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ANT-REPELLENT TERPENOIDS FROM *MELAMPODIUM DIVARICATUM*

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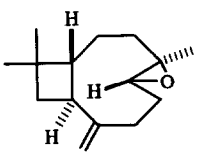
Key Word Index—*Melampodium divaricatum*; Compositae; leafcutter ants; *Atta cephalotes*; ant-repellent; caryophyllene oxide; terpenoids.

Abstract—The leaves of *Melampodium divaricatum* have been systematically fractionated by following biological activity in an assay which measures repellency to the leafcutter ant *Atta cephalotes* (Formicidae, Attini). Several terpenoids have been isolated which show significant ant-repellency.

INTRODUCTION

The leafcutter ants (Formicidae, Attini) are polyphagous herbivores which are considered pests in most of Latin America [1]. Nonetheless, they seldom or never attack many of the native plant species available to them, instead having a special fondness for agriculturally important species [2]. Guided by a bioassay that monitors ant choices among an array of treated and control food flakes [3], we have been pursuing the isolation of ant-repellent compounds from tropical plants. Recently we have reported the isolation of a number of ant-repellent terpenoids from several Costa Rican plant species that escape leafcutter ant attack [4–7]. The present study is concerned with the repellent components of a Panamanian plant species, *M. divaricatum*. We herein report the isolation of four potent ant-repellents from this species.



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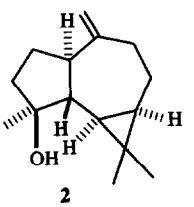

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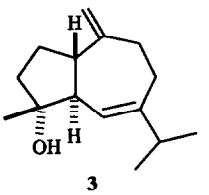

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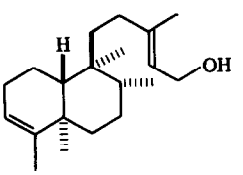

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RESULTS AND DISCUSSION

The chloroform extract of air-dried stems and leaves of *M. divaricatum* shows remarkable ant-repellent activity against our laboratory colonies of *A. cephalotes*. In fact, this extract is significantly active at a concentration (30 μg/flake, $p \leqslant 0.01$) far below the activity of our other crude plant extracts [4–7]. The chloroform extract was fractionated by column chromatography on silica gel, eluting with mixtures of ethyl acetate in hexane followed by methyl alcohol in ethyl acetate. Ant-repellent activity was found in the early, less polar fractions. Further purification of these fractions (by preparative TLC, column chromatography, or Kugelrohr distillation) gave the active compounds **1**–**4**.

The active component of the least polar fraction, isolated as a pure oil, contained in its mass spectrum M^+ at *m/z* 220, suggesting a formula of $C_{15}H_{24}O$. The ^1H NMR spectrum revealed the presence of a terminal olefinic group and a tertiary methyl bonded to an oxygen-bearing carbon atom. Examination of the ^{13}C NMR data showed signals at 63.7 and 59.7 ppm suggesting an epoxide linkage for the single oxygen in the compound. Comparison of the ^1H and ^{13}C NMR spectral data with that recorded for caryophyllene oxide, which we had isolated from *Hymenea coubaril* [8], revealed the two compounds to be identical.

Kugelrohr distillation of the second active fraction gave compound **2** as a colorless oil (*m/z* 220, suggesting $C_{15}H_{24}O$). The ^1H NMR suggested the presence of a terminal olefin and a tertiary methyl bonded to an oxygen-bearing carbon. Also evident in the proton spectrum were two 1H *ds* at 0.45 ppm indicating the presence of a cyclopropane ring. Comparison of the MS, ^1H and ^{13}C NMR data obtained for compound **2** with those reported for spathulenol [9–11] indicated the two were identical.

The third active compound, isolated as a yellow oil, also showed a molecular ion at *m/z* 220, again consistent with a $C_{15}H_{24}O$ formula. The ^1H NMR spectrum was similar to that of compound **2**, again showing a tertiary methyl bonded to an oxygen-bearing carbon and a terminal methylene. However, an additional vinylic proton was clearly visible and the presence of an isopropyl moiety (δ0.99 and 1.00, each 3H, *d*, $J = 7$ Hz) was apparent. This suggested to us a compound similar to spathulenol, but bearing an isopropyl group instead of the cyclopropane ring. Direct comparison with an authentic sample of the guaianol **3**, obtained from the soft coral *Nephthea chabrolii* [12], revealed the two compounds to be identical.

