DEFENSIVE SUBSTANCES FROM THE FRONTAL GLAND SECRETION OF *Nasutitermes nigriceps* TERMITE SOLDIERS

Irena VALTEROVÁ, Miloš BUDĚŠÍNSKÝ and Jan VRKOČ

Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, 166 10 Prague 6

Received October 2, 1990
Accepted January 21, 1991

Dedicated to Professor Alois Vystrčil on the ocassion of his 70th birthday.

The components of the defence secretion of the soldiers of *Nasutitermes nigriceps* termite species *(Isoptera: Termitidae: Nasutitermitinae)* have been identified. In the volatile fraction the following components were found: α- and β-pinene, camphene, myrcene, α-terpinene, *p*-cymene, limonene and terpinolene. Ten diterpenic compounds (*I*—*X*), mostly derivatives of the tricyclic trinervitane, were found in the non-volatile fraction. The structure of the so far undescribed 1(15),8(19)-trinervitadiene-2β,3α,14α-triol triacetate (*X*) was determined on the basis of its mass and infrared spectra and a detailed analysis of ^1H and ^{13}C NMR spectra. Considerable differences were found in the composition of the non-volatile fraction of the secretion of the species of termites studied from various localities.

The caste of termite soldiers of the subfamily *Nasutitermitinae* is morphologically well adapted to the chemical method of defence of the termite colony. In the defence reaction the soldiers squirt a toxic secretion from the frontal gland on the attacker, containing monoterpenic hydrocarbons together with polycyclic diterpenes[1]. Among the diterpenes from termites ever new compounds are being discovered, derived from structures unique among natural compounds.

The termites of the *Nasutitermes nigriceps* species were obtained from two very distant localities on the American continent, from Peru and Mexico. The defence substances of termites of this species, collected in Panama, have already been described by Gush and co-workers[2]. In this paper we discuss the differences observed in composition of the defence secretions of termites from all 3 localities.

Eight monoterpenic and two sesquiterpenic hydrocarbons were identified in the volatile fraction by means of GC-MS. Their content in the secretions of termites from various localities is surveyed in Table I. For comparison the values found by Gush and co-workers for his Panamanian colony of the same species are also included in Table I. From the table it is evident that the composition of the monoterpenic fraction does not differ very much for termites from individual localities.

Small amounts of sesquiterpenic compounds, such as caryophylene and γ-gurjunene, were identified only in Peruvian colonies of the species investigated.

In the non-volatile fraction we identified ten diterpenic compounds (*I*–*X*) by comparison of mass, infrared and NMR spectra, mostly derivatives of the tricyclic trinervitane. 1(15),8(19)-Trinervitadiene-2α,3α-diol (*I*) and 1(15),8(19)-trinervitadiene-2α,3β-diol (*II*) were isolated for the first time from the *Nasutitermes costalis* species[3]. 1(15),8(19)-Trinervitadiene-2β,3α-diol (*III*) and 1(15),8(19)-trinervitadien-3α-ol (*IV*) were detected for the first time in the species *Trinervitermes bettonianus*[4]. 1(15),8(9)-Trinervitadien-3α-ol (*V*) was described for the first time for the species *Trinervitermes gratiosus*[5] by Prestwich, with an incorrect localization of the hydroxy group into position 2β. Recently we published[6] a revision of the original Prestwich structure in favour of structure *V* on the basis of a detailed analysis of the NMR spectra of this compound, in which the hydroxy group is located in position 3α (ref.[6]). 1(15),8(9)-Trinervitadiene-2β,3α-diol[4] (*VI*) is also known to occur in the species *Trinervitermes gratiosus*. Another isolated alcohol is 15(16)-ripperten-3α-ol (*VII*), occurring in *Nasutitermes rippertii* species[7]. 9α-Acetoxy-3α-hydroxy-1(15),8(19)-trinervitadien-2-one (*VIII*) was described earlier for the species *Trinervitermes geminatus*[8]. The remaining two compounds discovered are the isomeric triacetates 1(15),8(19)trinervitadiene-2β,3α,13α-triol triacetate (*IX*), known from the species *Nasutitermes rippertii*[9], and the so far undescribed 1(15),8(19)-trinervitadiene-2β,3α,14α-triol triacetate (*X*), the structure determination of which is presented in this paper.

