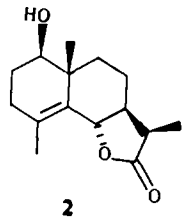

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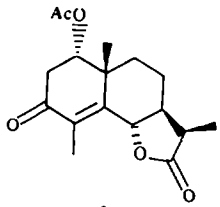

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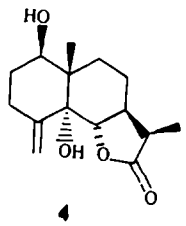

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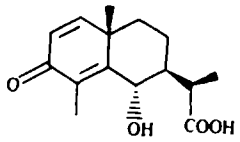

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Table 1. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra of artegallin (3) ($CDCl_3$; TMS as internal standard)

H	δ	multi.	J (Hz)	C	δ	C	δ
1	5.01	*dd*	3.8; 2.9	1	76.2	10	41.6
2	2.84	*dd*	17.0; 2.9	2	34.0	11	37.8
	2.69	*dd*	17.0; 3.8	3	195.1	12	178.1
6	4.90	*dq*	12.0; 1.8	4	129.0	13	9.7
7	2.42	*dddd*	12.0; 11.7	5	149.4	14	24.7
			7.7; 3.6	6	80.8	15	11.2
8	1.70–2.00	*m*		7	47.6	AcO-	170.4
9				8	21.0		20.8
11	2.74	*dq*	7.7; 7.7	9	39.0		
13	1.25	*d*	7.7				
14	1.40	*s*					
15	2.04	*d*	1.8				
AcO	2.05	*s*					

double bond. Furthermore, an ABX system constituted by a methylene group vicinal to a ketone and a hydrogen atom geminal to an axial acetate was observed. These data prompted us to assign the structure of 1α-acetoxy-3-oxo-Δ^4-*trans*-6,7-eudesmanolide to artegallin.

The absolute stereochemistry of **3** was supported by the Cotton effects at $\lambda_{max} = 326$ nm ($\Delta\varepsilon = -0.46$) and $\lambda_{max} = 247$ nm ($\Delta\varepsilon = +3.67$), according to the situation of the double bond in the fourth quadrant of the ketone and with a positive dihedral angle between the carbonylic and olefinic bonds [11].

The structure proposed for artegallin was confirmed through treatment with bases. By reaction with 5% KOH–MeOH it was converted into β-santonin and the γ-hydroxyacid **5** while satd aq. $NaHCO_3$–MeOH (1:4) gave only **1**: KOH saponification of **1** afforded the γ-hydroxyacid **5** which regenerated β-santonin under acidic conditions.

Neither artegallin (**3**) nor its deacetylated derivative have been reported previously although they could be common compounds in *Artemisia*. However, their easy transformation into β-santonin (and similar R_f) would mask its existence in other species of this genus. Another fact to be noted is that, while *A. caerulescens* produces 11α-methyl (α-santonin and artemin) and β-methyl (β-santonin) eudesmanolides, the subspecies *gallica* only produces 11β-methyl eudesmanolides.

EXPERIMENTAL

Mps are uncorr and were determined in capillaries. Optical rotations were measured in $CHCl_3$. UV were recorded in EtOH. IR spectra in film or KBr. ^{1}H NMR (200.13 MHz) and ^{13}C NMR (50.3 MHz) spectra in $CDCl_3$ (TMS int. standard) on a Bruker WP 200 SY spectrometer. EIMS were obtained at 70 eV. CD were measured in EtOH.

The plant was collected in Aldeamayor de San Martin (Valladolid, Spain) in September 1984. Voucher specimens are deposited in the Botany Dept. (register number SALAF No 4854).

The air dried plant material (779 g) was extracted in a Soxhlet with 6 l. hexane. The soln maintained overnight at −20° yielded an insoluble part (18.0 g) that was defatted with MeOH and extracted with 10% Na_2CO_3 to give neutral (4.8 g) and acidic (0.6 g) fractions.

Crystallization of the neutral fraction from EtOAc afforded **1** (450 mg). The mother liquors after silica gel (hexane–EtOAc of increasing polarity) CC and repeated flash CC and/or cryst. of the resulting fractions yielded **2** (52 mg), **3** (135 mg), **1** (250 mg) and **4** (192 mg).

