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QUADRANGOLIDE, A HELIANGOLIDE FROM *EUPATORIUM QUADRANGULARAE*

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Key Word Index—*Eupatorium quadrangularae*; Compositae; leafcutter ants; *Atta cephalotes*; feeding deterrent; ant-repellent; heliangolide; terpenoids.

Abstract—A new heliangolide, quadrangolide, was isolated from the chloroform extract of *Eupatorium quadrangularae*, together with three known sesquiterpene lactones.

INTRODUCTION

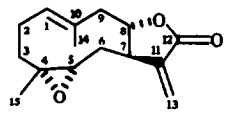
The leafcutter ants (Formicidae, Attini) are polyphagous herbivores which inhabit the American tropics [1]. As a result of their abundance and special fondness for agriculturally important plants, they have been classified as important pests throughout Latin America and in some parts of the southern United States. However, studies of the ants' behaviour in their natural habitat have revealed that they seldom or never attack many of the plant species available to them [2]. Utilizing a laboratory bioassay that monitors ant choices among an array of treated and control food flakes [3], we have been pursuing the isolation and characterization of chemical repellents and feeding deterrents from various tropical plant species [4–9]. We report herein on the isolation of a new heliangolide from *Eupatorium quadrangularae*.

RESULTS AND DISCUSSION

Eupatorium quadrangularae [10] (also known as *Critonia quadrangularae* [11]) is a composite plant described as being a huge herb with a flanged square stem in cross-section. The chloroform extract of air-dried leaves showed activity in our laboratory assay [3] using a captive colony of *Atta cephalotes*, and so it was divided by partitioning between hexane and methanol–water (1:1). Both of the resulting fractions showed biological activity, but the activity was strongest in the more polar material. Standard column chromatography (methanol–dichloromethane gradient) of the crude methanol–water soluble gum (1.8 g) resulted in the isolation of five compounds from the least polar fractions [7]. A sixth compound, 1, was obtained in small quantity (16 mg) from a. later fraction.

Compound 1 crystallized as prisms from methanol (mp 118–120°). High-resolution mass spectrometry revealed a molecular ion at *m/z* 248.1406, corresponding to a formula of $C_{15}H_{20}O_3$. The IR and NMR spectra contained signals characteristic of an α-methylene-γ-lactone (1760, 1660 cm^{-1}; δ5.60, 6.34 each *d*, *J* = 2.6 and 3.0 Hz, respectively) and suggested the presence of a second olefinic group (1620 cm^{-1}; δ5.45, *br t*). Furthermore, signals characteristic of protons geminal to a lactone oxygen (δ4.43, *dd br*) and to an epoxide (2.52, *dd*, *J* = 4.1, 10.1 Hz) were apparent. The presence of these functional groups was further substantiated by the data obtained from the broad-band and delayed decoupling ^{13}C NMR experiments. Finally, extensive ^1H homonuclear decoupling experiments established several extended spin systems (Table 1). The summation of these data can be best accommodated by the gross structure of a germacranolide derivative.

The results obtained from differential NOE experiments (Table 1), with the aid of Dreiding models, confirm the position of the epoxide and allow a clear assignment of the relative stereochemistry of this compound. Irradiation at the H-5 frequency enhanced the H-8 signal as well as the C-15 methyl signal, indicating the proximity of these groups on one face of the molecule. Furthermore, irradiation at the resonance of the olefinic proton H-1 significantly enhanced the H-7 signal, suggesting that these groups are positioned on the opposite face of the molecule from the H-5/H-8/C-15 set. These observations, when confirmed by irradiation–observation experiments



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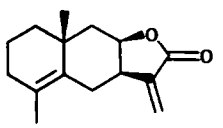

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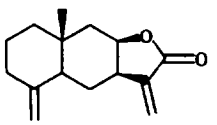

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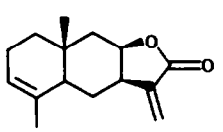