TABLE I

Content of mono- and sesquiterpenes in defence secretions of termites of *N. nigriceps* species from various localities

Compound	Locality		
	Mexico	Peru[a]	Panama[b]
α-Pinene	67·0	80·3	69·4
Camphene	0·6	0·25	0·4
β-Pinene	30·0	11·0	27·0
Myrcene	0·1	0·02	0·4
α-Terpinene	0·2	—	0·2
p-Cymene	0·3	—	—
Limonene	2·0	3·7	1·2
γ-Terpinene	—	—	0·02
Terpinolene	0·2	0·4	0·2
Caryophyllene	—	0·8	—
γ-Gurjunene	—	0·4	—

[a] Average value from 6 colonies; [b] the values are taken from literature[2].

The trinervitane skeleton for compound *X* followed both from the typical skeletal fragmentation in the mass spectrum (*m*/*z* 135), and the ^{1}H and ^{13}C NMR spectra. The signals of methyls, exomethylene and especially of H-7 and H-16 protons (see Table II) are characteristic of 1(15),8(19)-trinervitadiene skeleton. The NMR spectra were measured in deuteriochloroform solution, in order to compare the spectra

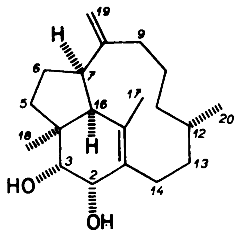
TABLE II

Proton NMR data of compound *X* in $CDCl_3$ and C_6D_6

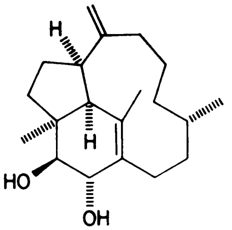
Proton	in $CDCl_3$		in C_6D_6	
	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)
H-2	5·96 dm	*J*(2, 3) = 7·7 *J*(2, 16) < 1 *J*(2, 17) < 2	6·43 dm	*J*(2, 3) = 9·1 *J*(2, 16) = 1·5 *J*(2, 17) = 2·0
H-3	5·38 d	*J*(3, 2) = 7·7	5·67 d	*J*(3, 2) = 9·1
H-7	3·13 m	*J*(17, 16) = 10·0 *J*(7, 6) = 9·4 *J*(7, 6′) = 7·0	2·95 dt	*J*(7, 16) = 12·2 *J*(7, 6) = 11·4 *J*(7, 6′) = 8·3
H-13	[a]	[a]	2·15 dt	*J*(13, 12) = 2·2 *J*(13, 13′) = 13·4 *J*(13, 14) = 12·5
H-13′	[a]	[a]	1·38 dt	*J*(13′, 12) = 6·0 *J*(13′, 13) = 13·4 *J*(13′, 14) = 5·8
H-14	4·92 dd	*J*(14, 13) = 10·2 *J*(14, 13′) = 4·6	5·10 dd	*J*(14, 13) = 12·5 *J*(14, 13) = 5·8
H-16	2·46 dd	*J*(16, 2) < 1 *J*(16, 7) = 10·0	2·19 dd	*J*(16, 2) = 1·5 *J*(16, 7) = 12·2
H-17	1·93 d	*J*(17, 2) = 2·2	1·97 d	*J*(17, 2) = 2·0
H-18	1·08 s		1·09 s	
H-19	4·90 t	*J*(19, 19′) = 2·0 *J*(19, 9) = 2·0	4·82 t	*J*(19,19′) = 2·2 *J*(19, 9) = 2·2
H-19′	5·03 bt	*J*(19′, 19) = 2·0 *J*(19′, 9) = 2·5	4·96 bt	*J*(19′, 19) = 2·1 *J*(19′, 9) = 2·6
H-20	0·96 d	*J*(20, 12) = 6·6	0·93 d	*J*(20, 12) = 6·6
3 × OAc	1·99 s 2·05 s 2·06 s		1·64 s 1·78 s 1·84 s	

[a] The value of the parameter was not determined.

