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AN ELEMANDIOLIDE FROM *ZINNIA CITREA**

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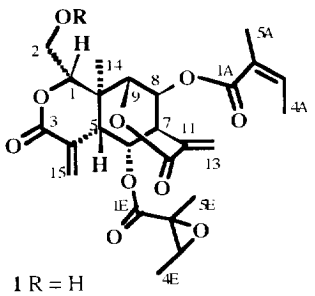
Key Word Index—*Zinnia citrea*: Heliantheae; zinnacitrin; sesquiterpene lactone; elemandiolide.

Abstract—The new elemandiolide zinnacitrin has been isolated from the aerial parts of *Zinnia citrea*, together with known compounds. Structure of zinnacitrin was determined as 8α-angeloyloxy-6β-epoxyangeloyloxy-2-hydroxyelema-11(13),4(15)-dien-1(3),9β(12)-diolide by spectroscopic means.

INTRODUCTION

Continuing our studies of *Zinnia* species [1] we describe the chemical constituents isolated from *Zinnia citrea* Torres (tribe Heliantheae, subtribe Zinninae, subgenus *Zinnia*), a perennial shrub from northeastern Mexico, which remained unknown for a long time, mainly due to its morphological similarity with *Zinnia acerosa* [2].

From the aerial parts of *Z. citrea* the known compounds sitosterol, stigmasterol and the flavone genkwanine have been isolated. In addition the new elemandiolide zinnacitrin (**1**), whose structural elucidation is described here, was also isolated.



1 R = H
2 R = Ac

RESULTS AND DISCUSSION

Repeated column chromatography of the acetone extract of *Z. citrea* yielded zinnacitrin (**1**), whose mass spectrum exhibited a $[M]^+$ at *m/z* 490 in accordance with the molecular formula $C_{25}H_{30}O_{10}$; 10 of these carbon atoms belong to esters (IR 1730 cm^{-1}) of the angelic and epoxyangelic acids. This was revealed by the fragments at *m/z* 391 $[M - AngO]^+$, 375 $[M - EpangOH]^+$ and 275 $[M - AngO - EpangOH]^+$ and the corresponding signals in the ^1H and ^{13}C NMR spectra [3] (see Tables 1 and 2). The remaining carbon atoms conform the skeleton of an elemane type sesquiterpene containing two α,β-unsaturated δ-lactones (IR 1730 cm^{-1}). The first one was characterized in the ^1H NMR spectrum by two doublets at δ6.54 and 5.22 for the C-15 protons conjugated with the 1(3)-δ-lactone. These signals were coupled with the H-5 signal at δ2.65. The signal for the proton under the lactone ring closure (H-1) was a triplet (δ4.51), which was part of an ABX system, whose AB part corresponded to the C-2 protons (δ3.78 *m*, 2H). These protons are geminal to a hydroxy group (IR 3598 cm^{-1}). The multiplicity of H-1 was consistent with C-1 being bonded to a hydroxymethylene and to a fully substituted carbon (C-10). The presence of the C-2 hydroxy group was supported by preparation of an acetyl derivative **2**, in whose ^1H NMR spectrum the H-2 and H-2′ signals were shifted to a lower field. This was confirmed by the ^{13}C NMR signals for carbons C-1 to C-5, C-10 and C-15 of compounds **1** and **2**, which were assigned by DEPT and HETCOR experiments. The chemical shift of the 3H singlet at δ1.36 (in $CDCl_3$), attributed to the methyl group at C-10, indicated that the elemandiolide **1** belongs to the H-5$_\beta$, C-14$_\alpha$ series (zinnolides) [4, 5].

Two broad singlets at δ6.58 and 5.53 characterized the second δ-lactone function and were assigned to the C-13 vinylic protons. The COSY spectrum revealed that they were coupled with the multiplet at δ3.32 (H-7), which in turn was coupled with the signals attributed to the protons under the above mentioned ester groups, H-6 (δ5.35), H-8 (δ5.46) and under the lactone ring closure H-9 (δ4.82). The $^4\sigma$ coupling (*W*-coupling) between H-7 and H-9 requires an equatorial disposition of these protons; therefore this second lactone is a 9β(12)-δ-lactone.

