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FULVENE LACTONES FROM *TANACETUM ANNUUM*

ALEJANDRO F. BARRERO, JUAN F. SÁNCHEZ, MA JOSÉ ZAFRA, A. BARRÓN and ARTURO SAN FELICIANO*

Department of Organic Chemistry, Faculty of Sciences of the University of Granada, Granada, Spain; *Department of Organic and Pharmaceutical Chemistry, Faculty of Pharmacy of the University of Salamanca, Salamanca, Spain

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Key Word Index—*Tanacetum annuum*; Compositae; fulvene sesquiterpene lactones; proazulenes; guaianolides.

Abstract—An investigation of *Tanacetum annuum* afforded two new guaianolides, tannunolides A and B, sesquiterpene lactones with a fulvene structure, elucidated by spectroscopic methods and chemical transformations.

INTRODUCTION

In this paper, we report the isolation of two new guaianolide sesquiterpene lactones from the hexene extract of flowers of the medicinal plant *Tanacetum annuum* [1]. We have called these new products tannunolides A and B (**1**, **2**) whose most interesting structural characteristic is the presence of a fulvene arrangement. These lactones are proazulenes and are responsible for the formation of a blue hydrocarbon on steam distillation to obtain the essential oil (unpublished results).

RESULTS AND DISCUSSION

Tannunolides A and B are yellow crystals, mp 108–109° and 102–103°, respectively; both present a molecular peak at *m/z* 230 in agreement with the molecular formula $C_{15}H_{18}O_2$. The ^{1}H NMR spectroscopic data (Table 1) show the presence of a guaiane skeleton and we can observe, for each of the substances, signals corresponding to two methyls bonded to an *sp*^{2} carbon, a methyl at a tertiary carbon and two olefinic protons whose deshielding indicates the presence of a conjugated system. On the other hand, the value of $J = 5.3$ Hz leads us to situate these hydrogens in a five-membered ring.

The existence of the fulvene arrangement is established by its IR absorption in the regions of 1600 cm^{-1} (1636 and 1630 cm^{-1} along with other weaker bands at 1595 and 1590 cm^{-1} for **1** and **2**, respectively) and 1360 (1340, 1365 and 1370 cm^{-1} for **1** and 1360 and 1375 cm^{-1} for **2**) characteristic of fulvenes [2] accompanied in both compounds by another medium band at 1500 cm^{-1}, which at first indicated a benzene structure. We rejected this structure because of the strong UV absorption at 278 and 279 nm (for **1** and **2**, respectively) and also because of its easy hydrogenation, principally to compound **3**. The γ-lactone system is observed in IR, with strong absorptions at 1755 and 1760 cm^{-1}, and also in the mass spectrum by the presence of fragments at *m/z* 186 corresponding to $[M - CO_2]^+$. Compound **1**, when injected into a capillary column of carbowax, is partially decomposed due to the formation of two isomers **4** with a molecular peak at *m/z* 186, resulting from decarboxylation.

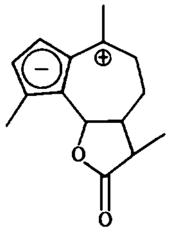
The ^{13}C NMR spectra agree with the proposed structures (Table 2). The assignment of ^{13}C NMR signals and some of ^{1}H NMR has been done by various experiments with tannunolide A. The 2D spectrum of direct H–C correlation [3] permits the assignments of C-7, C-11 and C-13 carbon atoms, while the study of 2D spectrum of indirect correlation through various H–C bonds (Table 3) leads to the remaining assignments leaving ambiguous the positions of C-4 and C-5, which have been resolved by heteronuclear NOE experiments [4] performed under selective irradiation of H-6 and H-15 protons, permitting the observation of a NOE difference response for the signals at 121.80 and 145.29 ppm, respectively, which are assigned at C-5 and C-4.

Considering an α-orientation of H-7, as in all the sesquiterpene lactones of higher plants [5], and taking in consideration the $J_{6,7}$ values, the α-position H-6 is established for both compounds. The configurations of C-11 are established by the values in ^{13}C NMR (Table 2) of the C-13 and C-8 carbon. The H-11 should have an α-position in **1** and a β-position in **2**, since in the first

Table 1. ^{1}H NMR spectral data of compounds **1**, **2** (200 MHz for **1** and 80 MHz for **2**; $CDCl_3$, TMS as internal standard)

	1	2
H-2	6.55 *d*	6.53 *d*
H-3	6.29 *d*	6.28 *d*
H-6	5.55 *d*	5.71 *d*
H-7	2.70 *m*	2.25–3.00 *m*
H-8	1.85 *m*	1.95 *m*
H-9	2.42 *m*	2.50 *m*
H-11	2.96 *dq*	2.25–3.00 *m*
H-13	1.26 *d*	1.36 *d*
H-14	2.19 *s*	2.19 *s*
H-15	2.09 *s*	2.12 *s*

J (Hz): compound **1**: 2,3 = 5.3; 6,7 = 5.3; 7,11 = 7.4; 11,13 = 7.4; compound **2**: 2,3 = 5.3; 6,7 = 6; 11,13 = 7.