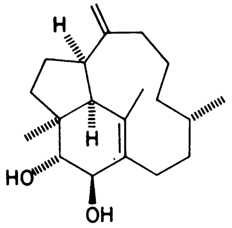
with those from literature, but owing to the low stability of compound *X* in chloroform solution the longer-lasting experiments were also measured in hexadeuteriobenzene solution. In this case the signals of one of the exomethylene hydrogen atoms and one of the CH-OAc hydrogens, which overlap in the measurement of the ^{1}H NMR spectrum in deuteriochloroform solution (δ 4·90 and 4·92), are also better resolved.



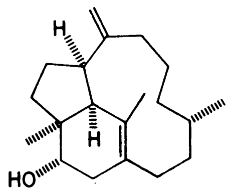
I



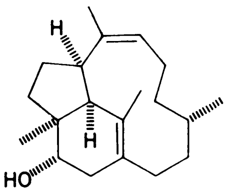
II



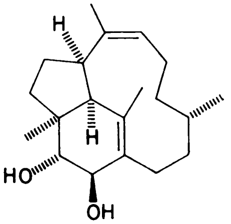
III



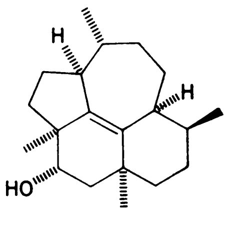
IV



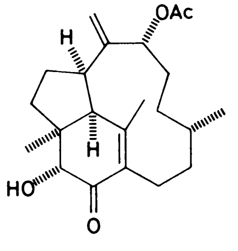
V



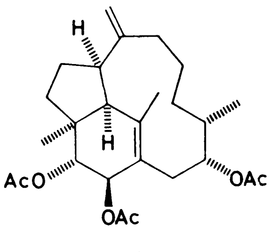
VI



VII



VIII



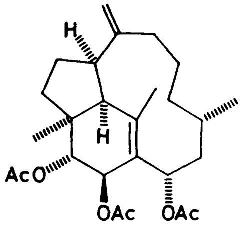
IX

The localization of two acetoxy groups into the vicinal position 2,3 clearly follows from the vicinal interaction (J = 9·1 Hz) of hydrogens at δ 5·76 and 6·43. The signal of C(2)**H**-OAc is shifted downfield under the effect of the neighbouring double bond 1(15). Neither of these two CH-OAc signals shows further vicinal interactions, because fully substituted atoms of carbons C(1) and C(4) are in the vicinity of the acetoxy groups. Hydrogen H-2 (δ 6·43) has a long-range coupling with the methyl group on C(15) (δ 1·97, $J(2, 17) = 2{\cdot}0$ Hz) and hydrogen H-16 ($J(2, 16) = 1{\cdot}5$ Hz). All these data represent proofs for the localization of the two acetoxy groups in positions 2 and 3.

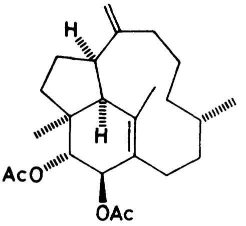
The configuration of the acetoxy groups in positions 2 and 3 was deduced from the value of the vicinal coupling constant and on the basis of analogy with the literature data. The value $J(2, 3) = 9{\cdot}1$ Hz indicates a *trans*-diaxial arrangement of hydrogens H-2 and H-3. *Cis*-diacetates have the value $J(2, 3) = 4{\cdot}5 - 5{\cdot}0$ Hz (ref.[10]). Hence, our compound *X* has the configuration of the acetoxy groups 2β,3α or 2α,3β. If comparing the chemical shifts values (in $CDCl_3$) of the signals H-3 (δ 5·38) and H-18 (δ 1·08) with the values given for analogous 2,3-diacetates in the literature, the best agreement is found for the configuration 2β,3α (δ 5·45 and 1·09 for 2β,3α-diacetate[9] *XI*, in contrast to δ 4·93 and 1·04 for 2α,3β-diacetate[10] *XII*).

