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ANT-REPELLENT SESQUITERPENE LACTONES FROM *EUPATORIUM QUADRANGULARAE*

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Key Word Index—*Eupatorium quadrangularae*; Compositae; sesquiterpene lactones; leafcutter ants; *Atta cephalotes*; ant-repellent.

Abstract—The chloroform extract from the leaves of *Eupatorium quadrangularae* has been systematically fractionated by following biological activity in a bioassay which measures repellency to the leafcutter ant *Atta cephalotes* (Formicidae, Attini). Several sesquiterpene lactones were isolated, two of which showed significant ant-repellency.

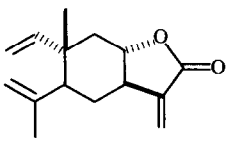
INTRODUCTION

The leafcutter ants of the tropical Americas (Formicidae, Attini) are considered polyphagous [1], but nonetheless they seldom or never attack many of the plants species available to them in nature [2]. Investigations conducted on the foraging behavior of Costa Rican colonies of *A. cephalotes* have shown that *E. quadrangularae* is one of the tree species seldom attacked [Hubbell S. P. and Howard J. J., personal communication]. To isolate repellent compounds from these plants, we rely upon a bioassay that monitors ant choices among an array of treated and control food flakes [3, 4]. We have recently reported the isolation of ant-repellent terpenoids from several other native plant species that escape the leafcutter attack [5–8]. In continuation of our studies of chemical defenses against insect herbivory, we here report the isolation of two potent ant repellents from *E. quadrangularae*, as well as the isolation of three other lactones with lesser biological activity.

RESULTS AND DISCUSSION

The chloroform extract of air-dried leaves of *E. quadrangularae* showed significant ant-repellent activity against our laboratory colonies of *A. cephalotes*. This extract was then partitioned between hexane and 50% aq. methanol, both fractions were bioassayed after concentration, and the activity was found to reside in the aqueous methanol layer. The residue from this layer was fractionated by column chromatography on silica gel, eluting with mixtures of methanol in methylene chloride. The least polar fraction from this column showed significant ant repellent activity and was further purified by preparative GC and TLC to yield compounds **1–3**. Compound **1**, which was most active, was obtained as a pure oil which later solidified on standing for several days. Its mass spectrum contained a molecular ion at m/z 232, suggesting a formula of $C_{15}H_{20}O_2$, and its IR and ^{1}H NMR spectra showed signals characteristic of an α-methylene-γ-lactone (1761 cm^{-1}, δ5.55, 6.34, each *d*, J = 1 Hz). The ^{1}H NMR spectrum also showed the presence of three terminal olefinic groups, one tertiary methyl group and one vinylic methyl group, and one proton (δ4.80 *br dd*) geminal to the lactone oxygen. Support for the presence of these groups was further obtained from the delayed decoupled ^{13}C NMR experiments, which also allowed determination of the carbon multiplicities (cf. Table 1). Comparison of the IR and ^{1}H NMR spectral data obtained for compound **1** with data recorded for the *seco*-eudesmanolide isolated from *Liatris cylindracea* [9] indicated that the two were identical.

Compounds **2** and **3** were not separated by GLC but were obtained pure by repeated preparative TLC on silica gel coated with 15% $AgNO_3$. Again, the presence of an α-methylene-γ-lactone was indicated by the IR and ^{1} NMR spectra. These data obtained for compounds **2** and **3** were found to correspond closely to those reported for alantolactone and isoalantolactone respectively [10]. Further support for the structures was obtained from the



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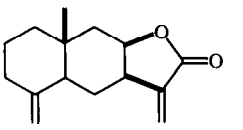

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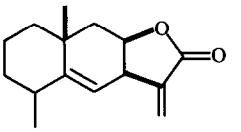

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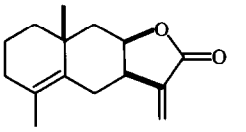

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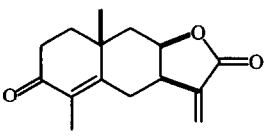

