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SESQUITERPENE LACTONES FROM *ARTEMISIA INCULTA*

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Key Word Index—*Artemisia inculta*; Compositae; Anthemideae; Seriphidium; sesquiterpene lactones; germacranolides; eudesmanolides; tetranorsesquiterpene lactone.

Abstract—The aerial parts of *Artemisia inculta* collected in three different locations in Egypt have been chemically investigated. As a result, a new eudesmanolide and a new tetranorsesquiterpene lactone have been isolated, together with several known compounds. The chemical data available suggest the existence of distinct chemotypes for this species, but do not support its identity with other North African members of the genus.

INTRODUCTION

The large genus *Artemisia* has been the object of numerous chemical studies [1]. *A. inculta* Dél. grows mainly in arid zones of several Middle East countries. Originally described in Egypt [2], this species, included into the section Seriphidium of the genus *Artemisia*, has thereafter been considered conspecific with *A. herba-alba* [3]. Some recent treatments even propose that all populations in North Africa referred to as *A. herba-alba* should be named *A. inculta* [4]. In our opinion, however, such a homogeneity does not exist within the North African Seriphidium complex. Thus, we have very recently proposed that different names at the specific or at least subspecific level should be maintained for the two aforementioned taxa [5].

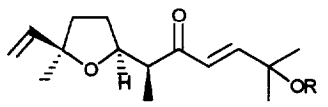
Only one previous chemical investigation on *A. inculta* has been published. Aerial parts of material collected in Saudi Arabia were shown to contain the flavonol artemetin, the eudesmanolide 11β,13-dihydrosantamarin and the new davanone-related sesquiterpene hydroperoxide arteincultone (**1a**) [6]. In continuation of our interest in the chemistry of North African *Artemisia* species [7–10], we now present the results of an investigation on *A. inculta* collected in three different locations in Egypt (L-1, L-2 and L-3, see Experimental). L-1 corresponds to the classic location cited by Délile in his first description of the species [2].

RESULTS AND DISCUSSION

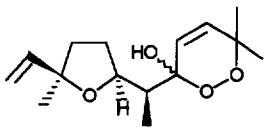
The aerial parts of material collected at location L-1 gave germacranolides **4** (gallicin) [11], **8** (11β,13-dihydrocostunolide) [12], **9** (balchanolide) [13], **12** (anhydroverlotorin) [14], **13** [15] and **14** (shonachalin A) [15, 16], and eudesmanolides **10** (α-santonin) [12], **11** [12], **15** (artemin) [8] and **19** (11β,13-dihydroreynosin) [16]. In addition, we isolated the new tetranorsesquiterpene lactone **16** as an inseparable mixture of epimers. The material collected at location L-2 afforded the davanone-like derivative **1b** [17], the related cyclic peroxide **2** [17], the truncated sesquiterpene acid **3** [17], the germacranolide **5** [18] and the eudesmanolides **6** [16, 18] and **7**, the latter being now described for the first time. Finally, the material from location L-3 yielded the germacranolides **4**, **8**, **9**, **13** and **14**, the eudesmanolides **10**, **15**, **18** [16], **19**, **20** [19], **21** [8] and **22** (artemisin) [12], and the monoterpene diol **17** [16].

The IR and NMR data (Table 1) of compound **7**, $C_{15}H_{22}O_4$, indicated the presence of a hydroxylated sesquiterpene lactone. The NMR data were very close to those of **6** [16], but an additional signal at δ 3.96 pointed to the presence of a second hydroxyl group. Spin decoupling allowed the allocation of this hydroxyl at C-8α. Together with the values of the coupling constants, the configurations of all stereogenic carbon atoms were definitively established with the aid of NOE difference measurements. For example, marked NOEs were visible between H-14, H-6 and H-8; between H-6 and H-13; and between H-1 and H-5. Compound **7** is thus the epimer at C-11 of the known lactone artapshin [20] and may therefore be named 11-*epi*artapshin.

Almost all of the hydrogen and carbon signals in the NMR spectra of **16** were duplicated, which sug-



1a R = OH
1b R = H



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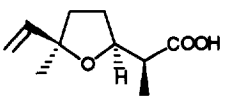

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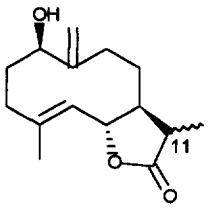
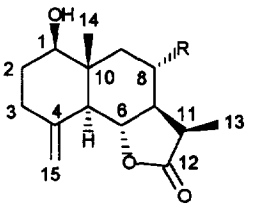
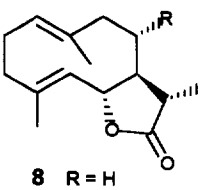