The stereochemistry of the cyclohexane ring in **1** was deduced by comparing the observed coupling constants with those of similar compounds such as juniperin,

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Table 1. ^{1}H NMR spectral data of compounds **1** and **2**

H	1*	1‡	2¶
1	4.51 *t*	4.64 *t*	4.82 *dd*
	5.4	6	6.7, 4
2,2′	3.78§ *m*	3.88§ *m*	4.32§ *m*
5	2.65 *dt*	2.91 *br dt*	2.92 *dt*
	3.6, 2.7	3.5, 3	3.5, 3
6	5.35 *t*	5.50§	5.57 *t*
	3.6		3.5
7	3.32 *m*	3.36 *m*	3.40 *br dt*
			3.5, 2.3
8	5.46 *dd*	5.50§	5.53†
	3.2, 2.1		
9	4.82 *t*	4.82 *t*	4.56 *t*
	2.1	2	2.1
13	5.53 *br s*	5.95 *br s*	6.79 *br s*
13′	6.58 *br s*	6.75 *br s*	5.98 *br s*
14	1.24 *s*	1.36 *s*	1.35 *s*
15	6.54 *d*	6.72 *d*	6.76 *d*
	2.7	3	3
15′	5.22 *d*	5.50§ *d*	5.53† *d*
	2.7	3	2.6
3_A	5.85 *qq*	6.17 *qq*	6.22 *qq*
	7.4, 1.4	7, 1.5	7.3, 1.5
4_A	1.88 *dq*	1.92 *dq*	1.95 *dq*
	7.4, 1.4	7, 1.5	7.3, 1.5
5_A	1.76 *t*	1.82 *t*	1.84 *t*
	1.4	1.5	1.5
3_E	2.79 *q*	3.10 *q*	3.13 *q*
	5.4	5.5	5.4
4_E	1.30 *d*	1.38 *d*	1.36 *d*
	5.4	5.5	5.4
5_E	1.48 *s*	1.54 *s*	1.55 *s*

*Determined at 300 MHz in $CDCl_3$–C_6D_6–DMSO-d_6.
†Superimposed signal.
‡Determined at 80 MHz in $CDCl_3$.
§Intensity two protons.
¶Determined at 200 MHz in $CDCl_3$.

Table 2. ^{13}C NMR spectral data of compounds **1** and **2**

C	1*	2†	C	1	2
1	83.6 *d*	80.8 *d*	Ang		
2	60.2 *t*	61.4 *t*	1	166.1 *s*	166.1 *s*
3	162.7 *s*	162.2 *s*	2	126.4 *s*	126.3 *s*
4	130.8 *s*	130.7 *s*	3	141.5 *d*	141.5 *d*
5	38.7 *d*	38.6 *d*	4	15.9 *q*	15.9 *q*
6	73.4 *d*	73.3 *d*	5	20.3 *q*	20.2 *q*
7	43.1 *d*	43.1 *d*	Epang		
8	63.2 *d*	63.2 *d*	1	169.3 *s*	169.4 *s*
9	80.2 *d*	80.0 *d*	2	59.6 *s*	59.5 *s*
10	41.2 *s*	41.1 *s*	3	60.2 *d*	60.2 *d*
11	130.9 *s*	130.7 *s*	4	13.7 *q*	13.7 *q*
12	161.9 *s*	161.7 *s*	5	19.2 *q*	19.2 *q*
13	134.9 *t*	135.0 *t*	Ac		
14	13.2 *q*	12.9 *q*	1		170.6 *s*
15	128.9 *t*	129.1 *t*	2		20.7 *q*

*Determined at 300 MHz in $CDCl_3$–C_6D_6–DMSO-d_6.
†Determined at 200 MHz in $CDCl_3$.

Table 3. Long range correlations detected in the COLOC spectrum of **1**

H	C
5	4*, 10
6	10, 11, 1E
7	6*, 11*
8	10, 11
9	8*, 12
13	7, 11*, 12
	13†
15	3, 15†

*Two-bond interactions.
†One-bond interaction.
Unlabelled carbons: three-bond interactions.

whose structure is based on an X-ray analysis or zinaflorins II and IV acetates [4]. This was confirmed by the observed NOEs between H-8 and H-14 on the α-face of the molecule, and between H-5 and H-1 on the β-face. This experiment also established the stereochemistry at C-1. At this point, the relative position of the ester groups could not be determined unambiguously. Therefore a COLOC NMR experiment was carried out. The results, summarized in Table 3, showed that the epoxyangelate was attached to C-6, because a three-bond interaction between H-6 and C-1 of the epoxyangelate was observed. This experiment also confirmed the assignment for C-4 and C-11.

The chemical composition of *Z. citrea* was thus different from that found in the closely related species *Z. acerosa*. Nevertheless, the composition of both species is consistent with the chemical profile observed in this genus.

