

SESQUITERPENES FROM TERMITE SOLDIERS. STRUCTURE OF AMITEOL,  
A NEW 5 $\beta$ , 7 $\beta$ , 10 $\beta$ -EUDESMAE FROM AMITERMES EXCELLENS

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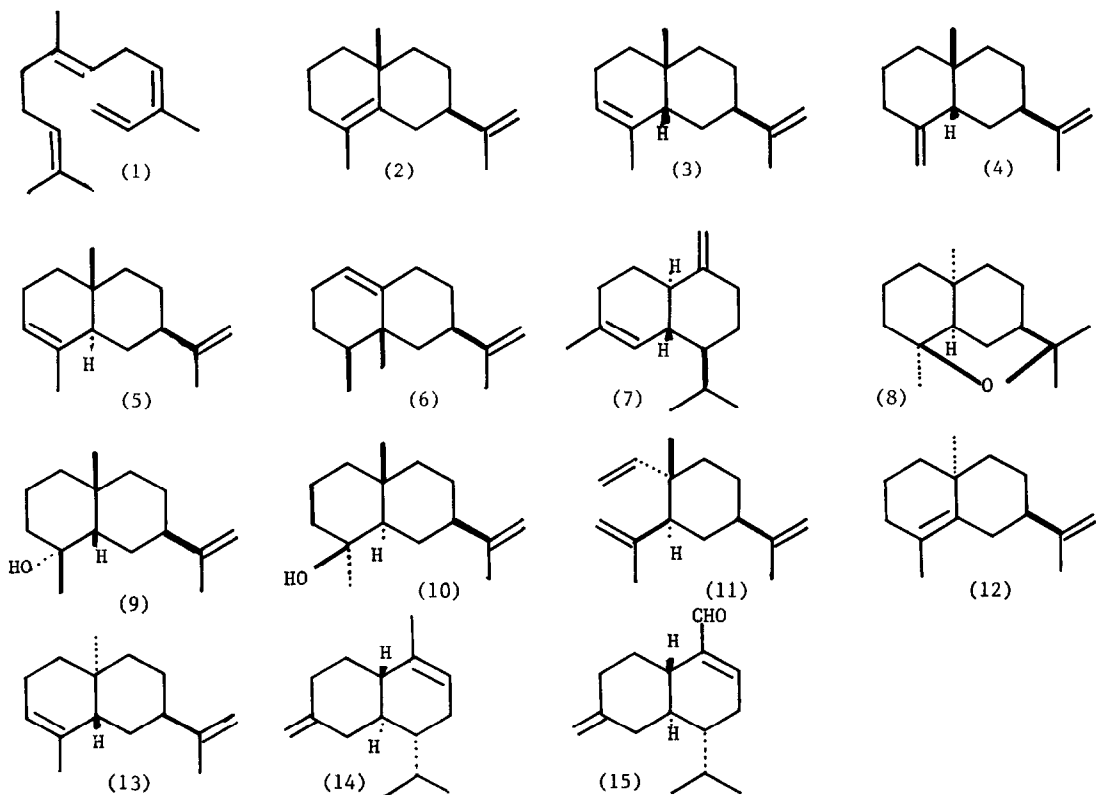
SUMMARY. The defense secretions of termite soldiers in the genera Amitermes, Noditermes, and Prorhinotermes contain sesquiterpene hydrocarbons, alcohols and ethers. Three new eudesmanes possessing the hitherto unknown 5 $\beta$ , 7 $\beta$ , 10 $\beta$  configuration were identified from Amitermes excellens.

Termite soldiers have provided a rich harvest of novel terpenes<sup>1,2</sup> which are employed as antihealants, repellents, glues, and irritants for the defense of the colony against predators. We now report the identification of bicyclic sesquiterpenoid hydrocarbons, ethers, and alcohols from two higher termites (Termitidae, Termitinae), Amitermes excellens and Noditermes wasambaricus, and of  $Z, E$ - $\alpha$ -farnesene (1) from a lower termite (Rhinotermitidae) Prorhinotermes simplex. We also report the discovery of a "missing" configurational isomer of the eudesmane skeleton, and describe three new members of the 5 $\beta$ , 7 $\beta$ , 10 $\beta$ -eudesmane class.

