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CHEMICAL COMPOSITION OF THE FRONTAL GLAND SECRETION FROM SOLDIERS OF *NASUTITERMES LUJAE* (TERMITIDAE, NASUTITERMITINAE)

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Abstract—The front gland secretion of soldiers of the West African Nasute termite *Nasutitermes lujae* contains the monoterpene terpinolene 1, and five diterpenes, four of them (5 to 8) belonging to the trinervitane skeleton while the fifth (3) is a secotrinervitane derivative. 3 and 8 are new derivatives whose structure has been established by chemical correlation and by X-ray diffraction analysis respectively.

Most of the termite societies possess a soldier caste which is morphologically specialized to protect the colony against its predators. Amongst the various possibilities of defensive strategies, chemical defense plays a prominent role. This is specially the case for the soldiers of the highly-evolved nasute termites, (Nasutitermitinae) which eject an irritating viscous secretion from a cephalic pear-shaped frontal gland, through a nozzle-like elongation of their rostrum called the nasus.[2] The chemical composition of the secretion from several species of Nasutitermitinae have been recently investigated. These secretions consist of a mixture of cembrane-derived diterpenes dissolved in monoterpene hydrocarbons.[3,4] In particular, the structure of selected monoterpenes and diterpenes of termites of the tropical genus *Nasutitermes* has been established.[4-6] This reveals that the diterpene composition of the secretions is complex and differs markedly from one species of *Nasutitermes* to another. It has been suggested by Prestwich[5] that these chemical variations may be viewed as indirect measures of the genetic variance and used as chemosystematic characters within the genus.

Behavioral aspects of the *Nasutitermes* secretions have also been analyzed. For example, alarm activity elicited by species specific monoterpene hydrocarbons has been conclusively demonstrated in *N. exitiosus*,[7] *N. costalis* and *N. rippertii*.[8] Moreover, in the case of the African species *N. lujae*, it has been clearly shown that the secretion of the soldiers regulates their number in the colony by inhibiting the formation of new soldiers.[9] In order to shed some light on the nature of the compounds responsible for this activity, we have investigated the terpene composition of the secretion of *N. lujae* soldiers, and we describe our results hereunder.

The capillary GLC trace of the dichloromethane extract of 100 soldier heads of *N. lujae* is shown in Fig. 1. The major monoterpene hydrocarbon (X) which represents 98% of the volatile fraction was identified as terpinolene (1, 12μg/soldier) on the basis of its mass spectrum (GC/MS) and by coelution with a standard sample when co-injected on several GLC columns. Terpinolene has been found in several other *Nasutitermes* species and specially in *N. octopilis* where it also accounts for 98% of the volatile fraction.[5]

The compounds A to E are diterpenes and account for 63% of the dry weight of the dichloromethane extract. They are present in the ratio 1 : 5 : 1 : 2 : 3 as shown by GLC. Silica gel chromatography of about half the extract obtained from 5250 soldiers led to three main fractions: F1 containing A + B, F2 containing C and F3 containing D + E. Further fractionation of F1 by preparative GLC led to homogeneous samples of A and B.

Compound A. A molecular formula $C_{20}H_{32}O$ was assigned to A from a consideration of the mass spectrum and a molecular ion at *m/e* 288. The IR spectrum indicated the presence of an hydroxyl group (ν_{OH} at 3400 cm^{-1}) and of an exomethylene function (δ C=CH_2 at 890 cm^{-1}). The ^1H NMR confirms the presence of an exomethylene function and shows signals assignable to 2 HC=C-CH_3 groups, a tertiary methyl and a secondary alcohol. Furthermore, the absence of the HC-7 and HC-16 signals, typical of the 1(15),8(19)-trinervitadiene derivatives,[6] suggests that compound A is a secotrinervitane derivative. Indeed, a careful comparison of the spectral characteristics of A with those of 3α-acetoxy-15β-hydroxy-7,16-secotrinervita-7,11-diene (2)[10] led us to propose structure 3 for A.