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Table 1. NMR spectral data for compounds 1–5

	^{1}H NMR of 5	C	^{13}C NMR of 1	2	3	4	5
1α-H	2.06 *dt*	1	148.0 *d*	41.3 *t*	41.0 *t*	41.4 *t*	42.5 *t*
1β-H	2.47 *dd* (*br*) (12.3, 3)*	2	113.0 *t*	22.7 *t*	22.2 *t*	22.7 *t*	34.7 *t*
2α-H, 2β-H	2.65 *m*	3	111.0 *t*	37.9 *t*	36.8 *t*	37.2 *t*	197.8 *s*
6α-H	3.06 *dd* (13, 7.2)	4	137.3 *s*	148.9 *s*	41.1 *d*	135.0 *s*	130.0 *s*
6β-H	1.95 *dd* (13.8, 4.5)	5	46.1 *d*	44.0 *d*	133.0 *s*	145.0 *s*	158.7 *s*
7α-H	3.23 *m*	6	26.5 *t*	27.5 *t*	122.3 *d*	27.6 *t*	29.7 *t*
8α-H	4.62 *ddd* (8.4, 8.4, 2.6)	7	40.2 *d*	40.6 *d*	46.3 *d*	40.0 *d*	39.6 *d*
9α-H	1.76 *dd* (11.1, 3)	8	75.8 *d*	76.8 *d*	77.0 *d*	76.4 *d*	74.6 *d*
9β-H	2.23 *t* (12.2)	9	39.2 *t*	41.0 *t*	42.2 *t*	40.1 *t*	35.2 *t*
13-H	6.37 *d* (3.0)	10	†	34.3 *s*	34.3 *s*	34.0 *s*	33.9 *s*
13'-H	5.72 *d* (2.5)	11	145.7 *s*	142.3 *s*	142.1 *s*	140.3 *s*	138.6 *s*
14-H	1.25 *s*	12	†	170.6 *s*	170.6 *s*	170.4 *s*	169.7 *s*
15-H	1.83 *br s*	13	120.3 *t*	120.1 *t*	119.9 *t*	120.6 *t*	123.1 *t*
		14	16.4 *q*	17.7 *q*	17.2 *q*	17.3 *q*	11.2 *q*
		15	19.3 *q*	106.6 *t*	17.6 *q*	21.1 *q*	25.0 *q*

*Coupling constants are in parentheses.
†Signals not observed.

^{13}C NMR broadband and delayed decoupled experiments (Table 1).

Compounds **4** and **5** were obtained from a slightly more polar column fraction which also showed ant-repellent activity, and they were further purified by preparative TLC. Compound **4** gave a molecular ion at *m/z* 232, corresponding to a formula of $C_{15}H_{20}O_2$. Its ^{1}H NMR spectral data differed from those obtained for **2** and **3** by the presence of a vinylic methyl group. The IR and ^{1}H NMR spectral data agreed well with those obtained for 4-desoxy-8-epi-ivangulin, isolated from *Inula helenium* [10], allowing this assignment of structure to compound **4**.

Compound **5** was easily shown to be an oxidized derivative of compound **4**. Its mass spectrum shows a molecular ion at *m/z* 246, corresponding to a formula of $C_{15}H_{18}O_3$. The presence of both an α-methylene-γ-lactone and an α,β-unsaturated carbonyl group was indicated from the IR and ^{1}H NMR spectral data. Homonuclear decoupling experiments allowed assignments of the ^{1}H NMR signals obtained for compound **5**. The signal at δ4.62 (*ddd*, J = 8.4, 8.4, 2.6 Hz) can be attributed to a proton geminal to the lactone oxygen and the coupling constants require a *cis*-fused lactone ring [10]. The chemical shifts and J-values observed for the other bridgehead proton (H-7) are also characteristic of *cis*-fused lactones [10, 11]. The position of the α,β-unsaturated carbonyl group follows from the delayed decoupled ^{13}C NMR spectra. The two singlets at 130.0 and 158.7 ppm can only be assigned to C-4 and C-5 respectively, and thus compound **5** is established as the 3-oxo derivative of compound **4**. Compound **5** is technically a new natural product, although its enantiomer has been reported [12] as a constituent of liverworts.