EXPERIMENTAL

General. Mps uncorr. IR spectra were determined in a Perkin-Elmer-283B spectrometer. MS were obtained in a Jeol JMS-AX505 HA mass spectrometer. ^{1}H NMR spectra at 80, 200 or 300 MHz and ^{13}C NMR spectra at 50 or 75 MHz were recorded in Nicolet FT-80, Varian Gemini-200 or Varian Unity 300 spectrometers. NOE and COLOC experiments were performed in a Varian Unity 300 spectrometer.

Zinnia citrea. Torres was collected in San Luis Potosí, México (a voucher specimen was deposited in the Herbarium of the Instituto de Biología, UNAM, MEXU 371687).

Isolation of the constituents of Z. citrea. Ground and dried aerial parts of the plant (2.1 kg) were extracted with

Me_2CO, then with MeOH to give after solvent evaporation 70.0 and 61.6 g of extracts, respectively. The Me_2CO extract was decolorized by percolation through bentonite. The resulting residue (63.7 g) was fractioned by CC (hexane–EtOAc gradient elution). Frs eluted with hexane–EtOAc (19:1) gave 289 mg of a mixture of sitosterol and stigmasterol. Frs eluted with hexane–EtOAc (17:3) gave 209 mg of genkwanin (5,4′-dihydroxy-7-metoxyflavone), identified by comparison with literature data [6]. Frs eluted with hexane–EtOAc (7:3) contained **1**. These were combined and purified by extensive CC (hexane–Me_2CO, 4:1) and crystallization from EtOAc–hexane to obtain 220.2 mg of zinnacitrin (**1**), mp 232–235°, $[\alpha]_D = -17.3$ ($CHCl_3$, *c* 0.208); UV λ_{max}^{MeOH} nm (ε): 207 (19 960), 209 (19 890), 211 (19 600); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3598, 3449, 1730, 1631, 1380, 1260, 1135, 1082, 1044, 968. EIMS (probe) 70 eV, *m/z* (rel. int.): 490 $[M]^+$ ($C_{25}H_{30}O_{10}$) (13); 472 $[M - H_2O]^+$ (< 1); 391 $[M - C_5H_7O_2]^+$ (2.5); 374 $[M - C_5H_8O_3]^+$ (7.5); 275 $[M - C_5H_7O_2 - C_5H_8O_3]^+$ (29); 257 $[275 - H_2O]^+$ (10); 83 $[C_5H_7O]^+$ (100); 55 $[C_4H_7]^+$ (57); 43 $[C_2H_3O]^+$ (67).

Acetylation of **1**. A soln of **1** (70.7 mg) in pyridine (1 ml) and Ac_2O (1 ml) was left to stand at room temp. for 10 min. After the usual work up 70.7 mg crude **2** was obtained and purified by percolation through activated charcoal with Me_2CO and further crystallization from benzene–Et_2O. Mp 158–160°, IR ν_{max}^{Nujol} cm^{-1}: 1748, 1721, 1647, 1630, 1451, 1378, 1233, 1137, 1084, 1069, 966. EIMS (probe) 70 eV, *m/z* (rel. int.): 532 $[M]^+$ ($C_{27}H_{32}O_{11}$) (15); 490 $[M - 42]^+$ (7); 472 $[M - HOAc]^+$ (< 1); 391 $[M - C_2H_2O - C_5H_7O_2]^+$ (12); 374 $[M - C_2H_2O - C_5H_8O_3]^+$ (2); 275 $[M - C_2H_2O - C_5H_7O_2 - C_5H_8O_3]^+$ (5); 257 $[275 - H_2O]^+$ (7); 229 $[257 - CO]^+$ (6); 83 $[C_5H_7O]^+$ (100); 55 $[C_4H_7]^+$ (38); 43 $[C_2H_3O]^+$ (24).

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REFERENCES

1. Ortega, A. and Maldonado, E. (1985) *Phytochemistry* **24**, 2635.
2. Torres, A. M. (1963) *Brittonia* **15**, 1.
3. Budesínksy, M. and Saman, D. (1987) *Coll. Czech. Chem. Commun.* **52**, 453.
4. Maldonado, E., Soriano-García, M., Guerrero, C., Romo de Vivar, A. and Ortega, A. (1985) *Phytochemistry* **24**, 991.
5. Ortega, A., Romo de Vivar, A., Toscano, R. A. and Maldonado, E. (1983) *Chem. Letters* 1607.
6. Sakakibara, M., DiFeo, Jr D., Nakatani, N., Timmermann, B. and Mabry, T. (1976) *Phytochemistry* **15**, 727.