This hypothesis was confirmed by chemical correlation with 2. Indeed, treatment of 2 with $POCl_3$ in pyridine affords a mixture of 2 dehydro-compounds as observed

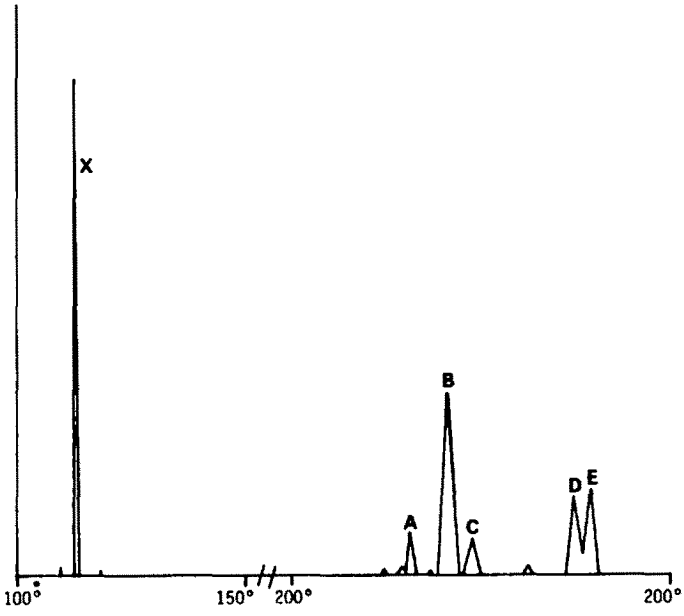


Fig. 1. Capillary GLC trace of the CH_2Cl_2 extract of soldier heads of *Nasutitermes lujae* (OV1, T° = 100° to 200°, 5°/min).

by capillary GLC. The retention time and the R_f of one of them are identical to those of the monoacetate **4** obtained by acetylation of A. Moreover, the mass spectrum of the dehydration mixture is superimposable to that of **4** whereas its ^{1}H NMR spectrum is identical to that of **4** except for small signals assignable to the 1(15)-dehydro isomer. A is thus 3α-hydroxy-7,16-secotrinervita-7,11,15(17)-triene **3**. This derivative has already been isolated by Prestwich[11] from *Longipeditermes longipes*, a Nasutitermitinae originating from Malaysia. Nevertheless, this is the first unambiguous proof of its structure. It is the second example of a secotrinervitane derivative which is a likely intermediate in the biosynthesis of the kempane and trinervitane diterpenes.[10]

Compound B. The spectral properties of B were found to be identical to those reported for 2β-hydroxy-1(15),8-trinervitadiene **5**,[12] a diterpene already isolated from several secretions of nasute soldiers (e.g. *Grallatotermes africanus*,[13] *Trinervitermes gratiosus*,[13] *Nasutitermes infuscatus*,[13] *N. octopilis*,[5] *N. gagei*[14] *and Subulitermes parvellus*[14]).

Compounds D and E. Despite several trials, we were unable to isolate pure D and E from fraction F3 neither by silica gel column chromatography nor by preparative GLC. Nevertheless, compound D could be successfully separated by chromatography on $AgNO_3$ impregnated silica gel. Under these conditions compound E underwent degradation and could not be recovered.

Compound D was identified as 2α,3β-dihydroxy-1(15),8(19)-trinervitadiene **6**[5] on the basis of its spectral properties and by coelution with an authentic sample when co-injected on two different capillary GLC columns. This diol is already known as a constituent of the secretions of several *Nasutitermes*[4,5,14,15] and *Subulitermes*[16] species.

