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CRITHMIFOLIDE: A SESQUITERPENE LACTONE FROM ACHILLEA CRITHMIFOLIA

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Key Word Index—*Achillea crithmifolia*; Asteraceae; sesquiterpene lactones; 1,2-*seco*-guaianolide hemiacetal.

Abstract—Crithmifolide, a novel 1,2-*seco*-guaianolide hemiacetal has been isolated from the aerial parts of *Achillea crithmifolia*, in addition to 11 known sesquiterpene lactones. The structure of the new compound has been elucidated by spectroscopic methods. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In our search for biologically active compounds from traditional medicinal plants, we have undertaken an investigation of *Achillea crithmifolia* Wald. & Kit. The genus *Achillea*, well-known for the medicinal properties of some members, has received much attention. However, a survey of the literature revealed only a small number of papers dealing with the chemical constituents of *A. crithmifolia* [1–7]. This might be due to the restricted distribution of the species to the Balkan peninsula, extending northwards to south-east Slovakia [8]. We now report on the isolation and structural elucidation of a new sesquiterpene lactone, named crithmifolide (**1**) from the aerial parts of *A. crithmifolia*. In addition, 11 known lactones, **2–12**, were also identified.

RESULTS AND DISCUSSION

The chloroform extract of the plant material afforded one new (**1**) and 11 known sesquiterpene lactones (**2–12**). The latter were identified by spectral data and TLC comparison using authentic samples as references. Crithmifolide (**1**) was obtained as a colourless gum. Its molecular formula, $C_{15}H_{18}O_6$, was deduced by CIMS ($[M + NH_4]^+$ *m/z* 312). The ^1H and ^{13}C NMR spectra of **1** (Table 1) showed it had one exomethylene double bond and two carbonyl groups, thus it possessed four rings based on the calculation of the degrees of unsaturation ($n = 7$). An α-methylene-γ-lactone ring was indicated by the carbon signals at δ 169.4, 137.7 and 121.1, and also by the characteristic ^1H NMR signals for H-13 (δ 5.52 and 6.27). The HMQC and ^1H-^1H COSY spectra established the connectivity of C-5/C-9 and the coupling patterns of the signals due to H-5, H-6 and H-7 allowed the assignment of the relative stereochemistry to give the structural fragment A (Fig. 1). The second ring in the structure of **1** was assumed to be an oxirane bearing a methyl group (δ 1.58) and a proton, which appeared as a sharp singlet at δ 3.20. Furthermore, the NMR data indicated the presence of a quaternary carbon carrying an oxygen function (δ 83.4) and a methyl group (δ 1.38 *s*, 20.4 *q*), and a carbonyl group (δ 207.5). The ^1H NMR spectrum displayed two broad signals at δ 2.90 and 5.50, attributable to a OH proton and a carbinol proton, as the former signal disappeared and the latter changed to a sharp singlet in a D_2O exchange experiment. These spectral features suggested the presence of four additional structural units, B–E (Fig. 1), which together with fragment A accounted for the Mr of crithmifolide (**1**). The connectivities of these structural fragments leading to the tricyclic partial structure F were determined by detailed analysis of the HMBC spectrum of **1** (Table 1). The carbonyl carbon at δ 207.5 exhibited a long-range C–H correlation with H-5 and H-9b, while H-9a, H-8 and H-5 showed correlation with the quaternary carbon at δ 83.4. Therefore, the presence of a seven-membered ring formed by the structural units

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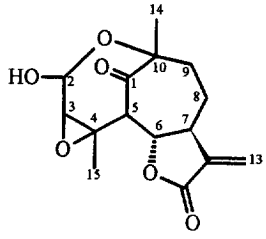

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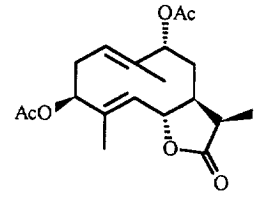

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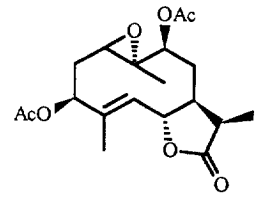

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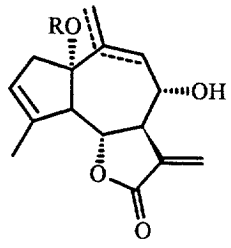
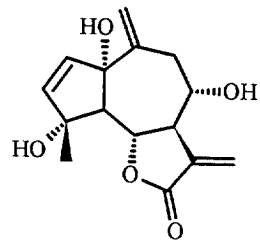


	R	Δ
4	H	9,10
5	H	10,14
6	OH	9,10
7	OH	10,14



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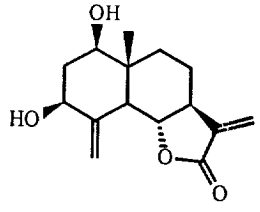

