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NEW SESQUITERPENE α -METHYLENE LACTONES FROM
THE EGYPTIAN PLANT *JASONIA CANDICANS*¹

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ABSTRACT.—Extracts of the plant *Jasonia candicans* possess antimicrobial activity. Et₂O/MeOH extracts were subjected to liquid chromatography, and several sesquiterpenes were isolated and identified including the known compounds confertin [**1**], 4,11(13)-eudesmadien-12-oic acid [**2**], and 11-eudesmen-4-ol [**3**]. Two new diol α -methylene lactone antimicrobial agents were identified from nmr and mass spectral data and X-ray crystallography as (4 α ,5 α ,8 β ,10 β)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide [**4**] and (4 α ,5 α ,8 β ,10 α)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide [**5**], which differ in stereochemistry at the C-10 tertiary alcohol center.

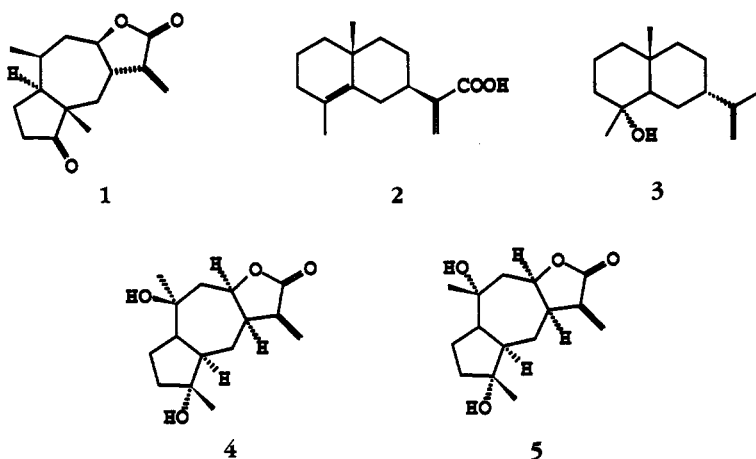
The small genus *Jasonia* (Asteraceae), which is mainly distributed in the Mediterranean region, has been placed in the *Inula* group (Family Asteraceae, Tribe Inuleae, subtribe Inulinae) (1). As part of our biochemical studies of this genus (2–5), we have investigated the terpenoid constituents of the plant *Jasonia candicans* (Del.) Botsch. Previous work on this species led to the isolation of three unidentified antibacterial sesquiterpene lactones (6), confertin, and an epimer of confertin (7).

RESULTS AND DISCUSSION

Air-dried plant parts were extracted in Et₂O/MeOH, and constituent chemicals were separated and purified using open-column liquid chromatography. Three known sesquiterpenoids, the pseudoguaianolide ketone confertin [**1**] (7), 4,11(13)-eudesmadien-12-oic acid [**2**] (8), and 11-eudesmen-4-ol [**3**] (9), were identified by comparison of their spectroscopic properties with those in the literature. Four further compounds were isolated, including the two new compounds whose identification through spectral and X-ray crystallographic data are reported herein, and two which are still under study.

The ¹³C-nmr spectra indicated that both new compounds possessed 15 non-equivalent carbon atoms, and similarities in the spectra indicated that the two unknown compounds were isomeric. General features of the spectra for both new compounds were peaks near δ 170 indicating conjugated acid or ester functionality, four peaks in the δ 120–152 range indicating presence of two double bonds, and three peaks in the δ 80–70 range indicating tertiary alcohol or ester (lactone) functionality. From DEPT experiments it was determined that both new compounds possessed two methyl groups, three alkyl and one aryl methylenes, three alkyl and one aryl methines, as well as two alkyl

¹Dedicated, with respect, to Professor Edward Leete, a fine scientist who will be greatly missed.



and two aryl tertiary centers. Combining the carbonyl carbon with one of the three C-O functions as a lactone would give a molecular formula of $C_{15}H_{20}O_4$, indicating six units of unsaturation, i.e., isomeric tricyclic structures with two double bonds. The information as a whole suggested sesquiterpene α -methylene lactones.

