erties of the parent compound, without inducing the interesting dissociation of activities displayed by the testosterone analogues[5,7].

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2 Present address: Farnex Laboratori S.p.A., Codogno, Italy.
3 Present address: Schering Corporation, Lafayette, New Jersey, USA.
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Termite soldier chemotaxonomy. A new diterpene from the Malaysian nasute termite *Bulbitermes singaporensis*[1]

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Summary. The defense secretion of the nasute termite *Bulbitermes singaporensis* consists of 1 new and 2 known tetracyclic diterpenes, spectrometrically identified as 14α-acetoxy-6,8-kempadien-3-one, 3α,14α- and 3β,14α-diacetoxy-6,8-kempadiene. The presence of these compounds supports the kinship of the oriental 'constricted-head' genera with *Nasutitermes* species in the Philippines and in East Africa.

Nasute termite soldiers (Isoptera: Termitidae: Nasutitermitinae) eject an irritating, viscous defense secretion when provoked. Progress has been reported in the elucidation of structures[3] of the mono- and diterpenoid constituents, instances of inter- and intraspecific variation[1,4] and the use of the secretion in defense[1,5]. Recently, we have analyzed defense secretions of nasute genera occupying intermediate phylogenetic positions in the hope of clarifying the evolution of diterpene biosynthesis in this advanced termite subfamily[1,6]. In this paper we describe the diterpenes of an oriental (Malaysian) nasute in the genus *Bulbitermes*, biogeographically and morphologically related to the Oriental-Ethiopian 'constricted-head' genera[7] including *Grallatotermes*[6]. A chemical connection to *Nasutitermes luzonicus*[1,3] (Philippines) and *Nasutitermes kempae*[1,3,8] (East Africa) is thereby established.

The crude defense secretion (15 mg) was obtained by hexane extraction of 1000 soldier heads of *Bulbitermes singaporensis* collected from a single spherical, hard carton arboreal nest in the Lesong Forest Reserve, Pahang, Malaysia. The secretion contained 2.9 µg monoterpene hydrocarbons per soldier (0.5% fresh weight %, which was predominantly α-pinene (89%) and β-pinene (7%) as established by GC-MS. Diterpenes (figure 1) comprised 1.6% fresh weight % of soldiers (10.6 µg/soldier). Chromatography of the crude secretion (Florisil, 10% ethyl acetate-hexane) gave 2 TLC-homogeneous (Polygram Sil UV, R_f 0.20 and 0.27 for 25% ethyl acetate-hexane) materials of 6 mg each. GLC (3% OV-17, 2 mm × 2 m glass column, T_i = 210 °C, T_p = 6 °C min^{-1}, T_f = 270 °C) examination of these materials showed the higher R_f spot (**I**) to be homogeneous; however, the lower spot was a 1:1 mixture of 2 closely-eluting compounds **II** and **III**. Analysis by GC/MS[9] indicated that compound **I** had a parent peak at m/z 342 and a base peak at 282 ($M^+ - HOAc$), and fragmented in an identical manner to kempene-2 (14α-acetoxy-6,8-kempadien-3-one) from *Nasutitermes kempae*[8]. This assignment was confirmed by TLC and GLC coelution and by the identity of the ^{1}H-NMR spectra of these 2 samples[10].

Compounds **II** and **III** gave virtually identical mass spectra which were consistent with that obtained for kempene-1[8]: m/z 386 (1%, M^+), 326 (18%, $M^+ - CH_3CO_2H$), 251 (100%, $M^+ - 2\ CH_3CO_2H - CH_3$). The 2nd peak **III** coeluted with kempene-1; however, the stereochemistry at C-3 had not been assigned in the original paper[8]. We suspected that **II** and **III** were epimeric at 1 of the 2 acetate centers; this was then determined by 2 independent methods as described below.