From the ^{1}H NMR spectrum of the mixture of D and E, it appears that the differences between these compounds are the position of one of the double bond, which is exocyclic in the 8(19) position in D (two 1H bs at 4.85 and 4.91 ppm respectively) and endocyclic in the 8(9) position in E (1H dd at 5.3 ppm and 3H d at 1.58 ppm), and the configuration of the hydroxy groups at C-2 and C-3 which are 2α,3β in D (1H d at 3.24 ppm and 1H bd at 4.21 ppm) and 2β,3α in E (1H d at 3.70 ppm and 1H bd at 4.05 ppm). Moreover, the mass spectrum of the mixture is superimposable to that of compound D. All these data suggest that E is 2β,3α-dihydroxy-1(15),8-trinervitadiene **7**. This was confirmed by coelution with an authentic sample when co-injected on two different capillary GLC columns. Compound E shows also the same R_f as the standard in tlc.

Compound **7** has already been isolated from *Trinervitermes gratiosus*,[16] *T. bettonianus*,[16] *Grallatotermes africanus*,[13] *Nasutitermes infuscatus*[13] and *N. kempae*.[5]

Compound C. Compound C showed M^+ et *m/e* 304, compatible with the formula $C_{20}H_{32}O_2$, and ν_{OH} at 3400 cm^{-1}. ^{1}H NMR showed C to be a diterpene presenting structural features not previously reported for the already known termite diterpenes. Therefore, and because of the small amounts of C available, its structure was solved by an X-ray diffraction analysis. The crystals of C belong to the space groups $P2_1$ with a unit cell of dimensions $a = 10.952(4)$, $b = 10.026(3)$, $c = 8.302(5)$ Å, $V = 872.3(6)$ Å^3 (MoKα radiation; $\lambda = 0.71069$ Å: 2θ max = 50°). If we assign two molecules to the unit cell, we find a calculated density of 1.16 g/cm^3.

The structure was solved using the *Multan* 80 system of programs.[17] The SHELX 76 program[18] was used to refine the structure to a final R value of 0.047 by a least-squares method on the basis of 1240 observed reflections. The final fractional coordinates for the non-hydrogen atoms are listed in Table 1. A computer-generated stereoscopic view of compound C is depicted in Figure 2[19]: the absolute configuration was arbitrarily chosen.

It follows that compound C is 2α-hydroxy-3β,8β-

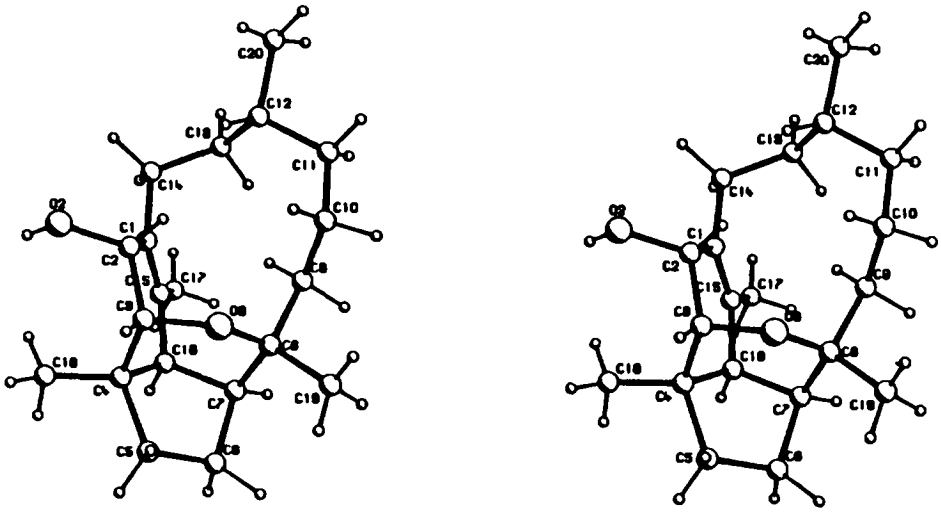
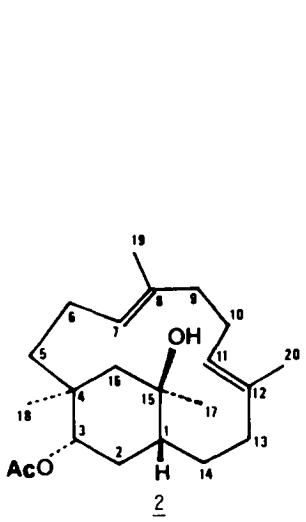
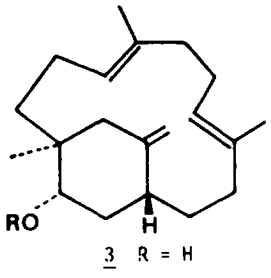