The general appearance of the 1H spectra supported this interpretation. Three vinyl proton peaks, two of which exhibited small vinyl couplings to complex multiplets near δ 3.3, were present for both compounds. Complex multiplets (d of d of d) at δ 4.75 for compound **4** and 5.14 for compound **5** were attributed to hydrogen at position 8 with upfield shift due to the lactone functionality. These were coupled to the peaks near 3.35 as well as to two d of d signals near δ 2 for both compounds exhibiting a large geminal coupling to each other. The peaks attributed to H-7 (3.23 for compound **4** and 3.35 for compound **5**) exhibited couplings to geminal protons near 1.3 and 2.1, which in turn were coupled to the signal for H-5 which occurs near 2.5. The other main features of the spectra were two methyl singlets for each compound at shifts indicating adjacent tertiary hydroxyl functionality, and a pair of near shift-equivalent geminal signals at 2.37 and 2.38 for the two compounds, showing a small coupling to the remaining vinyl proton. The basic structures were therefore as given, but stereochemistry was not fully determined. Coupling constants indicated a *cis* juncture for the lactone to a seven-membered ring system, and NOESY correlations suggested, but did not prove, the relative conformations given. Methyl resonances could be unambiguously assigned, however, and H-C correlation using HMQC spectroscopy was also useful, both in making ^{13}C assignments and in confirming the geminal nature of certain peaks. Crystals derived from the nmr samples were subjected to X-ray crystallography, which confirmed the structural assignments. Stereo views are given in Figure 1.

The new compounds identified, (4 α ,5 α ,8 β ,10 β)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide [**4**] and (4 α ,5 α ,8 β ,10 α)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide [**5**], are of the guaian-12,8-olide structural family, which constitutes a fairly small group to date. A structure similar to compound **4** with a hydroperoxide substituent at the 10 position has been reported by Rustaiyan and Faramarzi (10), and the 1H spectrum published is similar in many respects to those described here. Spectra reported for an analogous hydroperoxide structure with *trans* lactone to seven-membered ring juncture show a much lower correlation (11).