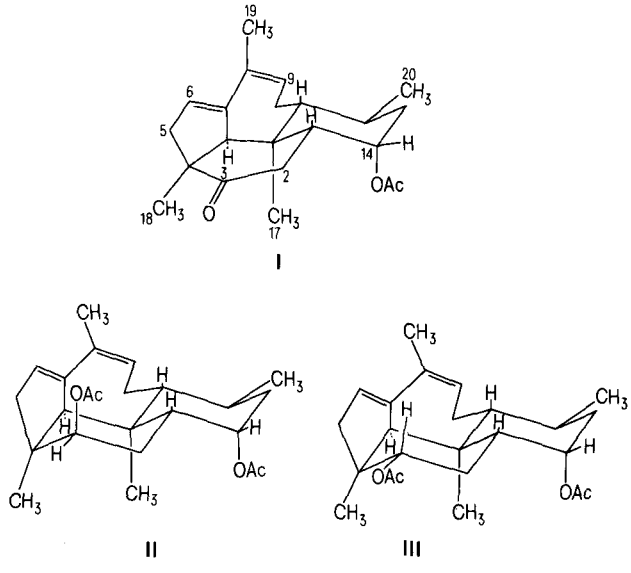

Fig. 1. Stereostructures of diterpenes from *Bulbitermes singaporensis*.

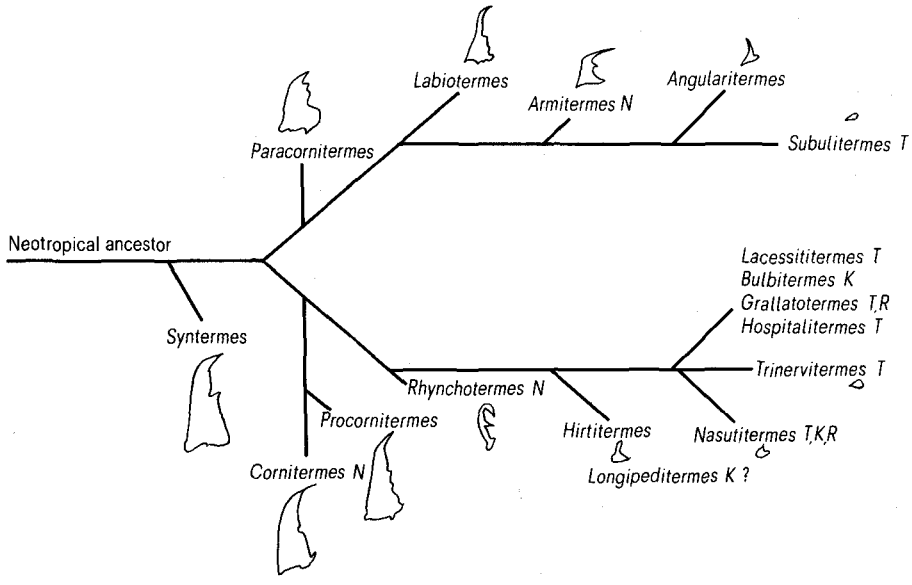

Fig. 2. Parallel diphyletic regressive evolution of soldier mandibles in the Nasutitermitinae[11], showing chemical defense secretion types found. Key: N, no terpenes isolated; T, trinervitanes; K, kempanes; R, rippertanes.

First, **II** and **III** were separated by liquid chromatography using 4 columns (4.5 mm × 25 cm) of μ Porasil in series, and eluting with 1.0 ml/min of 5% ethyl acetate-hexane to achieve baseline resolution as monitored at 254 nm and by GLC of collected fractions. Microcell ^{1}H-NMR[10] of these 2 compounds gave the following data, indicating the identity of **III** with kempene-1 and allowing the assignment of C-3 configuration in both **II** and **III**: **II**, δ 5.70 (br, dd, H-9), 5.65 (br s, H-6), 5.26 (dd, 9 Hz, 8 Hz, H-3α), 4.89 (br ddd, 3 Hz, 3 Hz, 3 Hz, H-14β), 2.72 (br d, 16 Hz, H-5α), 2.06 (s, 3-OAc), 2.04 (s, 14-OAc), 1.81 (d, 1 Hz, H-19), 1.11 (s, H-18), 1.01 (s, H-17), and 0.84 (d, 6 Hz, H-20); **III**, 5.75 (br dd, H-9), 5.60 (br s, H-6), 5.07 (d, 8.8 Hz, H-3β), 4.90 (br ddd, 3 Hz, 3 Hz, 3 Hz, H-14β), 2.45 (br d, 16 Hz, H-5α), 2.08 (s, 3-OAc), 2.04 (s, 14-OAc), 1.81 (d, 1 Hz, H-19), 1.23 (s, H-18), 0.99 (s, H-17), 0.85 (d, 6 Hz, H-20).

