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MACROCYCLIC LACTONES AS THE DEFENSE SUBSTANCES OF THE TERMITE GENUS ARMITERMES

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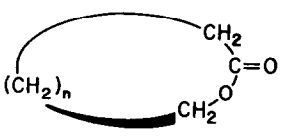
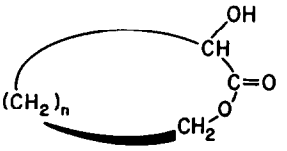
Abstract: Soldier termites of three species of Armitermes (Isoptera: Nasutitermitinae) contain macrocyclic lactones possessing from 22 to 36 carbon atoms and novel C_{24} and C_{26} α- and β-hydroxy macrolides as the primary components of their cephalic secretions.

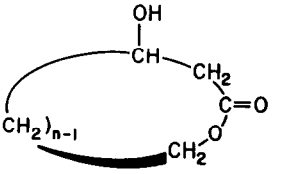
Soldiers of the advanced genera of the pantropical termite subfamily Nasutitermitinae (Isoptera: Termitidae) eject an irritating glue-like defensive secretion consisting of novel polycyclic diterpenoid 'resins' in a monoterpenoid 'solvent'[2]. Despite substantial variation in chemical structures found in over 50 different genera and species[3], all fully nasute soldiers possess terpenoid defense secretions which are biosynthesized in the soldier cephalic gland[4]. However, there are several genera of primitive nasute termites from which no defense secretions have been described[5]: those which are fully mandibulate (e.g. Syntermes, Cornitermes), and those which have hooked mandibles and a substantial nasus (e.g., Rhynchotermes, Armitermes; see Fig. 1). We now report the characterization of a homologous series of hitherto unknown twenty-two to thirty-six carbon macrocyclic lactones and of novel C_{24} and C_{26} 2- and 3-hydroxy macrolides from three species of Armitermes collected in Guyana.



1
n = 19, 21, 23, 25, 27, 29, 31, 33, (35)

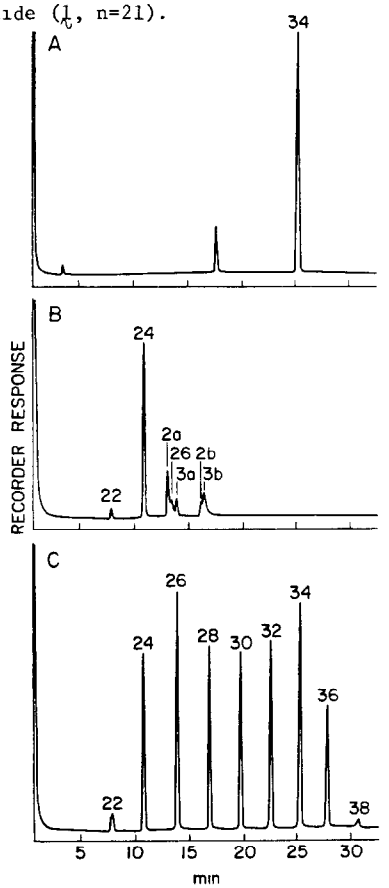


2 a, n=21
b, n=23



3 a, n=21
b, n=23

The heads of Armitermes soldiers[6] were crushed in hexane and analyzed by GLC and GC-MS[7]. Species-specific chromatograms are shown in Fig. 2. To obtain sufficient material for spectral examination, the heads of 600 soldier termites of Armitermes neotenicus (Holmgren) were crushed in hexane to give 45 mg of crude secretion. Pipette flash chromatography by elution with 5, 10, and 20% ethyl acetate in hexane on a 4 mm x 10 cm column of 230-400 mesh silica Gel 60 gave 15 mg of 1, 5 mg of 2 and 7 mg of 3. The following key spectral data was obtained for 1 (n=19, 21, 23): IR (neat), 1730 cm^{-1}, ^{1}H-NMR (80 MHz, $CDCl_3$), δ4.10 (t, J=6 Hz,-CH_2-CH_2-O-), 2.32 (t, J=7 Hz,-$CH_2CH_2CO_2R$); ^{13}C-NMR (20 MHz, $CDCl_3$) δ174.04 ($R'CO_2R$) 64.39 (RCH_2-O-R'), 34.66 ($R'CH_2CO_2R$).[8] Conspicuously absent from the NMR spectra were the proton and carbon resonances for methyl groups at the termini of long-chain compounds. GC-MS analysis indicated that the secretion components differed by 28 mass units, showed molecular ions M^+-H_2O peaks, and fragmented like long-chain alkenes.[7] On the basis of these data, we proposed the macrolide structure 1 for the least polar material; the major A. neotenicus compound was therefore tetracosanolide (1, n=21).