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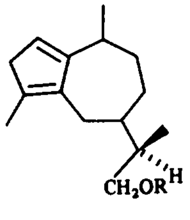

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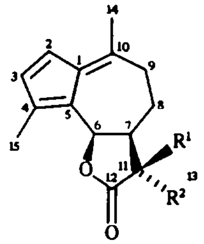
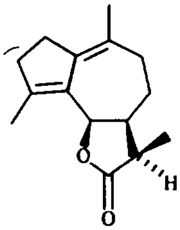
7 R = Ac



1 R^1 = Me, R^2 = H
2 R^1 = H, R^2 = Me



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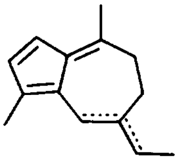

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compound these carbons are shielded 5 ppm, approximately, respective to the second position, owing to a *γ-gauche* interaction [6, 7]. The deshielding of C-10 in **1** and **2** is also interpreted according to known data [8], as a result of the contribution of polar structures **5** to the fundamental electronic state of the fulvene system.

The reduction of **1** with lithium aluminium hydride affords, among other substances, the compound **6** in which not only reduction with hydrogenolysis of lactone ring can be observed, but also the classical reduction of the fulvene system with its exocyclic carbon.

Table 2. ^{13}C NMR spectral data of compounds **1** and **2** (20.15 MHz, $CDCl_3$, TMS as internal standard)

	1		**2**	
Carbon	δ	Multiplicity	δ	Multiplicity
C-1	140.15	C	140.68	C
C-2	122.77	CH	123.06	CH
C-3	131.38	CH	132.50	CH
C-4	145.29	C	146.33	C
C-5	121.80	C	122.35	C
C-6	77.26	CH	78.14	CH
C-7	42.20	CH	41.27*	CH
C-8	24.44	CH_2	29.40	CH_2
C-9	33.95	CH_2	33.99	CH_2
C-10	153.20	C	152.89	C
C-11	39.10	CH	45.23*	CH
C-12	178.89	C	180.00	C
C-13	9.88	CH_3	15.17	CH_3
C-14	23.64	CH_3	24.19	CH_3
C-15	13.09	CH_3	14.00	CH_3

*These carbons may be interchanged.

EXPERIMENTAL

The plant was collected in September 1984 in Alfacar (Granada, Spain) and has been identificated by Professor F. Valle (Departamento de Botánica de la Universidad de Granada). A voucher specimen is available for inspection at the Herbarium of Faculty of Sciences of the University of Granada (GDAC 23365). The flowers, once air dried (546 g) were extracted with hexane in a Soxhlet apparatus and the resulting extracts (33.05 g) were

Table 3. Two-dimensional ^{13}C–^{1}H shift correlated spectra of compound **1** (50.3 MHz, $CDCl_3$, TMS as standard)

δ(ppm)	Observed correlations	Assignment
9.98	H-11	C-13
13.09		C-15
23.64		C-14
24.44	H-6, H-11	C-8
33.95	H-14, H-7	C-9
39.10	H-13, H-7	C-11
42.20	H-13, H-11	C-7
77.26	H-8	C-6
121.80	H-3, H-15, H-6, H-2	C-5*
122.77	H-3	C-2
131.38	H-15, H-2	C-3
140.15	H-14, H-6, H-3, H-2	C-1
145.29	H-15, H-6, H-3, H-2	C-4*
153.16	H-8, H-14, H-9	C-10
178.89	H-13, H-7, H-11	C-12

*Definitive assignment performed through heteronuclear {^{1}H} ^{13}C NOE difference experiments.

chromatographed on silica (150 g). From the fractions eluted with hexane–ethyl acetate (1 : 1), 1.050 g of **1** and 85 mg of **2** were separated by chromatography and crystallization in ether.

Tannunolide A (**1**). Yellow crystals, mp 108–109° (Et_2O); GC/MS m/z (rel. int.): 230 $[M]^+$ (27), 215 $[M-Me]^+$ (3), 186 $[M-CO_2]^+$ (5), 171 $[M-CO_2-Me]^+$ (5), 157 $[M-CO_2-Et]^+$ (100); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 278 (22.00); $[\alpha]_{589\,nm}$ $-100°$ ($CHCl_3$; c 1.0); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3060, 3000, 1755, 1634, 1595, 1500, 1200, 1170, 930.