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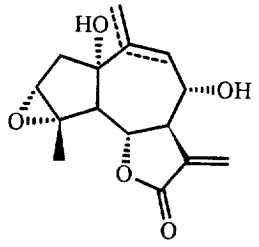
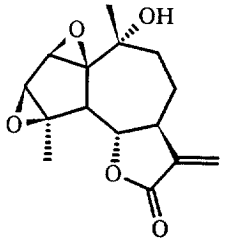


10 $\Delta^{9,10}$

11 $\Delta^{10,14}$



12

A, C and D was unequivocally established. Further, the connectivity C-2/C-5 was indicated by the observed correlations of the carbon at δ 90.1 with H-3 and the carbon at δ 64.9 with H-5 and H-15. The number of degrees of unsaturation for **1** meant that the only logical connectivity between C-2 and

Table 1. NMR data of crithmifolide (**1**) in $CDCl_3$ (250/62.9 MHz)

Position	$^{\delta}$H	$^{\delta}$C*	HMBC
1	—	207.5 *s*	H-5, H-9b
2	5.50 *brs*	90.1 *d*	H-3
3	3.20 *s*	64.9 *d*	H-5, H-15
4	—	63.4 *s*	H-5, H-6
5	3.35 *d* (8.0)	64.0 *d*	H-6
6	4.94 *dd* (9.8, 8.0)	81.1 *d*	H-5, H-8
7	2.53 *dddd* (10, 3.1, 3.4, 6.5, 3.5)	45.5 *d*	2H-13, H-8
8	1.80–2.00 *m*	23.9 *t*	H-6
9a	1.63–1.85 *m*	41.1 *t*	H-8, H-14
9b	1.95–2.05 *m*		
10	—	83.4 *s*	H-5, H-8, H-9a
11	—	137.7 *s*	2H-13
12	—	169.4 *s*	2H-13
13a	5.52 *d* (3.1)	121.1 *t*	—
13b	6.27 *d* (3.4)		
14	1.39 *s*	20.4 *q*	—
15	1.58 *s*	23.6 *q*	H-5
OH	2.90 *brs*		

* ^{13}C multiplicities were assigned from DEPT and HMQC spectra.

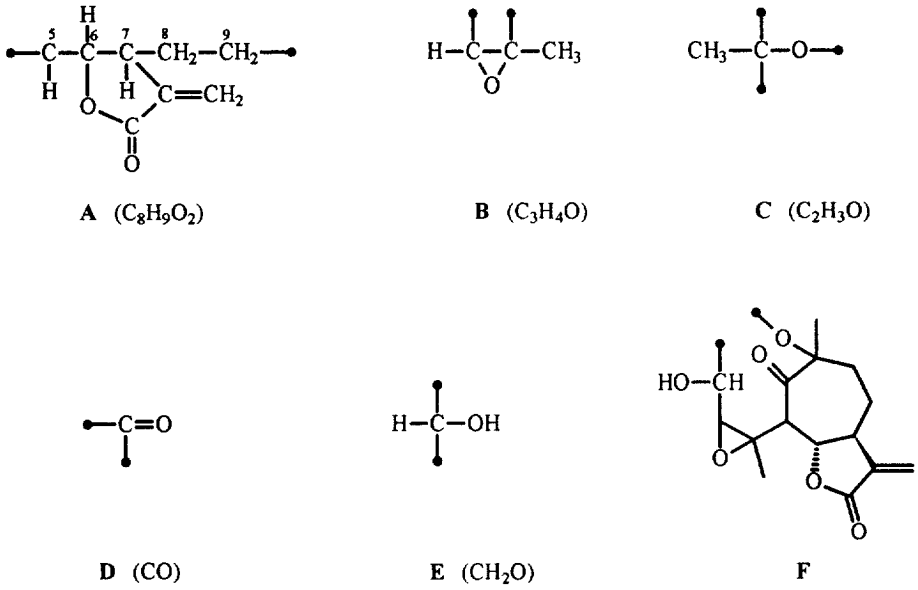


Fig. 1. Structural fragments of crithmifolide (**1**).

C-10 in F was by an ether-type linkage. The presence of the oxygen bridge was supported by the chemical shifts of C-2 (δ 90.1) and the hemiacetal proton H-2 (δ 5.50). Thus, the structure of crithmifolide (**1**) was unambiguously determined as a sesquiterpene hemiacetal of the 1,2-*seco*-guaianolide type. The structure of **1** contained seven stereogenic centres. These were assigned by NOE difference measurements and analysis of the ^1H–^1H coupling constants. The NOE connectivity pattern and the preferred conformation of crithmifolide (**1**) are depicted in Fig. 2. As mentioned above, the *trans*-closure of the lactone ring followed from the observed couplings $J_{6,7}$ (9.8 Hz) and $J_{7,13}$ (3.1 and 3.4 Hz). Irradiation of H-6 resulted in enhancement of only H-9a (δ 1.65), thus placing the two protons on the same β-face of the seven-membered ring. NOE interactions between H-5, H-7 and H-15; H-3, H-15 and H-2, as well as between H-14, H-2 and H-9b indicated their syn-α-orientation. The Dreiding model showed that the observed NOEs require the seven-membered ring to adopt a boat-like conformation with the carbonyl group, the epoxide ring and the ether bridge lying out of the plane. This conformation demonstrated the spatial proximity of the two oxygen atoms to H-6, which caused the latter's downfield shift to δ 4.94, and also explained the relative downfield shift of H-5 (δ 3.35), which is obviously affected by the anisotropy of the syn-oriented carbonyl group. Moreover, a