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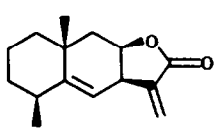

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Table 1. ^{1}H NMR assignments and DNOE data for compound 1

H	δ	Multiplicity	J (Hz)	Coupled to H	NOE with H (%)
1	5.45	*t(br)*		2	7 (7.8%)
2	2.41	*m*			
3	1.24	*m*			
5β	2.52	*dd*	4.1, 10.1	6α, 6β	8 (10.4%) 15 (9.3%)
6α	2.24	*m*		5, 6β, 7	
6β	1.65	*ddd*	4.1, 10.1, 10.6	5, 6α, 7	
7α	2.88	*m*		6α, 6β, 8, 13	1 (4.7%)
8β	4.43	*dd (br)*	4.3, 6.8, 7.6	7, 9	5 (7.0%)
9α	2.76	*d (br)*	11.1	8, 9	
9β	2.24	*m*			
13	6.34	*d*	3.0	7	
13'	5.60	*d*	2.6	7	
14	1.73	*s*			
15	1.30	*s*			5 (8.6%)

Table 2. Ant-repellency bioassay data

Compound	Conc.* (mg/ml)	No. of flakes taken: Control	Test	Probability
1	6.0	33	1	≤ 0.001
2 (A)	0.6	31	17	≤ 0.05
4 (B)	0.3	28	5	≤ 0.001
3 (C)	0.2	30	14	≤ 0.01

*A concentration of 1.0 mg/ml corresponds to an approximate final concentration of 20 μg/flake.

done in the inverse direction (Table 1), lead to the relative stereochemistry as assigned in structure 1.

While diastereomers of this structure have been reported [12–15], compound 1 itself is apparently a new compound. Among known heliangolides, compound 1 is unusual for its low degree of oxygen substitution [16]. In addition, this compound is a potent feeding deterrent as indicated by the assay results against our captive colony of *A. cephalotes* (see Biological Assays later).

Although less biologically active, the hexane fraction was also examined in the belief that it might be a source of other, even less oxidized heliangolides. The crude, hexane-soluble gum (69 g) was chromatographed over silica gel using a solvent gradient (ethyl acetate–hexane, methanol–ethyl acetate and finally methanol), and activity was found in the middle fractions. Further purification by flash chromatography and argentation radial chromatography eventually gave three pure compounds, A (32 mg), B (126 mg) and C (137 mg). Compound A was identified as 4-desoxy-8-epi-ivangustin (2) while compound C was identified as isoalantolactone (3) by comparisons with literature data [12].

Compound B was first obtained as a mixture with compound C, and the two compounds were difficult to resolve by normal phase chromatography. However, they were finally resolved by radial chromatography using silica gel impregnated with 10% silver nitrate. Compound B was identified as diplophyllolide A (4) by comparison with literature data [17]. With this pure material now in hand, the previous structural assignment of compound 5 from this plant [7] should be revised to 4.

Biological assays

The laboratory bioassay [3] consists of a forced choice test between pressed rye flakes treated with a solution of the test chemical and control flakes treated with the solvent alone. Our results are summarized in Table 2. All four compounds show significant activity, but the mechanism(s) responsible for this activity is (are) not yet clear.

EXPERIMENTAL

General procedures. Mps were obtained on a Thomas-Hoover melting point apparatus and are uncorr. Radial chromatography was conducted on a model 7924T Chromatotron (Harrison Research). The NMR spectra were recorded on a JEOL FX-90Q or Bruker WM 360 spectrometer. For the ^{1}H NMR spectra, $CDCl_3$ was used as solvent and chemical shifts are reported in ppm downfield from TMS as internal standard. For the ^{13}C NMR spectra, chemical shifts are reported in ppm downfield from TMS with $CDCl_3$ serving both as solvent and internal standard (77.0). The low-resolution mass spectra were recorded on a Hewlett–Packard 5985B GC/MS instrument at an ionization potential of 70 eV. High-resolution mass spectra were recorded on an AEI MS-902 instrument at Cornell University, Mass Spectrometry Laboratories.