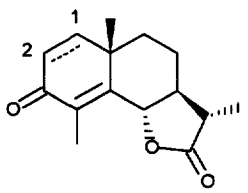
4 11αMe
5 11βMe



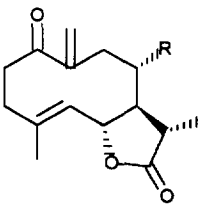
6 R = H
7 R = OH



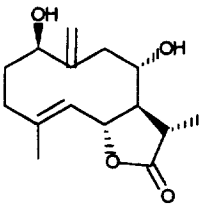
8 R = H
9 R = OH



10 1,2 double bond
11 1,2 single bond



12 R = H
13 R = OH



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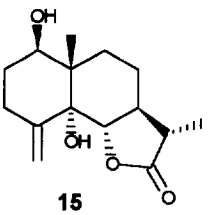

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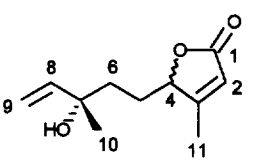
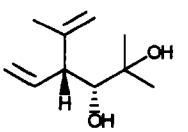
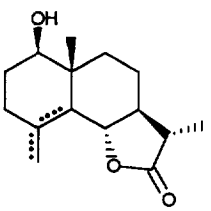


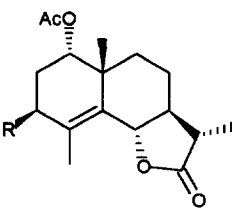
16



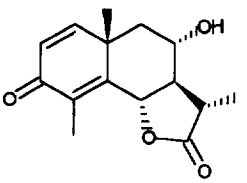
17



18 Δ4
19 Δ4(15)



20 R=H
21 R=OH



22

gested a mixture of two closely related isomers. The IR data indicated the presence of a hydroxyl group and a lactone ring (ν_{max} 3450, 1741 cm^{-1}). According to signal counting and multiplicity in the ^{13}C NMR spectrum (Table 1), a molecular formula $C_{11}H_{16}O_3$ (M_r = 196) was most likely. In fact, the mass spectrum showed the highest mass peak at m/z 181, in a good agreement with that expected for the peak due to methyl loss. Spin decoupling established the presence of a methyl vinyl carbinol moiety and of a β-substituted, five-membered conjugated lactone ring. In view of this, only structure **16** seemed suitable for both isomers. Heteronuclear long-range 2D-NMR correlations supported this conclusion. We have tentatively assumed that the configuration at C-7 is *S* for both compounds, as in natural (−)-nerolidol, the most likely biogenetic precursor. The two epimers should accordingly differ in their configuration at C-4.

Taken as a whole, these chemical data of *A. inculta* are well in line with that expected for members of section Seriphidium [1]. Furthermore, they suggest the existence of chemotypes of the species. The material from location L-1 and L-3, for instance, did not provide davanone-like sesquiterpenes, but mainly 11,13-dihydro germacranolides and eudesmanolides with an

Table 1. ^1H and ^{13}C NMR data of lactones **7** and **16**

H	7	16	C	7	16
1	3.52 *dd* (11.5; 4.5)		1	78.0	173.1
2α	1.82 *m*	5.80 *q* (1.5)/5.79 *q* (1.5)	2	30.9	117.0/116.9
2β	1.55 *dddd* (13; 13; 11.5; 5)		3	33.3	168.5/168.4
3α	2.14 *m**		4	142.3	84.5/84.4
3β	2.33 *ddd* (14; 5; 2)		5	52.1	26.4/26.1
4		4.85 *br m*	6	76.2	36.2/35.8
5	2.09 *br d* (11)	2.00 *m** (1H)	7	54.7	72.7/72.6
6	4.26 *t* (11)	1.65–1.50 *br m* (3H)	8	64.7	144.3/144.1
7	2.12 *m**		9	46.1	112.3/112.2
8	3.96 *ddd* (10.5; 10.5; 4.5)	5.87 *dd*/5.85 *dd* (17.3; 10.7)	10	42.2	28.6/28.2
9α	1.25 *dd* (12.5; 10.5)	5.22 *dd*/ 5.20 *dd* (17.3; 1)	11	37.1	13.8
9β	2.36 *dd* (12.5; 4.5)	5.08 *dd*/5.07 *dd* (10.7; 1)	12	179.4	
10		1.30 *s*/1.28 *s*	13	9.3	
11	2.85 *quint* (7.5)	2.04 *d*/2.03 *d* (1.5)			
13	1.31 *d* (7.5)		14	12.8	
14	0.81 *s*		15	110.8	
15	4.99 *br q* (1.2)				
	4.84 *br q* (1.2)				

J (parentheses) in Hz, 400 MHz (^1H) and 100 MHz (^{13}C), $CDCl_3$, 22°.
* Overlapped signal.

11βH configuration. In contrast, the material collected at location L-2 furnished lactones with the opposite, and less usual, configuration at C-11, in addition to davanone-like derivatives. Specific chemical differences between specimens collected in different geographical locations have already been found in other *Artemisia* species [1]. However, one most distinct chemical feature of the sesquiterpene lactones isolated from North African *A. herba-alba*, namely the presence of germacranolides and eudesmanolides oxygenated at C-9 [1, 7], is completely absent in the case under study. Except for the davanone-related compounds, *A. inculta* is chemically closer to *A. herba-alba* growing in Spain than to the North African counterpart [7]. We thus believe that the chemical data now available support our previous conclusions, based on morphological criteria [5], regarding the taxonomic differentiation of the North African species *A. herba-alba* and *A. inculta*.