Amitermes excellens.<sup>3</sup> The crude secretion (25 mg) was chromatographed on Florisil (10% ethyl acetate-hexane) to give four major fractions: A, C<sub>15</sub> hydrocarbons, B, C<sub>15</sub> cyclic ether, C and D, C<sub>15</sub> alcohols. Alcohol D was readily identified<sup>5</sup> (GC, GC/MS, FT-IR, NMR) as neointermediol (10), previously identified from Subulitermes baileyi cephalic secretion.<sup>4</sup> Prep GLC<sup>5</sup> of the major fraction C led to the isolation of a new sesquiterpene alcohol, amiteol (9) with the following spectral properties: GC/MS,  $m/z$  (rel. int.) 222 (4), 204 (44), 189 (22), 161 (22), 149 (30), 121 (27), 109 (100), 108 (84), 97 (34), 81 (36), 55 (30), 43 (46), 41 (32); IR, 3<sup>o</sup> hydroxyl absorption; PMR (CDCl<sub>3</sub>): 0.97 (3H, s), 1.25 (3H, s), 1.75 (3H, bs), 4.68, 4.71 (1H, each bs). These data indicated presence of an isopropenyl group, a tert.-methyl, and a

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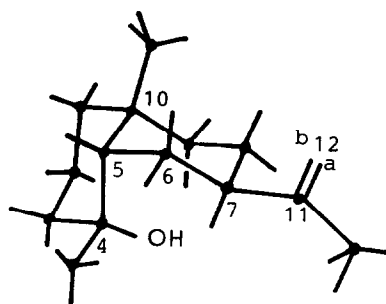
methyl carbinol group attached to an eudesmane skeleton. This structure was confirmed by micro-scale dehydration ( $\text{SOCl}_2$ , ether) to afford three hydrocarbons (2:5:1 ratio), which were shown to be identical to the three natural hydrocarbons ( $\lambda_2, \lambda_3, \lambda_4$ ) in the hydrocarbon fraction A, on the basis of comparative GC, combined GC/MS and direct comparison of spectroscopic data. In addition, the hydrocarbon  $\lambda_2$  coincided with authentic (+)-selina(4,5), 11-diene ( $\alpha$ -cyperene),  $[\alpha]_D^{24} + 30^\circ$  ( $\text{CHCl}_3$ ) in all respects. Amiteol  $\lambda_2$ ,  $[\alpha]_{365}^{24} + 8^\circ$  ( $\text{CHCl}_3$ ), thus has a 7 $\beta$ , 10 $\beta$ -eudesmane skeleton. Since hydrocarbon  $\lambda_3$  was not identical with  $\alpha$ -selinene having a 5 $\alpha$ -configuration, it has to be 5 $\beta$ , 7 $\beta$ , 10 $\beta$ -eudesma-3, 11-diene as supported by its PMR [0.99(3H, s), 1.66, 1.73 (3H, bs), 4.69(2H, bs), and 5.43(1H, bs)]. Hydrocarbon  $\lambda_4$  was a related dehydro product which exhibited MS fragmentation similar to that of  $\lambda_3$ , but insufficient quantities were available for complete characterization.



The detailed 360 MHz  $^1\text{H-NMR}$  analysis of  $\lambda_2$  (Table 1) reveals that the hydroxyl of amiteol  $\lambda_2$  is  $\alpha$ (R). The predominant conformation is illustrated in  $\lambda_2^a$  in which the deshielding of proton 9-H $\alpha$  (2.12) results from the anisotropy of the hydroxyl group. The methyl group at C-4

Table 1  $^1\text{H-NMR}$  Assignment for Amiteol (9) (360 M Hz, in  $\text{CDCl}_3$ )

Position	Configuration	$\delta$	J
4-Me		1.25	NOE 4.58% (6 $\alpha$ )
5	$\beta$	1.16	ddd 6,2,1
6	$\alpha$	1.89	ddd 14,4,2
	$\beta$	1.63	ddd 14,14,6
7	$\alpha$	2.67	dddd 14,14,4,4,1
8	$\alpha$	1.6-	
	$\beta$	1.48	ddd 14,14,14,4
9	$\alpha$	2.12	ddd 14,14,4
	$\beta$	0.99	ddd 14,4,4,1
10-Me		0.98	
11-Me		1.75	d 1
12a		4.68	ddq 2,1,1
12b		4.71	ddq 2,1,1



(9a)

exhibited NOE (4.85%) with 6-H $\alpha$  and W-type coupling between 5-H $\beta$  and 9-H $\beta$  (both eq in the B-ring) was observed.

Additional constituents in the hydrocarbon fraction A were demonstrated to be  $\alpha$ -selinene  $\xi$ , eremophyllene  $\zeta$  and  $\gamma$ -cadinene  $\eta$  on the basis of MS/GC and FT-IR; three additional unidentified sesquiterpene hydrocarbons have also been obtained as very minor constituents. A significant amount of sesquiterpene ether  $\theta$  was obtained pure as fraction B. Spectral data (GC/MS,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , IR) supported structure  $\theta$  for this ether; furthermore, it was identical to authentic samples isolated from *A. evuncifer*<sup>6-8</sup> and *A. messinae*.<sup>1</sup> It is noteworthy that the eudesmanes produced by these termites are products resulting from cyclization of all possible conformations of the biogenetic precursor, *i.e.*, the germacrenium cation, and that they include the last remaining configurational isomer, 5 $\beta$ , 7 $\beta$ , 10 $\beta$ -eudesmane.