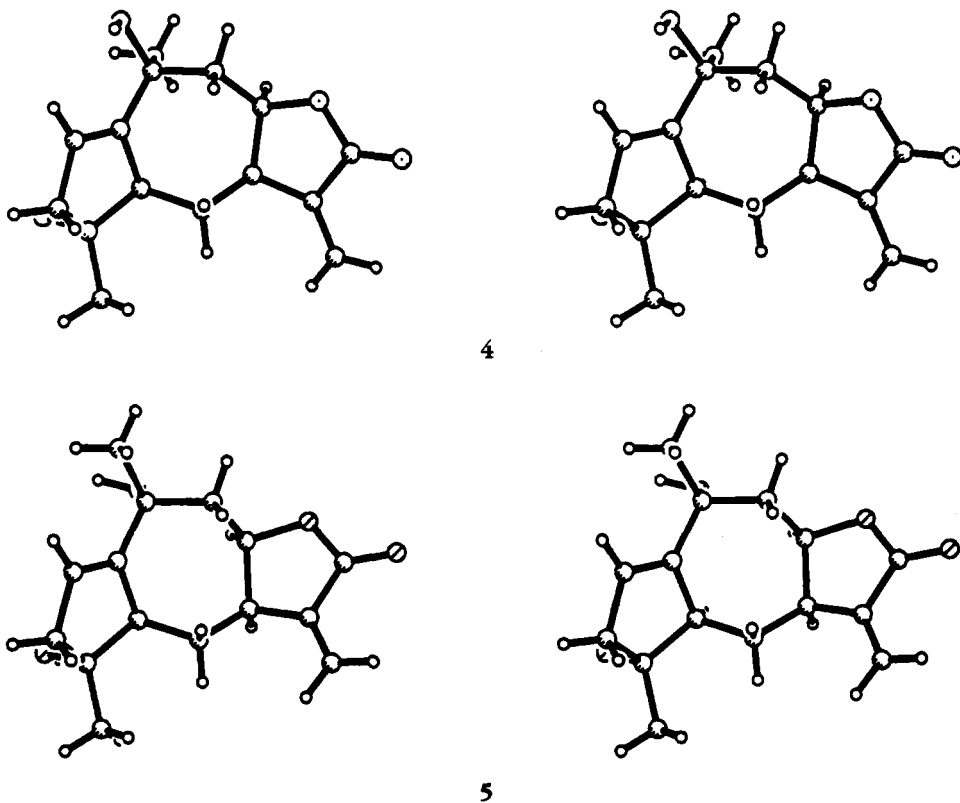


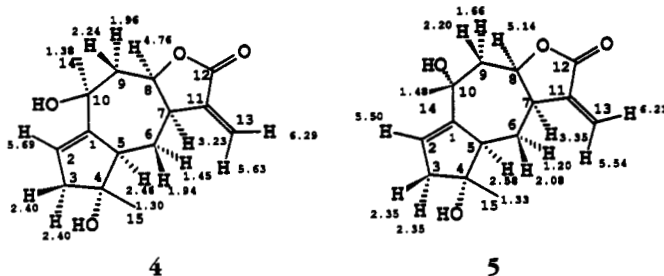
FIGURE 1. Stereo views of compounds **4** and **5**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—X-ray crystallography was performed on a Nicolet R3/M diffractometer (oriented graphite monochromator: MoK α λ =0.71073 Å radiation). All crystallographic calculations were performed with SHELXTL-PLUS rev 3.4 (G.M. Sheldrick, Institut für Anorganische Chemie der Universität, Tammannstraße 4, D-3400, Göttingen, Federal Republic of Germany) supplied by Nicolet Analytical X-ray Instruments, Madison, Wisconsin, on a microVaxII minicomputer. Nmr spectra were obtained on Bruker AM 500 and Varian UNITY 500 Fourier Transform Spectrometers in CDCl₃ solvent, and chemical shifts are referenced to solvent (7.24 δ for ¹H, 77.0 δ for ¹³C). ¹H assignments were made through selective decoupling experiments and COSY and double-quantum filtered COSY experiments, supplemented by NOESY data (600 msec mixing time) which allowed assignment of methyl groups at tertiary centers and individual hydrogens on geminal carbons. ¹³C assignments were made using data from DEPT experiments and carbon-decoupled HMQC experiments, with tertiary centers assigned using analogies to known compounds.

EXTRACTION AND PURIFICATION.—Air-dried aerial parts (1 kg) of *J. candicans* [syn. *Vartbemia candicans* (Del.) Boiss] collected from Marsah Matrouh, Egypt in April 1991 (voucher specimen is deposited in the Department of Botany, El-Minia University) were extracted with MeOH-Et₂O (1:1). The extract was defatted and chromatographed on Si gel in petroleum ether (bp 40–60°) and eluted with a petroleum ether/Et₂O step gradient. The fraction eluted with petroleum ether-Et₂O (4:1) was further chromatographed on a Sephadex LH-20 column with an initial solvent system of Et₂O-CH₂Cl₂-MeOH (7:4:0.5) and eluted with an increasing MeOH gradient to give in order of elution **1** (3 g) (**6**), **2** (30 mg) (**7**), and **3** (35 mg) (**8**). The fraction eluted by petroleum ether-Et₂O (1:1) was rechromatographed on a Si gel column with CH₂Cl₂ to give unknown compound A (25 mg), unknown compound B (20 mg), **4** (60 mg), and **5** (40 mg).

Compound 4.—[α]_D²⁰ +266.7° (c =0.03 CDCl₃); ¹³C nmr 169.8 (s, C-12), 152.4 (s, C-1), 139.7 (s, C-11), 123.0 (t, C-13), 120.2 (d, C-2), 81.9 (s, C-4), 77.0 (d, C-8), 71.2 (s, C-10), 55.8 (d, C-5), 46.6 (t, C-3), 43.9 (t, C-6), 41.9 (d, C-7), 31.9 (t, C-9), 27.0 (q, C-14), 24.2 (q, C-15); ¹H nmr see Figure 2 and Table 1; ms m/z [M-H₂O]⁺ 246 (40%), [M-2 H₂O]⁺ 228 (80%), [228-Me]⁺ 203 (45%).

FIGURE 2. ^1H -nmr peak assignments for compounds **4** and **5**.

Compound 5.— $[\alpha]_D^{20}$ -280.6° ($c=1.8$ CDCl_3); ^{13}C nmr 170.4 (s, C-12), 152.0 (s, C-1), 139.9 (s, C-11), 122.0 (t, C-13), 121.5 (d, C-2), 81.3 (s, C-4), 77.8 (d, C-8), 69.1 (s, C-10), 54.5 (d, C-5), 46.2 (t, C-3), 42.8 (t, C-6), 42.0 (d, C-7), 32.2 (t, C-9), 29.6 (q, C-14), 24.3 (q, C-15); ms m/z $[\text{M}-\text{H}_2\text{O}]^+$ 246 (40%), $[\text{M}-2\text{H}_2\text{O}]^+$ 228 (80%), $[\text{228}-\text{Me}]^+$ 203 (45%); ^1H nmr see Figure 2 and Table 1.