Fig. 2. Computer generated stereoscopic view of 2α-hydroxy-3β,8β-oxido-1(15)-trinervitene (8). PLUTO program.[19]



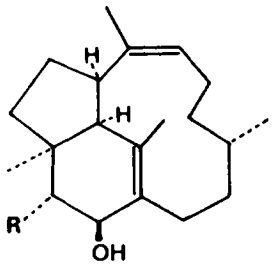
1

2



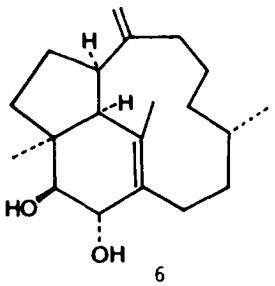
3 R = H

4 R = Ac

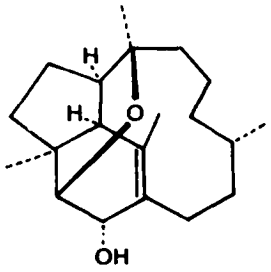


5 R = H

7 R = OH



6



8

Table 1. Final fractional coordinates (× 10^4) for the non hydrogen atoms of **8**

	x		y		z	
C1	3302(	4)	5039(	6)	-582(	6)
C2	4136(	4)	3895(	6)	-782(	6)
C3	4681(	4)	3064(	6)	794(	5)
C4	5162(	4)	3886(	6)	2396(	5)
C5	5506(	5)	2958(	8)	3987(	6)
C6	4335(	6)	2957(	9)	4628(	6)
C7	3251(	5)	3452(	7)	3116(	6)
C8	2776(	5)	2373(	6)	1743(	6)
C9	1505(	5)	2822(	7)	440(	6)
C10	1099(	6)	2037(	7)	-1189(	7)
C11	187(	5)	2793(	7)	-2669(	8)
C12	805(	5)	3820(	7)	-3569(	7)
C13	1075(	5)	5195(	7)	-2708(	8)
C14	2468(	5)	5659(	6)	-2184(	7)
C15	3251(	4)	5389(	6)	949(	6)
C16	3951(	4)	4618(	6)	2518(	6)
C17	2466(	6)	6524(	8)	1266(	10)
C18	6241(	5)	4811(	7)	2345(	7)
C19	2531(	7)	1027(	9)	2463(	8)
C20	-59(	8)	3974(	9)	-5370(	9)
O2	5121(	3)	4335(	5)	-1490(	4)
O3	3759(	3)	2067(	0)	939(	4)

oxido-1(15)-trinervitene **8**. Diol **6** is an obvious precursor of **8**. Indeed, the formation of an ether ring may easily be viewed as an electrophilic addition of the 3β-hydroxyl group to the exomethylene at C-8 leading to the formation of an extra 6-membered heterocycle.

In conclusion, like all the other *Nasutitermes* studied so far, *N. lujae* secretes its own specific blend of cembrane-derived diterpenes. The observed composition may be considered as characteristic of the species, since no variation of content was found between different batches of termites. Biological testing has shown that the monoterpene hydrocarbon terpinolene is not responsible for the soldier inhibition properties of the secretion. Testing of the other constituents is in progress.