Artegallin (**3**). Oil UV λ_{max}: 246 nm (ε 10770). IR ν_{max} cm^{-1}: 1790, 1745, 1685, 1650, 1245, 1040. EIMS *m/z* (rel. int.): 264 (10), 246 (24), 231 (13), 218 (7), 217 (10), 203 (7), 201 (11), 191 (15), 190 (11), 173 (23), 172 (16), 135 (20), 91 (61), 45 (40), 43 (100).

$$[\alpha]_\lambda = \frac{+14.1^\circ \quad +14.8^\circ \quad +16.7^\circ \quad +22.7^\circ}{589 \qquad 578 \qquad 546 \qquad 436} (c\,0.66).$$

Saponification of artegallin (**3**). Compound **3** (93 mg) in 5 ml 5% KOH–MeOH after 72 hr at room temp. afforded 75.6 mg of reaction product. Cryst. (hexane–EtOAc) gave 14 mg of **5** (mp 209°) and 58 mg of a mixture (4:1) of **1** and **5**.

Compound **3** (74 mg) in 5 ml aq. satd $NaHCO_3$–MeOH (1:4) maintained overnight with stirring at room temp. gave by cryst. of the reaction product 51.5 mg of **1**.

Hydroxyacid **5**. UV λ_{max} nm (ε): 253 nm (8131), 263 (6520). IR ν_{max}^{KBr} cm^{-1}: 3400, 3300–2300, 1700, 1660, 1615, 1595, 1280. ^{1}H NMR (C_5D_5N): δ 6.70 (1H, *d*, *J* = 9.8 Hz, H-1), 6.35 (1H, *d*, *J* = 9.8 Hz, H-2), 5.20 (1H, *d*, *J* = 9.6 Hz, H-6), 3.46 (1H, *dq*, *J* = 2.6, 7.3 Hz, H-11), 1.39 (3H, *d*, *J* = 7.3 Hz, H-13), 1.19 (3H, *s*, H-14), 2.69 (3H, *br s*, H-15). ^{13}C NMR (C_5D_5N): δ 156.5 (1), 125.9 (2), 187.2 (3), 135.0 (4), 161.1 (5), 72.8 (6), 50.6 (7), 22.3 (8), 39.6 (9), 41.2 (10), 42.4 (11), 177.9 (12), 12.0 (13), 23.5 (14), 14.9 (15).

$$[\alpha]_\lambda = \frac{-137.7^\circ \quad -144.8^\circ \quad -168.4^\circ \quad -339.5^\circ}{589 \qquad 578 \qquad 546 \qquad 436} (c\,1.09;\ \text{EtOH}).$$

Saponification of β-santonin (**1**). Compound **1** (130 mg) treated with KOH–MeOH as above yielded 36 mg of crystalline **5** ($CHCl_3$) and 72 mg of unchanged **1**.

Lactonization of hydroxyacid **5**. Treatment of **5** at room temp. with 5 mg of *p*-TsOH in 5 ml Me_2CO or **5** (40 mg) with 1 ml Ac_2O in 2 ml pyridine overnight at room temp. produced quantitatively **1** in both cases.

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IDENTIFICATION OF MOLLIC ACID α-L-ARABINOSIDE, A 1α-HYDROXYCYCLOARTENOID FROM *COMBRETUM MOLLE* LEAVES

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Key Word Index—*Combretum molle*; Combretaceae; 1α-hydroxycycloartenoids; mollic acid α-L-arabinopyranoside.

Abstract—A further novel 1α-hydroxycycloartane glycoside, mollic acid α-L-arabinoside, has been identified in the leaves of *Combretum molle*.

INTRODUCTION

In continuation of our work on the acetone extract from *Combretum molle* leaves [1], we recently reported the characterization of the 1α-hydroxycycloartenoid mollic acid (**1**) and its 3β-D-xyloside (**2**) [2]. We now wish to report the identification by comparative NMR studies of mollic acid α-L-arabinoside (**3**) as the minor constituent in the acid fraction from this extract.

RESULTS AND DISCUSSION

A slight difference in solubility between the glucoside (**4**) and the xyloside (**2**) of mollic acid (**1**) in ethanol enabled compound **4**, the least soluble, to be obtained pure by repeated recrystallization of the acid fraction from the acetone extract [2]. Preparative HPLC separation of the resultant mother liquors yielded xyloside **2** plus a fraction comprised of a mixture of this compound and a minor constituent of similar polarity. This HPLC separation was achieved by shaving fractions from the leading peak which contained the slightly less polar xyloside **2** [2]. Attempts at separating the mixed fraction **5** have thus for not been successful since band tailing of the xyloside **2** results in this compound coeluting with the minor constituent. It was evident from their response to TLC spray reagents in addition to their similarities in polarity that the two compounds were closely related.

This was confirmed by ^{1}H NMR and in particular ^{13}C NMR analysis, which showed that the mixture spectrum differed from the spectrum of the pure xyloside **2**

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