

## HELMINTHOGERMACRENE, A MAJOR COMPONENT IN THE DEFENSIVE SECRETION OF THE NEARCTIC TERMITE, *AMITERMES WHEELERI*

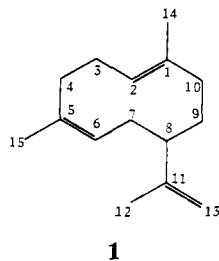
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The chemistry of soldier cephalic secretions in the cosmopolitan genus *Amitermes* (Isoptera; Termitidae) has been examined in at least ten species (1,2). Some of these terpene-derived exudates act as alarm pheromones (3); others are released during defensive encounters as close-range predator repellents (2,4). The soldier secretion from Wheeler's desert termite, *Amitermes wheeleri* Desneux, was reported to consist of a mixture of three bisabolene hydrocarbons, of which one component was given the preliminary structural assignment of the  $\alpha_2$  isomer based on circumstantial data (4). Upon spectrometric comparison with synthetic  $\alpha_2$  bisabolene (5), we determined this component was not  $\alpha_2$  bisabolene but a germacrene isomer.

Attention was again focused on the nature of the *A. wheeleri* defensive secretion when it was discovered that its chemical composition was extremely variable among termite populations in the southwestern U.S. (6). Although it was verified that the original California population contained only the three sesquiterpenes, one Arizona colony produced a mixture of six sesquiterpenoids of which only the unspecified germacrene isomer was common to both groups. The predominance of the germacrene in six allopatric *A. wheeleri* populations and its absence in other *Amitermes* species rendered chemotaxonomic utility to this compound as a species marker (6). Furthermore, glc examination of secretions

liberated from individual soldiers collected from a single colony revealed two chemically distinct, and possibly sex-linked, soldier types occurring in equal proportion. This chemical dimorphism was most pronounced for the germacrene compound which constituted 3% of the total secretion in A-type soldiers and 92% in B-type soldiers (Scheffrahn, unpublished). We now report the structure of this pivotal termite product as the recently elucidated germacrene isomer (8), (-) helminthogermacrene (**1**).



Glc of a crude soldier head extract revealed a major component (50%) having identical retention time to the previously isolated " $\alpha_2$  bisabolene" (4). The compound was isolated by preparative glc to yield ca. 2 mg of pure oil. The room temperature <sup>1</sup>H-nmr trace (4) showed broad, poorly resolved absorbance bands, unlike those observed among known bisabolene isomers (4,5). We, therefore, suspected a monocarbocyclic structure which was undergoing conformational rotation. At probe temperatures below -20°, the <sup>1</sup>H-nmr spectrum was sharp, and coupling constants could be precisely measured.

Of the known germacrene hydrocarbons (7,8), only two isomers fit the structural constraints imposed by the

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spectral data. Germacrene A was ruled out due to  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr trace dissimilarities (9) and thermal liability via Cope rearrangement (10, 11). (-) Helminthogermacrene (**1**), isolated and characterized from the hydrocarbon fraction of the plant pathogenic fungus, *Helminthosporium sativum* Pam., King & Bakke (8), yielded matching ambient temperature  $^1\text{H}$ -nmr (Arigoni, personal communication), ms, and optical rotation data (4) with the termite compound. More recently, McMurry and Kočovský (12) used a titanium induced dicarbonyl coupling reaction to synthesize ( $\pm$ ) helminthogermacrene. Unpublished  $^{13}\text{C}$ -nmr data (McMurry personal communication) were identical ( $<1$  ppm) to those for (-) helminthogermacrene isolated in this study as well as those previously reported (4) and are given in Table 1. The ambient temperature 300 MHz  $^1\text{H}$ -nmr trace (McMurry, personal communication) was also identical. The report that **1** is thermally stable (12), and our finding that the termite

product is not converted during glc analysis (4,6), further confirms structural congruency between these two compounds.

## EXPERIMENTAL

*A. wheeleri* soldiers ( $n=130$ ) were collected 28 July 1984, from a single colony in Peña Blanca Recreation Area, 18 km WNW of Nogales, Arizona. Soldiers were identified from morphological description (13) and deposited in the collection of RHS. Live detached heads were extracted in 1 ml  $\text{CS}_2$  and stored at  $-20^\circ$ . The extract was concentrated, and **1** collected by preparative glc (isothermal  $150^\circ$ ) from a 2 mm  $\times$  6 mm ID column packed with 10% Silar 10C on Chromosorb W. A  $^1\text{H}$ -nmr spectrum was obtained in  $\text{CDCl}_3$  with a Nicolet NMR-1280 300 MHz instrument at a probe temperature of  $-20^\circ$  to yield the following data:  $\delta$  1.36 (1H, m), 1.46 (1H, m), 1.62 (2H, m), 1.66 (3H, s, Me-12), 1.70 (6H, s, Me-14 and Me-15), 1.74 (2H, m), 1.96 (2H, m), 2.17 (1H, td,  $J=14.9, 4.3$  Hz), 2.30 (1H, m,  $J=5.2$  ave.), 2.45 (1H, dt,  $J=15.6, 5.9$ ), 4.58 (1H, s, H-13), 4.66 (1H, d,  $J=2.8, \text{H-13}$ ), 5.21 (1H, d,  $J=12.2, \text{H-1 or H-5}$ ), 5.33 (1H, t,  $J=9.5, \text{H-1 or H-5}$ ). A broadband decoupled  $^{13}\text{C}$ -nmr spectrum ( $\text{CDCl}_3$ ) was obtained at 75 MHz on the same instrument (Table 1). Gc/ms of the crude extract was run at

TABLE 1.  $^{13}\text{C}$ -nmr Shifts (ppm) Obtained for Helminthogermacrene (**1**)

Carbon No. Assignment	From <i>Amitermes wheeleri</i>		Synthetic <sup>b</sup>
	Arizona	California <sup>a</sup>	
14	16.15	16.05	16.20
12	19.79	20.17	20.29
15	24.09	23.97	24.10
3	25.27	25.09	25.27
7	— <sup>c</sup>	(23.97) <sup>d</sup>	29.71
9	30.44	30.34	30.49
4	32.33	32.00	32.14
10	40.25	39.76	40.04
8	48.44	48.10	48.28
13	108.71	108.95	108.74
2	124.19	124.20	124.25
6	126.09	125.92	125.90
1	131.96	131.22	131.95
5	— <sup>c</sup>	134.32	135.07
11	150.57	149.57	150.57

<sup>a</sup>Scheffrahn *et al.* (4). Run in acetone- $d_6$ .

<sup>b</sup>McMurry, personal communication.

<sup>c</sup>Weak resonance indistinguishable from background due to sample quantity.

<sup>d</sup>Originally judged to be two coincidental resonances due to strong signal. Disregard.

70 eV on a VG-ZAB-2F ms connected to a Supelcowax 10 column (30 m, SCOT). The peak corresponding to **1** produced the following fragmentation: *m/z* (% rel. int.), 204 (21), 189 (33), 175 (7), 162 (9), 161 (32), 148 (17), 147 (60), 135 (15), 134 (12), 133 (25), 122 (12), 121 (46), 120 (11), 119 (30), 109 (16), 108 (32), 107 (58), 106 (12), 105 (37), 95 (29), 94 (31), 93 (92), 92 (14), 91 (30), 81 (57), 80 (13), 79 (42), 77 (19), 69 (18), 68 (100), 67 (55), 65 (7), 55 (26), 53 (27), 43 (8), 41 (33), 40 (8).

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