The configuration at C-3 was assigned as 3β-acetoxy in **II** and 3α-acetoxy in **III** (kempene-1) on the basis of 3 observations. First, the chemical shift of equatorial H-3α is downfield of axial H-3β. Second, H-3α bisects the angle of H-2α and H-2β to give virtually equal coupling constants, while axial H-3β is nearly orthogonal to H-2β and couples only to H-2α with a reduced axial-axial coupling. Finally, the axial nature of the 3β-acetate is sterically permissible while maintaining the chair conformation for that 6-membered ring. In this configuration, the axial acetate does not deshield the H-18 methyl protons (1.11 ppm in **II**) while the equatorial acetate does affect the shift of that methyl (1.23 ppm in **III**).

To confirm these assignments, a 3-mg sample of crystalline kempene-2 (**I**) from *N. kempae* was reduced with excess $LiAlH_4$ (THF, 25 °C, 1 h) and the resulting diol was acetylated (Ac_2O, Py, 2 days). The diacetates (1:2 ratio of **II**:**III**) thus obtained were identical (GC/MS, TLC) with the naturally-occurring samples of **II** and **III**.

The Nasutitermitinae comprise the largest, most widely distributed, most abundant, and most advanced subfamily of higher termites. The nasutes arose from a neotropical mandibulate ancestor which then underwent reduction of the mandibles with concomitant development of the squirt-gun apparatus[11]. It is believed that this occurred independently along 2 phyletic branches, thus providing a classic example of parallel evolution[7,11] (figure 2). Our investigations into termite chemical evolution indicate that the development of terpenoid compounds lags behind the mandibular regression/nasus elongation[1]; moreover, the occurrence of trinervitanes in *Subulitermes* draws the diphyletic hypothesis into question[13]. In fact, neither *Armitermes* nor *Rhynchotermes* possess diterpenes despite their well-developed elongate nasi. A major evolutionary step was taken between *Rhynchotermes* and *Longipeditermes;* the latter genus has been shown to possess 2 diterpenes not yet found in other nasute genera[14].

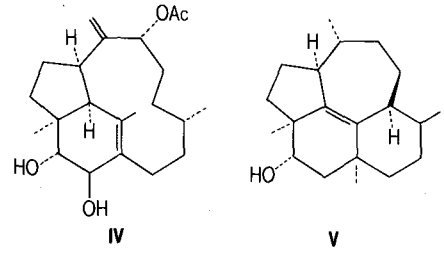

Fig. 3. Trinervitane and rippertane diterpenes from other nasute soldiers.

Sands[7] places *Bulbitermes* in the 'constricted-head' genera which includes the predominantly oriental genera *Grallatotermes, Hospitalitermes,* and *Lacessititermes.* We have shown[6] that the African termite *Grallatotermes africanus* possesses trinervitanes (e.g., **IV**) similar to the African termites *Trinervitermes* spp. and *Nasutitermes infuscatus,* and that *Lacessititermes* and *Hospitalitermes* [3] secretions also contain trinervitanes. *G. africanus* also possesses the methyl-shifted rippertenol **V**[12] first isolated from the neotropical termites *Nasutitermes rippertii* and *N. ephratae* (figure 3). *Bulbitermes* is the first member of the constricted-head genera in which kempanes have been isolated; previously, kempene-1 and -2 were known only from *N. luzonicus* from the Philippines[1,3] and from *N. kempae* from East Africa[1]. It is now clear that the diversity of diterpenes found in the genus *Nasutitermes* also occurs in the somewhat more primitive 'constricted-head' genera. We are confident that continued research into the evolution of diterpene biogenesis will provide results of utility in deducing the phyletic history of these advanced termites.

1 For 2 earlier papers in this series, see G.D. Prestwich, Sociobiol. *4*, 127 (1979); and G.D. Prestwich, Biochem. Syst. Ecol. *7*, 211 (1979).
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Antimicrobial metabolites of the marine sponge *Axinella polycapella*

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Summary. Extracts of the marine sponge *Axinella polycapella* contain 1,2,4-trihydroxybenzene (**1**) and 2,2′,4,4′,5,5′-hexahydroxybiphenyl (**3**) as antimicrobial constituents. Methods of synthesizing **3** by oxidative dimerization of **1** were examined.