Isolation procedures. Eupatorium quadrangularae was collected in Santa Rosa National Park, Costa Rica in July 1981 and preserved by air-drying. A 1 kg sample (dry wt) was ground and extracted exhaustively with 2 l. $CHCl_3$. After evaporation of the solvent, the resulting crude gum was further partitioned between hexane and 50% aq. MeOH, and bioassay of the two fractions revealed both to be active. The aq. MeOH partition (1.8 g) was fractionated by means of silica gel CC (20 g; CH_2Cl_2–MeOH gradient). Activity was found in one early band and one more polar band. The isolation of five sesquiterpene lactones from the early fraction has been described [7]; compound 1 (16 mg) crystallized, upon standing, from the later fraction.

The hexane partition (69 g) was subjected to CC over silica gel (600 g; EtOAc–hexane, followed by MeOH in EtOAc gradients). Twenty-three fractions were taken, each was assayed, and activity was found in the fractions of intermediate polarity. Fraction 9 was subjected to flash CC and then to radial chromatography on 10% $AgNO_3$-impregnated silica gel (eluting with first 10% EtOAc–hexane followed by 25% EtOAc–hexane) to give the active compound A (32 mg). Fraction 11 was subjected to flash chromatography (MeOH–$CHCl_3$–hexane, 3:97:100), and the only active subfraction (445 mg) was finally purified by radial chromatography on 10% $AgNO_3$-impregnated silica gel (15% EtOAc–hexane followed by 50% EtOAc–hexane) to give compounds B (126 mg) and C (137 mg).

Compound 1. $[\alpha]_D^{27}$ +227°; ^{1}H NMR: see Table 1; ^{13}C NMR: δ15.4 (*q*), 18.5 (*q*), 22.6 (*t*), 34.6 (*t*), 36.4 (*t*), 40.9 (*d*), 41.0 (*t*), 59.8 (*s*), 61.7 (*d*), 80.2 (*d*), 123.2 (*t*), 126.0 (*d*), 131.1 (*s*), 138.7 (*s*), 169.3 (*s*); EI GC/MS *m/z* (rel. int.): 248 (3), 233 (5), 230 (13), 215 (13), 190 (56), 179 (25), 163 (35), 145 (38), 135 (35), 119 (39), 109 (48), 91 (70), 79 (56), 67 (100), 53 (94), 43 (86); HRMS: Found, 248.1406; calc. for $C_{15}H_{20}O_3$: 248.1412.

Compound A (2) gave ^{1}H NMR and mass spectra identical to those previously reported [7]. The ^{13}C NMR spectrum for A should be corrected to: 18.71 (*t*), 19.15 (*q*), 26.77 (*q*), 27.61 (*t*), 31.86 (*t*), 33.46 (*s*), 37.06 (*t*), 41.01 (*d*), 42.48 (*t*), 76.36 (*d*), 121.2 (*t*), 127.1 (*s*), 131.2 (*s*), 140.3 (*s*), 171.0 (*s*).

Compound C (3) gave ^{1}H NMR, ^{13}C NMR and mass spectral data identical to those previously reported.

Compound B (*diplophyllolide A*, 4). $[\alpha]_D^{27}$ +18.6°; ^{1}H NMR agreed with literature data [17]. ^{13}C NMR: δ17.17 (*q*), 21.04 (*q*), 22.13 (*t*), 27.46 (*t*), 30.85 (*s*), 37.84 (*t*), 40.93 (*t*), 41.18 (*d*), 43.86 (*d*), 76.93 (*d*), 120.10 (*t*), 122.2 (*d*), 132.9 (*s*), 142.0 (*s*), 171.0 (*s*); EI GC/MS *m/z* (rel. int.): 232 (37), 217 (100), 199 (9), 187 (7), 171 (66), 145 (36), 131 (47), 117 (25), 105 (47), 91 (88), 79 (53), 65 (20), 53 (28), 41 (12).

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