EXPERIMENTAL

NMR: 400 MHz (^1H) and 100 MHz (^{13}C) in $CDCl_3$ (22°). The solvent signals were used as the ref. NOE measurements were carried out by the 1-D difference method. Optical rotations at 22°. CC: silica gel Merck (particle size 50–200 μ), gradient elution with the solvent mixts indicated in each case. HPLC: LiChrosorb RP-8 (250 × 8 mm), elution with MeOH–H_2O mixts.

Plant material. Aerial parts of *A. inculta* were collected in Egypt during April 1995 at three different locations: L-1. Wadi Gindali (El-Qahira province), near the Bir Gindali (*locus classicus*, voucher BCF-41112). L-2. Saint Katherine (peninsula of Sinai), near the Wadi Sheik (voucher BCF-41111). L-3. Umma-er-Raham (Ed-Diffa), near the Wadi Habis (voucher BCF-41110). The voucher specimens have been deposited in the herbarium of the Laboratory of Botany, Faculty of Pharmacy, University of Barcelona, Spain (Prof. J. Vallès-Xirau).

Extraction and chromatography. The plant material from all three locations was processed according to the described protocol [21]. The defatted extract was prefractionated by CC on silica gel (fr. A, hexane–EtOAc 3:1, fr. B, hexane–EtOAc 1:3; fr. C, EtOAc–MeOH 9:1). The three frs were subjected to further chromatographic sepns as described below. The compounds were eluted in the indicated order, which corresponds to increasing polarity on normal-phase silica gel.

Material from location L-1. CC of fr. A (hexane–EtOAc 10:1 → 1:5), followed where necessary by prep. TLC, allowed the isolation of **8** (12 mg), **11** (15 mg) and **12** (10 mg). Fr. B (CC with CH_2Cl_2–EtOAc 10:1 → 1:2) afforded **10** (20 mg), **19** (13 mg), **9** (14 mg), **4** (72 mg), **16** (8 mg), **15** (8 mg) and **13** (14 mg). Fr. C by CC (CH_2Cl_2–MeOH 50:1 → 1:5) yielded **14** (34 mg).

Material from location L-2. CC of fr. A (hexane–EtOAc 10:1 → 1:1), followed where necessary by prep. TLC, allowed the isolation of **2** (218 mg) and **1b** (268 mg). Fr. B (CC with hexane–EtOAc 5:1 → 1:3) afforded more **1b** (33 mg), **6** (5 mg) and **5** (2 mg). Finally, CC of fr. C (CH_2Cl_2–MeOH 50:1 → 5:1), followed by HPLC, furnished **7** (15 mg) and **3** (10 mg).

Material from location L-3. CC of fr. A (hexane–EtOAc 15:1 → 1:1), followed where necessary by prep. TLC, allowed the isolation of **8** (12 mg), **20** (5 mg) and **17** (55 mg). CC of fr. B (CH_2Cl_2–EtOAc 10:1 → EtOAc) afforded **10** (2.1 g), **18** (14 mg), **19** (64 mg), more **17** (190 mg), **9** (22 mg), **4** (280 mg), **15** (2

mg), **21** (7 mg), and **22** (14 mg). CC of fr. C (CH_2Cl_2–MeOH 50:1 → 1:5) yielded **13** (7 mg) and **14** (25 mg).

11-*Epiartapshin* (**7**). Oil, $[\alpha]_D + 31°$ ($CHCl_3$, *c* 1.5). IR ν_{max}^{film} cm^{-1}. 3450 (*br*, OH), 3060, 1770 (lactone C = O), 1585, 1500, 1450, 1370, 1260, 1120, 1035, 970, 950, 875, 840, 810; EIMS (probe) *m/z* (rel. int.): 266.1532 $[M]^+$ (10), 248 $[M-H_2O]^+$ (82), 230 $[M-2H_2O]^+$ (20), 204 (18), 181 (35), 175 (30), 160 (100), 145 (34), 133 (58), 119 (31), 107 (80), 91 (51), 79 (33), 69 (44), 56 (32). Calc. for $C_{15}H_{22}O_4$, M = 266.1518; NMR, Table 1.

(4*R**, 7*S**)- *and* (4*S**, 7*S**)-7-*Hydroxy*-3,7-*dimethylnona*-2,8-*dien*-1,4-*olide* (**16**). Oil. IR ν_{max}^{film} cm^{-1}: 3450 (*br*, OH), 3071, 1741 (lactone C = O), 1650, 1437, 1282, 1160, 948, 725; EIMS (probe) *m/z* (rel. int.): 181.0857 $[M-Me]^+$ (30), 178 $[M-H_2O]^+$ (6), 169 $[M-CH{=}CH_2]^+$ (15), 163 $[M-Me-H_2O]^+$ (18), 126 (67), 111 (75), 98 (100), 81 (23), 71 (90), 55 (38). Calcd for $C_{10}H_{13}O_3$, M = 181.0864; NMR, Table 1.

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