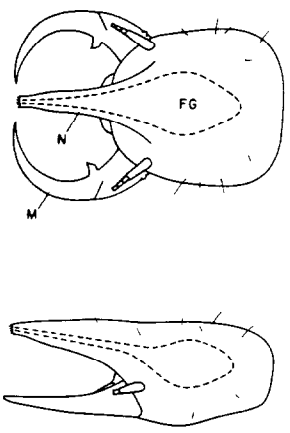


Figure 1. (above) Dorsal and side views of Armitermes neotenicus soldier head showing location of nasus (N), frontal gland reservoir (FG), and mandibles (M).

Figure 2. (left) Gas chromatograms of Armitermes soldier cephalic secretions: Varian 3700, 2m x 2mm I.D. glass column, 1% SP-2100 on Supelcoport, T_i = 200° (delay 2 min), T_p = 4°/min, T_f = 300°(hold 20 min). A, A. holmgreni; B, A. neotenicus; C, A. teevani. Numbers over peaks indicate total carbon atoms in the unsubstituted macrolides possessing structure 1. Hydroxy lactones 2a,b ($C_{24}H_{46}O_3$) and 3a,b ($C_{26}H_{50}O_3$) were only found in A. neotenicus.

Lactones possessing 16, 18, and 20, 22, and 24 carbons have been previously found in the Dufour's glands[9] and brood cell linings[9c,d] of solitary bees, and their mass spectra have been extensively studied[9a]. Long chain ω-hydroxy fatty acids, which are the ring-opened hydrolysis products of the corresponding lactones, have been previously identified from the hydrolysis of Douglas fir bark (up to C_{26})[10], and from the alkaline hydrolysis of lanolin (C_{22} to C_{36})[11]. We were unable to find any reported examples of the corresponding C_{26} to C_{36} macrolides. Confirmatory evidence for the structures of the Armitermes macrolides was obtained by acid-catalyzed methanolysis of 1 from A. teevani followed by trimethylsilylation, thereby providing the homologous methyl ω-trimethylsiloxy alkanoates. The mass spectra[7,8] of the methyl esters of the trimethylsilyl ethers of unbranched ω-hydroxy fatty acids gave highly characteristic mass spectra[12]. Intense peaks at M^+-15 and M^+-47 were evident, as were rearrangement fragments at m/z 146 and 159 indicative of aliphatic compounds with both trimethylsiloxy and carbomethoxy moieties.

Compound 2a (m/z 382.3429, $C_{24}H_{46}O_3$) and the minor C_{26} compound 2b gave NMR data suggestive of an α-hydroxy macrolide, since the resonances of the methylene adjacent to the carbonyl were shifted downfield: ^{1}H-NMR, δ2.32 absent, δ2.7 (d, J = 6 Hz, -OH, D_2O exchangeable), δ4.1-4.2 (m, H-2 and H-ω); ^{13}C-NMR, δ175.73 (C-1), 70.50 (C-2), 65.89 (C-ω), 34.45 (C-3). Lactone opening in methanol-hexane-HCl followed by silylation gave C_{24} and C_{26} bis(trimethylsiloxy) alkanoates which showed only weak M^+-15, M^+-31, and M^+-47 fragments, small M^+-32 (2%) and M^+-59 (4%) fragments, and large peaks at m/z 73, 75, 129, 147, 163, 411, and 453. The presence of the M^+-59 fragment is diagnostic for α-trimethylsiloxy methyl esters[12]. It is noteworthy than the TMS ether of the parent α-hydroxymacrolide also shows an M^+-59 (12%) ion with m/z 129 as the base peak.

Compounds 3a and 3b did not give parent ions in the mass spectrum, showing only a weak M^+-18; the TMS ethers of 3 clearly show M^+-15 and M^+-33 fragments. NMR evidence suggested a β-hydroxy macrolide structure. The ^{13}C spectrum showed two carbinyl carbons at δ68.26 (C-3) and 64.93 (C-ω) and the appearance of two downfield alkyl carbons at 41.37 (C-2), and 36.48 (C-4). The 80 MHz proton spectrum showed a triplet for H-ω, a broad multiplet at δ4.1-4.2 for H-3 and an ABX system for the diastereotopic H-2 protons. Methanolysis and silylation gave C_{24} and C_{26} bis(trimethylsiloxy) methyl esters isomeric to those from 2. The mass spectra showed small M^+-15 and M^+-47 ions, the base peak at m/z 175, and large m/z 73 and 75 ions. The major fragment at m/z 175 in conjunction with substantial m/z 89, 133, 131, and 159 peaks provide diagnostic evidence[12] for the β-OTMS methyl ester.