For the localization of the third acetoxy group we made use of decoupling experiments, NOE-difference spectra and 2D-H,H-COSY spectra. The corresponding hydrogen atom CH-OAc has two vicinal interactions ($J = 12{\cdot}5$ and $5{\cdot}8$ Hz), from which it may be judged that only two hydrogens are present in vicinal positions. In the trinervitane skeleton with occupied positions 2,3 this fact is in agreement with positions 5, 9 and 14. Position 9 may be eliminated on the basis of decoupling experiments. The exomethylene hydrogens H-19 display homoallylic coupling. However, this coupling is not caused by hydrogens H-7 or the discussed CH-OAc hydrogen. Therefore a methylene group must be in position 9 and for our acetoxy group the decision lies between positions 5 or 14. In order to solve this problem we used the NOE-difference spectra. During the saturation of the signal of the discussed hydrogen CH-OAc (δ 5·10) we observed the NOE enhancement of H-2 (8%) together with the other ones (5% in the region δ 1·38 and 3% for secondary methyl H-20 at δ 0·93). Using differential decoupling and 2D-COSY spectra hydrogens H-13 were detected in the region δ 1·38 and δ 2·15, which have vicinal couplings with the discussed CH-OAc hydrogen. From these facts a spatial proximity of the hydrogen in CH-OAc and the hydrogens H-2 and H-20 may be deduced, which is in favour of the localization of the third acetoxy group in position 14.

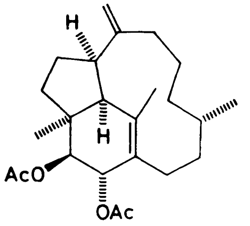
The determination of the configuration of the acetoxy group on C(14) is complicated by partial flexibility of the eleven-membered ring. However, the flexibility of the ring is considerably restricted by the annellation with the two further cycles and by steric hindrance. The configuration of the acetoxy group was determined on the basis of conformational analysis carried out on a model of the compound, the values of the coupling constants of the CH-OAc hydrogen and the NOE experiments. The discussed hydrogen in CH-OAc has zero homo allylic interactions with H-16 and 17-CH_3, which indicates its proximity to the C=C bond plane. The coupling constants values with the vicinal methylene group ($J = 12{\cdot}5$ and $5{\cdot}8$ Hz) correspond to the *trans* or *gauche* orientation of hydrogens on C(14)–C(13) bonds, respectively. The observed NOE with hydrogens H-2, H-13 and H-20 indicated a mutual spatial closeness, which, according to models, suggests the localization of the acetoxy group into position 14α unambiguously.



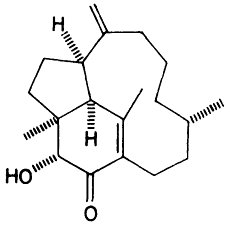
X



XI



XII



XIII

Differences in Composition of the Defence Secretions of N. nigriceps from Various Localities

In this paper we compare the quantities of individual mono-, sesqui- and diterpenes in the defence secretions of termites from Peru and Mexico (Table III). For comparison we also give the results of Gush and co-workers^2 from the same species, collected in Panama. While in the case of monoterpenes from all localities their proportions differ very little, qualitatively and quantitatively, in the case of diterpenes these differences are considerable. The Peruvian termites produce only hydroxy derivatives of trinervitane *I*, *II*, *IV*, *V* and 2-rippertenol *VII*, which agrees approximately with the results by Gush and co-workers^2. In contrast to this in termites from Mexican colonies acetoxy derivatives of trinervitane (*VIII*–*X*) were found in addition to diols *III* and *VI*, which represents a distinct difference from termites from Peru and Panama. Considerable differences between individual colonies of the same species were already observed earlier in the species *Nasutitermes ephratae*^{11}. The explanation of this fact would require a broader chemotaxonomic study with a large number of samples from various localities.