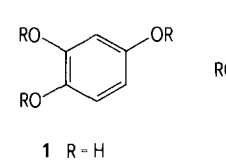
Marine organisms have yielded a number of antibiotics bearing novel functionality[2]. Antimicrobial screening of sponges collected near St. Petersburg, Florida, revealed that extracts of *Axinella polycapella* inhibited *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Enterobacter aerogenes.* Chromatography of the methanol-soluble extract on silica gel provided 2 antimicrobial compounds. The less polar substance (0.1% dry weight) was indistinguishable (TLC, ^{1}H-NMR, IR) from an authentic sample of 1,2,4-trihydroxybenzene (**1**)[3], whose isolation from *A. polypoides*[4] and whose antibiotic properties[5] have been described.

The more polar compound (0.03% dry weight) was obtained in an impure state as a dark purple solid. The molecular formula $C_{12}H_{10}O_6$ was established for this material from its high resolution mass spectrum (M^+ 250.0487, calculated 250.0476). Acetylation yielded a hexaacetate ($\nu_{CH_2Cl_2}$ 1775 cm^{-1}, M^+ 502), suggesting that the antibiotic was a hexahydroxy-biphenyl. Since the ^{1}H-NMR spectrum (d_6 acetone, D_2O) of the natural compound consisted of 2 singlets of equal intensity (δ 6.70 and 6.50) and the ^{13}C-NMR showed 6 signals (δ 146.8, 145.2, 138.9, 117.6, 117.5 and 104.5), the structure was assigned as 2,2′,4,4′,5,5′ hexahydroxybiphenyl (**3**). Quantitative antimicrobial testing of pure **3** is difficult because **3** is air sensitive and decomposes significantly during the assay.

To verify this structural assignment, an authentic sample of **3** was sought. Although many polyhydroxylated biphenyls are known, Forrest et al. reported the only direct synthesis of **3**, via an oxidative dimerization of **1**[6]. Thus, treatment of **1** with 0.5 equivalent of benzoquinone in 10% H_2SO_4 gave a 75% yield of **3** as a light gray solid (m.p. 273–275 °C) which was indistinguishable (TLC, ^{1}H-NMR) from the natural compound. Other experiments showed that aqueous solutions of $FeCl_3$ or $K_3Fe(CN)_6$ also convert **1** into **3**.

A report that trimethyl ether **2** could be dimerized to produce hexamethyl ether **4** using $AlCl_3$ in nitrobenzene[7] led us to examine the reactions of **1** with Lewis acids in several solvents which contain a nitro group. When **1** is heated overnight in nitrobenzene, nitromethane, or 2-nitropropane containing 0.2 equivalents of BF_3 etherate, 50–80% yields of **3** can be obtained from the dark purple product mixtures. The reaction fails if BF_3 etherate is omitted, or if p-dioxane is used as solvent. These results suggest that the nitro groups serve a crucial role in these rather novel reactions. Although nitroso compounds might be the expected by-products of these oxidations, careful examination of the mixture resulting from the dimerization reaction in 2-nitropropane failed to reveal the presence of either acetone oxime, the stable tautomer of 2-nitrosopropane, or its Beckmann rearrangement product, N-methylacetamide. Similarily, nitrosobenzene could not be detected when nitrobenzene was used as solvent. The mechanism of coupling under these conditions remains unclear.

Since **3** can arise by oxidative dimerization of **1**, it is possible that this conversion may have occurred during workup of the sponge, which had been stored in aqueous methanol-acetone (pH ~ 5.5) for 1 week after collection. Experiments showed that **1** decomposes rapidly at pH 9, but it is quite stable at acidic pH's, even when O_2 is bubbled through the solution. Thus, it would appear that **3** occurs in the living sponge rather than arising in vitro after collection.



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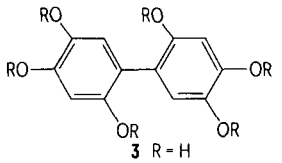
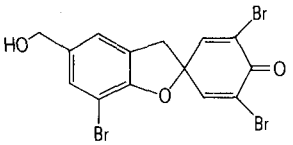
2 R = CH_3



3 R = H
4 R = CH_3



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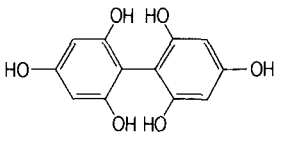

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