Tannunolide B (**2**). Yellow crystals, mp 101–102° (Et_2O); GC/MS m/z (rel. int): 230 $[M]^+$ (100), 215 $[M-Me]^+$ (24), 186 $[M-CO_2]^+$ (19), 171 $[M-CO_2-Me]^+$ (29), 157 $[M-CO_2-Et]^+$ (71); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 279 (20.00); $[\alpha]_{589\,nm}$ $-29.8°$ ($CHCl_3$; c 1.52); IR $\gamma_{max}^{CHCl_3}$ cm^{-1}: 3000, 3010, 1755, 1632, 1590, 1500, 1205, 1170, 930.

Partial hydrogenation of tannunolide A. 250 mg of **1** were dissolved in 10 ml Et_2O; 35 mg 5% $Pd/BaSO_4$ were added and it was hydrogenated at atmospheric pressure and room temperature during 3.5 hr. The ^1H NMR spectrum shows signals assignable to **3**. ^1H NMR (80 MHz, $CDCl_3$): δ5.31 (1H, *d*, $J = 5$ Hz, H-6), 1.84 (3H, *s*, H-14), 1.62 (3H, *s*, H-15).

Chamazulenogenes **4**. They were detected in GC/MS, with He as carrier gas at 1 ml/min in a capillary column WCT of carbowax 20 M HP 19091/W-02, 25 m, 0.31 mm of ID, split ratio 60 : 1 and injection vol. of 0.2 l.; the injector and detector heater temps were 220 and 150°, respectively; temp. programmed 100–230° at 12°/min; Rt_1 8.433 min, Rt_2 8.684 min; GC/MS m/z (rel. int.): 186 $[M]^+$ (86), 171 $[M-Me]^+$ (82), 157 $[M-Et]^+$ (42), 156 $[M-Me-Me]^-$ (44), 142 $[M-Me-Et]^+$ (100) and 186 $[M]^+$ (92), 171 $[M-Me]^-$ (100), 157 $[M-Et]^+$ (42), 156 $[M-Me-Me]^+$ (45), 142 $[M-Me-Et]^+$ (80), 129 $[M-Me-Me-Et]^+$ (64).

Reduction of tannunolide A with lithium aluminium hydride. To 248 mg of **1** (1.08 mmol) dissolved in 100 ml dry Et_2O, 102 mg $LiAlH_4$ (3.20 mmol) was added and the mixture stirred at room temp. in N_2 for 10 min. After this, Et_2O satd with H_2O and then an NH_4Cl soln were added. The decanted solution was dried and the solvent evaporated. The residue was acetylated with AC_2O (2 ml) and pyridine (2 ml) at room temp. for 14 hr followed by purification on silica gel coumn with hexane–Et_2O (95 : 5); 48 mg of **7** was obtained: oily product; IR ν_{max}^{film} cm^{-1}: 1740, 1235; ^1H NMR (80 MHz, $CDCl_3$): δ0.83 (3H, *d*, $J = 7$ Hz, H-13), 1.09 (3H, *d*, $J = 7$ Hz, H-14), 1.93 (3H, *s*, H-15), 2.04 (3H, *s*, OAc), 2.70 (1H, *br s*, H-3), 3.85 (1H, *dd*, $J_{12,12'} = 12$ Hz, $J_{11,12} = 6$ Hz, H-12), 4.00 (1H, *dd*, $J_{12',11} = 7$ Hz, H-12'), 5.65 (1H, *br s*, H-2).

Two-dimensional ^{13}C–^1H *shift correlated spectra of* **1**. Magnetization transfer from ^1H to ^{13}C was made via the ^1J(CH) coupling and also between ^1H and ^{13}C more than one bond separated coupling, by using the pulse sequence XHDEPT.AU [3]. In the first case, 128 FIDs of 32 scans were acquired, $\Delta t_1 = 8.06$ msec, recycle delay = 1 sec and by adjusting the maximum polarization transfer for $J_{C,H} = 135$ Hz. Both time domains were multiplied with a sine-bell function and a matrix of 128 × 1024 data points, with a digital resolution = 13.0 Hz/pt in f_2 and 4.84 Hz/pt in f_1 was obtained. The second experiment was performed with 128 FIDs of 128 scans, $\Delta t_1 = 3.6$ msec, recycle delay = 1.2 sec and the polarization transfer adjusted for $J_{C,H} = 10$ Hz. The carrier wave in the proton frequency domain was placed at the end of the same to avoid a signal duplication. The Fourier transform made under a sine-bell multiplication with one degree of zero-filling in f_1 and a matrix of 256 × 1024 data points with a digital resolution of 18.4 Hz/pt in f_2 and 5.4 Hz/pt in f_1 was obtained.

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