*Noditermes wasambaricus*.<sup>9</sup> The crude soldier cephalic secretion was chromatographed on Florisil to give a mixture of three sesquiterpene hydrocarbons which were isolated by preparative GC and identified as  $\beta$ -elemene  $\lambda\lambda$  (a minor constituent), (-)-7 $\beta$ , 10 $\alpha$ -eudesma-4, 11-diene  $\lambda\lambda$  and (+)-5 $\beta$ , 7 $\beta$ , 10 $\alpha$ -eudesma-3, 11-diene  $\lambda\lambda$  (1:3 ratio) on the basis of PMR, IR, and GC/MS.<sup>10</sup> As in the sympatric termitid *Cubitermes*,<sup>1</sup> we believe these sesquiterpenes to act as oily anti-healants when added to wounds made with the elongated mandibles. Compounds  $\lambda\lambda$  and  $\lambda\lambda$  have previously been isolated from *Syntermes* soldiers (Termitidae, Nasutitermitinae).<sup>11</sup>

*Prorhinotermes simplex*. Chromatography of crude secretion on silica gel with hexane allowed separation of the minor component, a sesquiterpene, from the major component (>90%)

1-nitro-1-(E)-pentadecene<sup>13</sup> which is known to be a contact poison.<sup>14</sup> Further purification of the minor component by preparative GC<sup>5</sup> has now allowed identification of ( $\zeta,\epsilon$ )- $\alpha$ -farnesene  $\mu$  based upon the GC retention time, GC/MS, PMR and FT-IR. Only one other lower termite (Reti- culitermes flavipes, another rhinotermitid) has been shown to possess sesquiterpenes,<sup>15</sup>  $\gamma$ -1-cadinene  $\mu$  and  $\gamma$ -1-cadinene aldehyde  $\mu$ . These authors found no evidence for defensive or alarm functions of these chemicals in encounters with ants. Likewise, although the defensive value of the nitroalkene is understood,<sup>14</sup> no evidence is available for the function of the farnesene in vivo.

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#### References and Notes

1. G.D. Prestwich, J. Chem. Ecol. 1979, 5, 459-470; G.D. Prestwich, Sci. American, in press (1982).
2. G.D. Prestwich, Tetrahedron 1982, 38, Symposium in Print, "Organic Chemistry of Animal Chemical Defense", in press.
3. Amitermes excellens colonies were obtained from conspicuous chevron-like colonies on tree trunks at Makuri and at Kartabo Point, Guyana. Soldiers were collected individually, cooled, and decapitated and their heads were crushed in hexane. Approximately 25 mg was obtained from 110 individuals.
4. G.D. Prestwich and M.S. Collins, Biochem. System. Ecol. 1981, 9, 83-89.
5. Preparative GLC was performed using 10% Thermon 1000 (4mm x 3m). Analytical GLC and GC/MS were performed on 0.25mm x 50m SCOT column of CPTm-Wax 51.
6. L.J. Wadhams, R. Baker, and P.E. Howse, Tetrahedron Lett. 1974, 1697-1698.
7. R. Baker, D.A. Evans, and P.G. McDowell, Chem. Comm. 1977, 111.
8. R. Baker, D.A. Evans, and P.G. McDowell, Tetrahedron Lett. 1978, 4073-4076.
9. Noditermes wasambaricus colonies were collected from the Makadara Forest, Shimba Hills near Kwale, Kenya. Soldier termites were individually removed from the excavated 0.5m high mounds, cooled to 0 °C on moist filter paper, and decapitated. The heads were crushed under hexane to yield 31 mg of crude secretion from 920 individuals.
10. E. Klein and W. Rojahn, Tetrahedron Lett. 1970, 289-292.
11. R. Baker, H.R. Coles, M. Edwards, D.A. Evans, P.E. Howse, and S. Walmsley, J. Chem. Ecol. 1981, 7, 135-146.
12. Prorhinotermes simplex was obtained from rotten logs near Miami, Florida, by P. Luykx and M. Melcer. Whole soldiers were homogenized in hexane to extract both the frontal gland and the abdominal gland reservoirs. Approximately 8.4 mg of crude secretion was obtained from 36 soldiers.
13. J. Vrkoč and K. Ubik, Tetrahedron Lett. 1974, 1463-1464.
14. S.G. Spanton and G.D. Prestwich, Science 1981, 214, 1363-1365.
15. L.H. Zalkow, R.H. Howard, L.T. Gelbaum, M.M. Gordon, H.M. Deutsch, and M.S. Blum, J. Chem. Ecol. 1981, 717-731.

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