These compounds are reported here for the first time from *Eupatorium*. Furthermore, our bioassay results suggest that compounds **1** and **4** have appreciable value as defensive agents with respect to discouraging leafcutter attack (Table 2).

Biological assays

The laboratory bioassay technique, described in detail elsewhere [3, 4] consists of a forced choice test between pressed rye flakes treated with solutions of a potential repellent and control flakes treated with solvent alone. Our results are summarized in Table 2. Compounds **1** and **4** are among the most active compounds we have isolated, while the closely related sesquiterpenoids **2**, **3** and **5** do not show statistically significant repellency even at 5–10 fold higher concentrations. The features responsible for the biological activity are not known, but through isolation of larger numbers of natural ant repellents it may be possible to draw such generalizations.

Table 2. Repellency tests of constituents of *E. quadrangularae* in laboratory bioassay

Compound	Conc.* mg/ml	No. of flakes taken: Control (C)	Test (T)	(T/C) 100	Probability
1	0.5	29	14	48.3	$P < 0.05$
2	5.0	32	25	78.1	n.s.†
3	5.0	29	21	72.4	n.s.
4	1.0	32	20	62.5	$P < 0.05$
5	5.6	30	23	76.1	n.s.

*A concentration of 0.5 mg compound 1/ml corresponds to an approximate final concentration of 10 μg/flake.
†n.s.—not statistically significant at or below the 0.05 level.

EXPERIMENTAL

All mps are uncorr. The IR spectra were recorded in $CHCl_3$. The ^1H NMR spectra were obtained on a Bruker 360 spectrometer while the ^{13}C NMR spectra were recorded on a JEOL HX-90E instrument, using $CDCl_3$ as solvent with TMS as internal standard. Mass spectra (70 eV) were recorded with Hewlett Packard 5985B instrument. Preparative GC was performed with a TCD detector on a glass column (50 × 0.4 cm) packed with OV-17 (10%), column temperature was programmed linearly between 160° and 220° at 5°/min.

Isolation procedures. Air dried leaves of *E. quadrangularae* (1 kg, collected at Santa Rosa Park, Costa Rica in July 1981) were extracted successively with 2 l. $CHCl_3$ (24 hr) and then with 2 l. EtOH (24 hr) in a Soxhlet extractor. After both extracts were concentrated *in vacuo* and bioassayed, the ant repellent activity was associated with the $CHCl_3$ extract. This extract was further partitioned into polar (50% aq. MeOH) and nonpolar (hexane) fractions, both were concentrated *in vacuo* and bioassayed. Only the polar fraction showed significant activity (at a concentration of 6.3 mg/ml, $p < 0.001$).

After column chromatography of the polar residue (1.8 g) on silica gel (18 g); (CH_2Cl_2–MeOH gradient) a band of activity was located at fractions A (100% CH_2Cl_2) and B (99:1). Further purification of fraction A by preparative GC and repeated preparative TLC (silica gel, coated with 15% $AgNO_3$) yielded compounds **1** (12.5 mg) mp 65–67°, $[\alpha]_D^{27}$ +40.6° ($CHCl_3$); **2** (8.6 mg) and **3** (7.8 mg). Fraction B was also purified by preparative TLC and compounds **4** [6.0 mg; $[\alpha]_D^{27}$ +43.8° ($CHCl_3$)] and compound **5** (12 mg) were obtained.

Compound **5**, (+)-3-oxodiplophyllin: crystals from MeOH, mp 145–147° $[\alpha]_D^{27}$ +161°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1660, 1620, EIMS *m/z* (rel. int.): 246 (100) $[M]^+$, 228 (37) $[M-H_2O]^+$, 218 (22) $[M-CO]^+$, 204 (60) $[M-H_2C{=}C{=}O]^+$, 185 (40), 159 (50), 145 (53), 135 (33), 91 (59). NMR, see Table 1.

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