The discovery of the even-carbon macrolides from docosanolide to octatriacontanolide and the α-and β-hydroxy C_{24} and C_{26} macrolides 2 and 3 in a genus of primitive mandibulate nasute termites is both chemically and biologically intriguing. In contrast to the sticky terpenoid secretions of the advanced nasute termites,[2,3] the Armitermes macrolides probably function as oily antihealants in the manner described for C_{21}-C_{33} alkanes in Macrotermes and for diterpene hydrocarbons in Cubitermes.[2] The chemistry and ecology of Armitermes are now under closer scrutiny to determine the role of these novel macrolides in colony defense.[13]

References and Notes

1. Fellow of the Alfred P. Sloan Foundation, 1981-1983.

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5. Following completion of this work, R. Baker et al. reported the identification of several known monoterpenes and sesquiterpenes from five Brazilian Syntermes species (R. Baker, H.R. Coles, M. Edwards, D.A. Evans, P.E. Howse, and S. Walmsley, J. Chem Ecol. 1981, 7, 135 and from Curvitermes, a close relative of Armitermes (R. Baker, M. Edwards, D.A. Evans, S. Walmsley, J. Chem. Ecol. 1981, 7, 127.

6. Armitermes holmgreni (Snyder), A. neotenicus (Holmgren), and A. teevani (Emerson) colonies were collected from soil mounds on tree trunks in rainforest at Kartabo Point, Guyana. Soldiers were removed from the nest material, decapitated, and the heads were crushed in hexane. Gas chromatograms of the high molecular weight volatiles obtained for the secretions 'milked' from the tip of the nasus with a microcapillary were chemically indistinguishable from the crude hexane extracts, so that further chemical work was performed on the latter. Total hexane extract of heads: A. holmgreni, 10 µg/soldier; A. neotenicus, 80 µg/soldier; A. teevani, 230 µg/soldier.

7. High-resolution mass spectra of the more volatile lactones (MW<430) were obtained by Dr. C. Iden on an MS-30 interfaced to an HP7120A gas chromatograph, and low resolution spectra of the less volatile (430<MW<620) macrolides and their derivatives were obtained by Mr. P. Chang (Stony Brook). Diagnostic high-resolution data for the A. neotenicus and A. teevani secretion are given: docosanolide (1, n=19), m/z 338.3183 ($C_{22}H_{42}O_2$ requires 338.3185); tetracosanolide (1, n=21), 366.3502 ($C_{24}H_{46}O_2$ requires 366.3498); hexacosanolide (1, n=23), 394.3801 ($C_{26}H_{50}O_2$ requires 394.3811; octacosanolide (1, n=25). 422.4108 ($C_{28}H_{54}O_2$ requires 422.4124). The only major high mass fragments in each spectrum are the less intense M^+-18 and M^+-60 peaks; the low mass regions are dominated by m/z 55.0555 ($C_4H_7^+$). Additional low-resolution data for A. teevani: triacontanolide (1, n=27), m/z 450 (3.8), 422 (2.5); dotriacontanolide (1, n=29), 478 (3.5), 460 (2.8); tetraoontanolide (1, n=31), 506 (2.4), 488 (1.9); hexatriacontanolide (1, n=33), 534 (1.0), 516 (0.6); octatriacontanolide (1, n=35) could not be detected by mass spectrometry and its identification is based solely on GC retention time. Fragmentation of the macrolides 1 and of the TMS ethers of the corresponding ω-hydroxy methyl esters (M^+-15, M^+-47; m/z 75, 103, 129, 146, 159) indicated no branching of the aliphatic chains.

8. NMR spectra were obtained in deuteriochloroform solutions using Varian CFT-20 instruments operating at 80 MHz for ^1H and 20 MHz for ^{13}C. Complete experimental and spectral details, including 360 MHz spectra, will be published separately. (G.D. Prestwich, Tetrahedron Symposium-in-Print, "Animal Chemical Defenses", in press, 1981.)

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