X-RAY CRYSTALLOGRAPHY.—Colorless crystals of isomer **4** [0.02 mm \times 0.04 mm \times 0.46 mm] and of isomer **5** [0.14 mm \times 0.26 mm \times 0.56 mm] were mounted on glass fibers with epoxy cement at room temperature and cooled to 193°K. Formula of **4** and **5** $\text{C}_{15}\text{H}_{20}\text{O}_4$, formula weight = 264.3 amu. Preliminary examination and data collection for isomers **4** and **5** were performed on a Nicolet R3m/V X-ray diffractometer (oriented graphite monochromator, $\text{MoK}\alpha=0.71073$ Å radiation). Cell parameters were calculated from the least-squares fitting of the setting angles for 25 reflections. Isomer **4** [monoclinic, $P2_1$ (No. 3)] $a=7.944(4)$ Å, $b=6.258(4)$ Å, $c=13.576(8)$ Å, $\beta=97.79(5)$ deg, $V=674.8(16)$ Å³, $D_x=1.301$ $\text{g}\cdot\text{cm}^{-3}$, $\mu=0.087$ mm^{-1} , $Z=2$, $F(000)=284e$. Isomer **5** [orthorhombic, $P2_12_12_1$ (No. 18)] $a=9.037(2)$ Å, $b=10.166(2)$ Å, $c=14.839(3)$ Å, $V=1363.3(4)$ Å³, $D_x=1.288$ $\text{g}\cdot\text{cm}^{-3}$, $\mu=0.086$ mm^{-1} , $Z=4$, $F(000)=568e$. Omega scans for several intense reflections indicated acceptable crystal quality for both crystals.

For isomers **4** and **5** the data were collected between $4.0 \leq 2\theta \leq 50.0$ deg, ω (Wyckoff) scans at 193°. Indices for isomer **1** $-9 \leq b \leq 9$, $0 \leq k \leq 7$, $-16 \leq l \leq 0$; indices for isomer **2** $0 \leq b \leq 10$, $0 \leq k \leq 12$, $-17 \leq l \leq 0$. For isomers **4** and **5** the scan width, on ω , for the data collection was 1.00 deg, with a variable scan rate of 1.50 to 14.65 $\text{deg}\cdot\text{min}^{-1}$. Three control reflections, collected every 97 reflections, showed no significant trends. Background measurement was by stationary crystal and stationary counter technique at the beginning and end of each scan for one half of the total scan time.

TABLE 1. ^1H Spectral Data for **4** and **5**.

Proton	Compound		Coupling	Compound	
	4	5		J 4	J 5
H-2	5.69	5.50	H-2-H-3	1.6, 2.1	1.8, 1.9
H-3 α	2.40	2.35	H-5-H-6 α	12.0	11.9
H-3 β	2.40	2.35	H-5-H-6 β	2.0	1.2
H-5	2.46	2.58	H-6 α -H-6 β	13.6	12.2
H-6 α	1.45	1.20	H-6 α -H-7	12.1	6.4
H-6 β	1.94	2.08	H-6 β -H-7	2.0	<1
H-7	3.23	3.35	H-7-H-13 cis	1.6	3.2
H-8	4.76	5.14	H-7-H-13 trans	1.9	3.4
H-9 α	1.96	1.66	H-7-H-8	4.8	5.1
H-9 β	2.24	2.20	H-8-H-9 α	12.2	11.8
H $_a$ -13	5.63	5.54	H-8-H-9 β	4.2	3.6
H $_b$ -13	6.29	6.21	H-9 α -H-9 β	13.2	13.9
Me-14	1.38	1.48			
Me-15	1.30	1.33			

²Atomic coordinates for compounds **4** and **5** have been deposited at the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

Lorentz and polarization corrections were applied to 1366 reflections and 1418 reflections for isomers **4** and **5**, respectively. No absorption correction was applied to either data set. A total of 849 unique reflections [$I > 2\sigma(I)$] for isomer **4** and 1286 unique reflections [$I > 2\sigma(I)$] were used in further calculations. Both structures were solved by direct methods. Full-matrix least-squares anisotropic refinement for all non-hydrogen atoms, $\omega^{-1} = \sigma(F)^2 + gF^2$, $g = 0.00100$ for isomer **4** and $g = 0.0026$ for isomer **5** (12) yielded for isomer **4** $R = 0.080$, $\omega R = 0.085$, and $S = 1.54$, and for isomer **5** $R = 0.034$, $\omega R = 0.045$, and $S = 1.88$ at convergence. The relative configuration of the unknown atoms for both isomers was based on the known configuration. The extinction coefficient χ {where $F^* = F_c / [1 + \chi^2 * 0.002 F_c^2 / \sin(2\lambda)]^{0.25}$ } was refined to 0.009(3) and 0.002(4) for isomers **4** and **5**, respectively (12). For both isomers the hydrogen atoms were placed in idealized positions with isotropic thermal parameters fixed at 0.08 \AA^2 . Neutral atom scattering factors and anomalous scattering correction terms were taken from "International Tables for X-ray Crystallography" (13).

ACKNOWLEDGMENTS

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