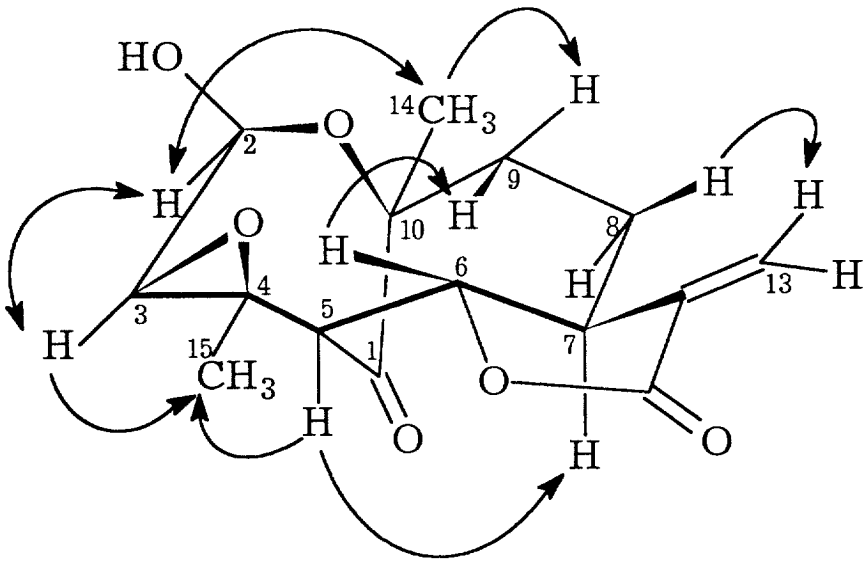
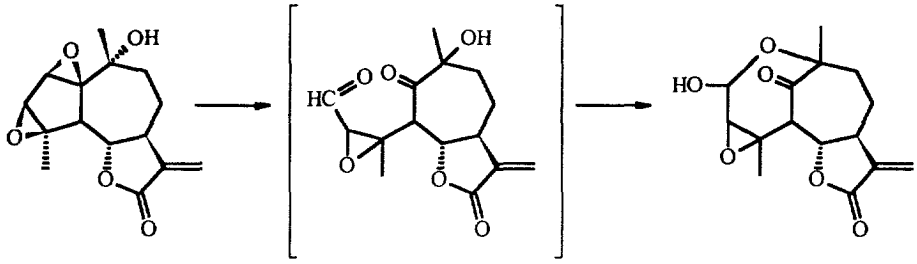


Fig. 2. Observed NOE of crithmifolide (**1**).



12 **1**

Scheme 1.

dihedral angle of ca 90° between H-2 and H-3 explained the lack of vicinal coupling in the ^{1}H NMR spectrum, and established the relative stereochemistry at C-2 as depicted in Fig. 2.

Crithmifolide (**1**) is most probably derived from a guaianolide precursor, and the lactone **12** is a likely candidate. As shown in Scheme 1, oxidative cleavage of the five-membered ring in **12** followed by intramolecular formation of the hemiacetal would give **1**.

EXPERIMENTAL

Plant material

The aerial part of *A. crithmifolia* were collected from Pirin mountain (south-western Bulgaria) in July 1996. The plant material was identified by Dr. D. Peev from the Institute of Botany, Bulgarian Academy of Sciences and a voucher specimen (SOM 153315) was deposited in the Herbarium of the same Institute.

Extraction and isolation

The air-dried plant material (130 g) was ground and extracted with $CHCl_3$ to give, after evaporation of the solvent under red. pres., a brownish gum (10 g). This was then separated into 7 frs. by vacuum–liquid chromatography on silica gel using CH_2Cl_2–Me_2CO mixtures as eluents. The lactone-containing frs. **4**–**6** (IR control) were subjected to repeated chromatography to yield **1** (5 mg) and the sesquiterpene lactones **2** (3 mg) [9], **3** (4 mg) [3], **4** (7 mg) and **5** (8 mg) [10], **6** (3 mg) and **7** (4 mg) [11], **8** (5 mg) [6], **9** (5 mg) [10], **10** (4 mg) and **11** (3 mg) [12], and **12** (5 mg) [13].

*Crithmifolide (**1**)*

CIMS (NH_3): *m/z* (rel. int.): 312 $[M + NH_4]^+$ (100), 296 $[M + NH_4\text{-}16]^+$ (5), 295 $[M + NH_4\text{-}17]^+$ (3), 294 $[M]^+$ (6); EIMS (70 eV) *m/z* (rel. int.): 294 $[M]^+$ (5), 235 (10), 219 (23), 205 (25), 189 (35), 164 (44), 121 (62), 109 (92), 95 (95), 85 (100), 53 (85); NMR: in Table 1.

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