EXPERIMENTAL

The soldiers of termites of the *N. nigriceps* species were collected at the localities Iquitos (Peru) and Quinta "La Escondida" (Mexico) in 1981 and 1982. The collection and the extraction of the defence substances were carried out as described in ref.[11] and the extracts were separated to volatile and non-volatile fractions[12]. The volatile fractions were analysed by means of GC MS on a HP 5895A instrument with a 25 m SE-54 capillary column (1% methyl, 5% phenyl methyl silicone) and VGZAB-EQ connected with a gas chromatograph HP 5890, using a HP 5 column (with 5% phenyl methyl silicone). The compounds were identified by comparing their mass spectra with the spectra of standards. Non-volatile fractions were chromatographed on a silica gel column with 15% of water (Herrmann, Köln-Ehrenfeld). The final purification of the compounds was carried out by preparative TLC on silica gel 60 PF_{254} (Merck, Darmstadt). In some instances the same silica gel was used for the separation, impregnated by spraying it with a saturated aqueous $AgNO_3$ solution until wet, and drying overnight at room temperature. Detection was carried out by spraying with 0·2% methanolic morine solution. The purity of the compounds was checked by TLC on silica gel 60 G (Merck, Darmstadt) and detection was carried out by spraying with concentrated sulfuric acid and heating. In some special cases of compounds with close R_F values the compounds were detected by spraying with vanillin (90 ml of a 10% ethanolic solution of vanillin + 5 ml of concentrated sulfuric acid) and cautious heating. The purity of the compounds was also checked by gas chromatography on a Chrom 4 instrument (Laboratorní přístroje, Praha), using a column packed with 3% OV-17 (50% phenyl methyl silicone) on Gas Chrom Q (100—120 mesh), 2·5 m long and 3 mm i.d. The melting points were determined on a Kofler block and they are not corrected.

TABLE III

Presence of diterpenes in defence secretions of *N. nigriceps* termites from various localities. The sign + means the presence and the sign — the absence of a detectable amount of compound in the extract

Compound	Locality		
	Mexico	Peru	Panama[a]
I	—	+	+
II	—	+	+
III	+	—	+
IV	—	+	+
V	—	+	—
VI	+	—	—
VII	—	+	+
VIII	+	—	—
IX	+	—	—
X	+	—	—
XIII	—	—	+

[a] The data are taken from literature[2].

The compounds were identified on the basis of comparison of their mass, infrared and NMR spectra with the spectra of standards. The mass spectra of diterpenes were measured on a VG ZAB EQ instrument. The infrared spectra were measured in KBr pellets (1·5 mm diameter) on a Perkin–Elmer 621 spectrophotometer. The NMR spectra were recorded on a Varian XL-200 instrument (^{1}H at 200 MHz and ^{13}C at 50·3 MHz, resp.) in deuteriochloroform or hexadeuteriobenzene solution with tetramethylsilane as internal reference. Proton NOE experiments and 2D-COSY spectra were measured on a Varain VXR-400 (400 GHz) instrument.

1(15),8(19)-*Trinervitadiene*-2α,3α-*diol* (I) was obtained by elution with light petroleum–ether (30%), m.p. 70–73°C (from light petroleum–ether, ref.[3] gives 75–77°C). All spectra agreed with those of a standard sample.

1(15),8(19)-*Trinervitadiene*-2α,3β-*diol* (II) was eluted with light petroleum–ether (30%). Crystallization from ethanol gave diol *II*, m.p. 87–92°C (ref.[3] gives 93–96°C). All spectra were identical with the spectra of the standard compound.

1(15),8(19)-*Trinervitadiene*-2β,3α-*diol* (III) was eluted with a mixture of light petroleum and ether (30%). The product obtained by crystallization from light petroleum–ether had m.p. 172 to 175°C (ref.[9] gives m.p. 174–176°C). All spectra were identical with those of a standard.

1(15),8(19)-*Trinervitadien*-3α-*ol* (IV) was obtained by elution with light petroleum–ether (20%) and then crystallized from the same solvent mixture, affording a product with m.p. 119–122°C (ref.[9] gives 128–131°C). All spectra were identical with those of a standard.

1(15),8(9)-*Trinervitadien*-3α-*ol* (V) was eluted with a mixture of light petroleum and ether (20%), in the form of an amorphous oil. The separation from simultaneously eluted isomeric alcohols *IV* and *VII* was carried out by preparative TLC on a silica gel plate (20 × 20 cm) impregnated with silver nitrate. The spectral data and the structure proof of alcohol *V* have been published in our preceding paper[6].