EXPERIMENTAL

The following instruments were used for measuring the physical data: IR: Pye Unicam SP 1000 (NaCl film); ^{1}H NMR: Jeol J NM/MH 100 and Bruker HFX 270; MS: Finnigan 3000 D and Micromass 7070 F; Rotation power: Perkin Elmer 141; Capillary GLC: Varian 3700 (WCOT OV_1 column, H.L., 1 = 25 m, I.D. = 0.5 mm, T° = 210°). Preparative GLC: Hewlett Packard 402.

The ^{1}H NMR spectra were recorded in $CDCl_3$ solution. Chemical shifts are quoted in δ values (ppm) downfield from TMS as internal standard. The TLC were performed on Polygram Sil G pre-coated plates (Macherey-Nagel), spray reagent: ethanolic solution of phosphomolybdic acid (3%).

All described compounds are homogeneous by TLC and capillary GLC.

Collection of the material

The termites were collected in the forests of Banco and Yapo in Ivory Coast. 1100 soldiers were decapitated and the heads preserved in CH_2Cl_2 (batches of 50 heads in 0.5 ml of CH_2Cl_2). Moreover, 4200 intact soldiers were also preserved in CH_2Cl_2 (batches of 100 soldiers in 1 ml of CH_2Cl_2).

Extraction

Capillary GLC of the heads and the intact soldiers shows that their terpene composition is identical. A characteristic trace of the extract is represented in Fig. 1. The major monoterpene hydrocarbon (X) was identified as terpinolene by GC/MS (Carbowax 20M, T = 100°) and by coelution with a standard sample when co-injected on two different capillary GLC columns (0V1, Carbowax 20M, T = 100°).

1100 soldier heads and 4200 intact soldiers were extracted with CH_2Cl_2. The extract (460 mg) was filtered on silica gel using hexane/ethylacetate 7:3 as eluent. After evaporation of the solvents, an oily residue (372 mg) was obtained. 200 mg of this residue were chromatographed on silica gel, using hexane–ethylacetate 9:1 to 7:3 as eluent. The fractionation was monitored by TLC and capillary GLC.

This led to 3 fractions: F1 (60 mg) containing compound A and B; F2 (10 mg) containing C; F3 (80 mg) containing compounds D and E.

13 mg of F1 were submitted to preparative GLC chromatography (OV_3 3%, T = 190°). This yielded 3 mg of A and 8 mg of B.

Compound A (3). 3α-Hydroxy-7,16-secotrinervita-7,11,15(17)-triene. Oil; MS: M^+ at 288 (40), 273 (5), 270 (2), 255 (5), 175 (8), 135 (100); IR: 3400 cm^{-1} (ν_{OH}), 890 cm^{-1} (δ = CH_2); ^{1}H NMR (270 MHz): 0.82 (3H, s, H_3C-18), 1.57 (6H, bs, H_3C-19 and H_3C-20), 4.02 (1H, dd, J = 6, 10 Hz, HC-3), 4.56 and 4.65 (1H each, bs, H_2C-17), 4.83 and 5.29 (1H each, dd, J = 5, 8 Hz, HC-7 and HC-11).

Compound B (5). 2β-Hydroxy-1(15),8-trinervitadiene. Oil; $[\alpha]_{579}$: +23° ($CHCl_3$, c = 0.41); the MS and ^{1}H NMR spectra are identical to those reported[12] for 2β-hydroxy-1(15),8-trinervitadiene isolated from *Trinervitermes gratiosus*.