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The most polar column fraction yielded compound **4**, which was isolated as a yellow oil. Its mass spectrum contained M^+ at *m/z* 290, suggesting a molecular formula of $C_{20}H_{34}O$, and a signal at 272 corresponding to loss of water from the parent ion. Also noted in the MS was an intense signal at *m/z* 189 which suggested the presence of an alkyl side chain. The ^1H NMR spectrum contains signals for five methyl groups, two of which are vinylic, and two vinylic protons. The broadband decoupled ^{13}C NMR spectrum revealed the presence of a hydroxyl function, while a delayed-decoupling experiment established that both double bonds were trisubstituted. To accommodate these NMR data and the molecular formula, the molecule must be bicylic. Direct comparison with an authentic sample of kolavenol, isolated from *Hardwickia pinnata* [13, 14], proved the two compounds were identical.

These four compounds are reported here for the first time from *Melampodium*. In addition, as suggested by our bioassay data, these compounds may have considerable value as defensive agents against leafcutter ant attack (Table 1).

Biological assays

The laboratory bioassay technique, described in detail elsewhere [3], consists of a forced choice test between pressed rye flakes treated with a solution of a potential repellent and control flakes treated with the solvent alone. Our results are summarized in Table 1 below. All four compounds show significant activity. The mechanism(s) responsible for the ant-repellency of these compounds are not known. However, the leafcutter ants exist in a mutualistic association with a specific fungus, which is cultured on the collected leaves to serve as their major food [1]. In preliminary investigations, spathulenol (**2**) has shown activity as an antifungal agent. It is possible that these ants discriminate against this compound because of its antifungal properties. We plan to further explore this possibility in our continuing studies.

EXPERIMENTAL

The ^1H and ^{13}C NMR were recorded using $CDCl_3$ as the solvent and TMS as an internal standard. Mass spectra (70 eV) were recorded with a Hewlett–Packard 5985B instrument.

Table 1. Ant-repellency bioassay data for compounds **1**–**4**

Compound	Conc.* (mg/ml)	No. of flakes taken: Control	Test	Probability
1	3.0	31	6	$\leqslant 0.001$
2	4.0	31	4	$\leqslant 0.001$
3	2.0	32	12	$\leqslant 0.005$
4	5.5	28	10	$\leqslant 0.005$

*A concentration of 1.0 mg compound/1 ml corresponds to an approximate final concentration of 20 μg/flake.

Isolation procedure. Air dried stems and leaves of *M. divaricatum* (1 kg, collected near Barro Colorado Island, Panama in May, 1982) were extracted first with $CHCl_3$ and subsequently with EtOH in a Soxhlet. Concentration of the extracts *in vacuo*, followed by bioassay of the resulting gum, revealed the ant-repellent activity to be in the $CHCl_3$ extract.

Column chromatography of the crude $CHCl_3$ gum (16 g) on silica gel (220 g, EtOAc–hexane, MeOH–EtOAc gradients) gave several active fractions (numbered 5, 6, 7 and 8; eluting at 1–8% EtOAc–hexane). Fraction 5 was separated by prep. TLC (silica gel, 0.25% *i*-PrOH–$CHCl_3$, 2 devel.) which gave as the active component compound **1** (25 mg). Fraction 6 was purified by Kugelrohr distillation to give a colorless distillate of compound **2** [35 mg; $[\alpha]_D^{27} + 4.6$ ($CHCl_3$)]. Fraction 7 was purified by preparative TLC (silica gel, 3% *i*-PrOH–$CHCl_3$, 3 devel.) to give compound **3** (40 mg). Fraction 8 was first washed with aqueous base, to remove a fatty acid, and both the base soluble and neutral materials were bioassayed. The ant-repellent activity was found to lie in the neutral portion and column chromatography of this material afforded 57.2 mg of **4**.

Compound **4**, *kolavenol.* ^{13}C NMR 15.98 (*q*), 16.53 (*q*), 17.97 (*q*), 18.32 (*t*), 18.37 (*q*), 19.98 (*q*), 26.91 (*t*), 27.57 (*t*), 32.87 (*t*), 36.32 (*d*), 36.81 (*t*), 36.90 (*t*), 38.22 (*s*), 38.64 (*s*), 46.49 (*d*), 59.48 (*t*), 120.4 (*d*), 122.9 (*d*), 140.9 (*s*), 144.5 (*s*). MS *m/z* (rel. int.): 290 (M^+, 8), 272 $[M - H_2O]^+$ (3), 257 (10), 189 (89), 175 (21), 161 (14), 145 (17), 133 (27), 120 (76), 107 (80), 95 (100), 79 (41), 67 (31), 55 (37).

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