1(15),8(9)-*Trinervitadiene*-2β,3α-*diol* (VI) was eluted with light petroleum–ether mixture (30%). All spectra of this compound were in agreement with the spectra from literature[4].

15(16)-*Ripperten*-3α-*ol* (VII) was eluted and purified in the same manner as alcohol *V*. All spectra of this compound agreed well with those of a standard.

9α-*Acetoxy*-3α-*hydroxy*-1(15),8(19)-*trinervitadien*-8-*one* (VIII) was obtained as an amorphous oil by elution with light petroleum–ether (20%). All spectra agreed with those of a standard[8].

1(15),8(19)-*Trinervitadiene*-2β,3α,13α-*triol triacetate* (IX) was obtained by elution with light petroleum–ether (20%) and crystallized from the same mixture, m.p. 104–108°C (ref.[9] gives 110–112°C). All spectra were identical with the spectra of a standard.

1(15),8(19)-*Trinervitadiene*-2β,3α,14α-*triol triacetate* (X) was eluted with a mixture of light petroleum and ether (20%). Crystallization from a mixture of light petroleum and ether gave a compound of m.p. 157–161°C. Mass spectrum, *m/z* (%): 386 (M – 60, 4), 302 (7), 284 (40), 256 (13), 173 (7), 135 (19), 43 (100). Infrared spectrum, cm^{-1}: 3 080, 1 628, 906 ($>C=CH_2$), 1 741, 1 721, 1 232 (OAc), 1 646 (C=C). ^{13}C NMR spectrum (C_6D_6, δ, ppm): 169·82, 168·91, 168·55 (3 × C=O); 151·64, 136·52, 128·74 (3 × $>C=$); 112·19 ($=CH_2$); 72·67, 72·21, 70·16 (3 × $>CH-OAc$); 57·42, 52·43 (2 × $>CH-$); 45·88, 37·57, 35·81, 34·91, 29·49, 26·66, 25·07 (6 × CH_2); 24·25, 22·92, 21·79, 20·45, 20·03, 19·71, 19·60 (6 × CH_3 + $>CH-$).

The authors thank Dr D. Daloze, Université Libre de Bruxelles, Belgium, for the kind donation of the spectra of compound VIII *for comparison. We also thank Dr S. Vašíčková for the measurement and the interpretation of the* IR *spectra. Our thanks are due to Dr K. Ubik and Dr J. Křeček* *for the collection of biological material. The determination of the biological species was carried out by Dr J. Křeček.*

REFERENCES

1. Prestwich G. D.: Ann. Rev. Entomol. *29*, 201 (1984).
2. Gush T. J., Bentley B. L., Prestwich G. D., Thorne B. L.: Biochem. Syst. Ecol. *13*, 329 (1985).
3. Vrkoč J., Buděšínský M., Sedmera P.: Collect. Czech. Chem. Commun. *43*, 2478 (1978).
4. Prestwich G. D., Tanis S. P., Pilkiewicz F. G., Miura I., Nakanishi K.: J. Am. Chem. Soc. *98*, 6062 (1976).
5. Prestwich G. D.: Experientia *34*, 682 (1978).
6. Valterová I., Buděšínský M., Vrkoč J., Prestwich G. D.: Collect. Czech. Chem. Commun. *55*, 1580 (1990).
7. Prestwich G. D., Spanton S. G., Lauher J. W., Vrkoč J.: J. Am. Chem. Soc. *102*, 6825 (1980).
8. Braekman J. C., Daloze D., Dupont A., Pasteels J. M., Josens G.: J. Chem. Ecol. *10*, 1363 (1984).
9. Vrkoč J., Buděšínský M., Sedmera P.: Collect. Czech. Chem. Commun. *43*, 1125 (1978).
10. Valterová I., Buděšínský M., Tureček F., Vrkoč J.: Collect. Czech. Chem. Commun. *49*, 2024 (1984).
11. Valterová I., Křeček J., Vrkoč J.: Biochem. Syst. Ecol. *17*, 327 (1989).
12. Valterová I., Křeček J., Vrkoč J.: Acta Ent. Bohemoslov. *81*, 416 (1984).

Translated by Ž. Procházka.