Compound C **8**. 2α-Hydroxy-3β,8β-oxido-1(15)-trinervitene, crystallized out of a pentane solution of F2. m.p. 168–9°; $[\alpha]_{579}$: −36° ($CHCl_3$, c = 0.42); MS: M^+ at 304 (80), 289 (40), 286 (5), 271 (2), 261 (25), 243 (30), 233 (20), 220 (25), 159 (45), 149 (40), 135 (100); IR: 3400 cm^{-1} (ν_{OH}); ^{1}H NMR (270 MHz): 0.94 (3H, d, J = 6 Hz, H_3C-20), 0.99 (3H, s, H_3C-18), 1.08 (3H, s, H_3C-19), 1.78 (3H, bs, H_3C-17), 3.62 (1H, bs, HC-3), 4.38 (1H, bs, HC-2).

Chromatography of F3 (28 mg) on $AgNO_3$ (10%) impregnated silica gel (eluent hexane–ethylacetate 7:3) led to the isolation of compound D (18 mg). E could not be eluted from the column.

Compound D (6). 2α,3β-Dehydroxy-1(15),8(19)-trinervitadiene. Oil; $[\alpha]_{579}$: −9.5° ($CHCl_3$, c = 1.46). The MS and ^{1}H NMR spectra of D are identical to those reported[16] for 2α,3β-dehydroxy-1(15),8(19)-trinervitadiene isolated from *Nasutitermes costalis*.

The ^{1}H NMR spectrum of F3 shows, besides the signal attributable to compound D, signals at 0.85 (d, J = 6 Hz, H_3C-20), 0.95 (s, H_3C-18), 1.58 (d, H_3C-19), 1.68 (bs, H_3C-17), 3.70 (d, J = 10 Hz, (HC-3), 4.05 (bd, J = 10 Hz, HC-2), 5.30 (dd, J = 10, 5 Hz, HC-3) assignable to compound E. These values correspond to those reported for 2β,3α-dihydroxy-1(15),8-trinervitadiene **7**.[17] Moreover compound E shows the same retention time on two different capillary GLC columns (OV_1 and OV_{17}) as well as the same R_f in TLC (eluent hexane–ethylacetate 7:3 and hexane–acetone 8:2) as an authentic sample of **7** previously isolated from *Trinervitermes oeconomus*.[20]

Acetylation of compound A

Compound A (1 mg) was stirred for 24 h at room temperature in a 1:1 mixture of pyridine–acetic anhydride (0.5 ml). After addition of 2 ml of distilled water, the aqueous solution was extracted (3 times) with 5 ml of CH_2Cl_2. The CH_2Cl_2 fractions were combined and evaporated to dryness under reduced pressure. The acetylated compound **4** was then purified by chromatography on silica gel (eluent hexane–ethylacetate 9:1). **4**: 3α-Acetoxy-7,16-secotrinervita-7,11,15(17)-triene. Oil; MS: M^+ at 330 (3), 288 (8), 270 (15), 255 (10), 149 (100): ^{1}H NMR: 0.87 (3H, s, H_3C-18), 1.54 (6H, bs, H_3C-19 and H_3C-20), 2.04 (3H, s, acetate), 4.55 and 4.64 (1H each, bs, H_2C-17), 4.95 and 5.27 (1H each, m, HC-7 and HC-11), 5.34 (1H, m, HC-3).

Dehydration of 2

Compound **2** (2 mg) was dissolved in pyridine (0.5 ml) and treated with 1 drop of $POCl_3$. The mixture was stirred at room temperature for 20 h. After addition of 2 ml of distilled water, the aqueous solution was extracted (3 times) with 5 ml of CH_2Cl_2. The CH_2Cl_2 fractions were combined and evaporated to dryness under reduced pressure.

The compound having the same R_f in TLC as **4** was purified by chromatography on silica gel (eluent hexane–CH_2Cl_2 9:1 to 7:3).

By capillary GLC (OV_1, 210°) this compound proved to be a mixture of two dehydrated derivatives. One of them having the same retention time as **4**. The MS of the mixture was identical to that of **4**. Its ^1H NMR spectrum was identical to that of **4**, except for small peaks attributable to the 1(15)-dehydro isomer of **4**.

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