(8*Z*)-1(15),8(9)-TRINERVITADIEN-3α-OL FROM *Nasutitermes nigriceps* TERMITES. THE REVISED STRUCTURE FOR A DEFENSE COMPOUND OF *Trinervitermes gratiosus* TERMITES

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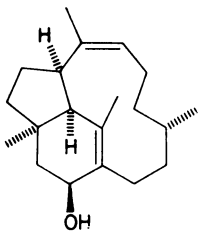
The structure of (8*Z*)-1(15),8(9)-trinervitadien-3α-ol (*IIa*) was established by a detailed analysis of its NMR spectra for a trinervitane derived alcohol isolated from the species *Nasutitermes nigriceps*. The previously suggested structure *I* for the same compound isolated from *Trinervitermes gratiosus* is now revised.

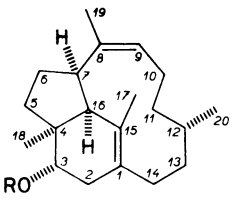
The caste of soldiers of the highly developed termites of the species *Nasutitermes nigriceps* produces a defense secretion in the frontal gland. From the chemical point of view, this secretion is formed by a mixture of monoterpenic and diterpenic, in some cases also of sesquiterpenic compounds. It was demonstrated that the components of the secretion from the termite frontal gland produce toxic effects on potential predators[1].

The chemical composition of the defense secretion of termite soldiers of the species *Nasutitermes nigriceps* from the central America region has been described by Gush et al.[2]. Recently, we have analyzed the secretion from soldiers of the same species originated in Peru. A detailed study of the intraspecific variations in the secretion composition of soldiers of this species will be presented elsewhere. This paper concerns only one of the isolated compounds, the spectral characteristics of which reveal a different structure to that presented previously[3].

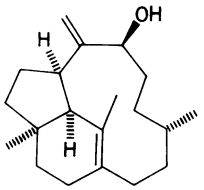
The mass, IR and ^{1}H NMR spectra of the alcohol *IIa* were identical with those of the compound isolated from *Trinervitermes gratiosus*, the structure of which was designated by one of us as *I* in the original paper[3]. The identity of the alcohol isolated by three of us from *N. nigriceps* was proved by comparing it with the authentic sample of the original compound from *T. gratiosus*. This complete the structure revisions of the monohydroxy trinervitadiene compounds using more powerful NMR techniques, which previously allowed one of us to revise the *Trinervitermes sp.* compound *III* (ref.[4]) to the structure *IV* (ref.[5]).



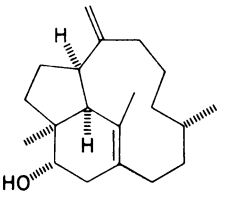
I



IIa, R = H
IIb, R = $CCl_3CONHCO$
IIc, R = (*S*) - MTPA
IId, R = (*R*) - MTPA



III



IV

The determination of the structure of the above mentioned diterpenic alcohol is based on a detailed analysis of its NMR spectra (Tables I and II). The position of the hydroxy group in alcohol *IIa* have been determined on the basis of proton decoupling experiments. On irradiation of the proton at C(9) the doublet at δ 1·64 collapses to a singlet so that this signal must belong to the methyl on the double bond in position 8. Hence, the other signal of sp^2-methyl (at δ 1·58) belongs to the methyl at position 15, in agreement with its multiplicity (homoallylic couplings with four protons). By irradiation of the **CH**—OH proton (δ 3·93) we could assign the signals of neighbouring methylene protons (δ 2·34 bdd and δ 1·79 bdd). For one of these methylene protons (δ 2·34) we have found also homoallylic coupling with C(15)--methyl. Therefore the structural fragment —C—CH(OH)—CH_2—C(=C)—CH_2— was proved and hydroxy group localized into position 3. The structure *I* (ref.[3]) with hydroxyl in position 2 doesn't agree with our decoupling experiments.

Hydroxy group in position 3 was further supported by the NMR measurement trichloroacetyl carbamoyl derivative (TAC) *IIb*. In addition to the characteristic downfield shift of the **CH**—OR proton (1·30 ppm) we have observed significant acylation shifts for methyl protons H(18) (0·12 ppm) and C(2)H_2 protons (0·21 and 0·14 ppm) which indicate their proximity to the acylated hydroxyl.

Further we have to solve the question of the configuration of the hydroxy group in position 3. As follows from vicinal couplings of the **CH**—OH proton (J = 10·6

resp. 6·3 Hz) the hydroxy group should be equatorial with the corresponding CH—OH hydrogen in axial position. However, as follows from molecular models, this situation can be achieved for both configuration at C(3) depending on the conformation of the cyclohexene ring. For solution of this problem we decided to use the diastereoisomeric esters of alcohol *IIa* with (*S*)- resp. (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA). Both esters *IIc* and *IId* were prepared from alcohol *IIa* and the corresponding Mosher acids with 2-chloro-1-methylpyridinium iodide as the condensation agent and in the presence of 4-dimethylaminopyridine as a base[6]. It is well known from the literature[7], that acidic part of these esters prefers the conformation with the planar arrangement of bonds —CH—O—CO—C—CF_3, shown in the scheme 1. The NMR method of determination of the absolute configuration then makes use of the different predictable shielding effects of the phenyl ring

TABLE I

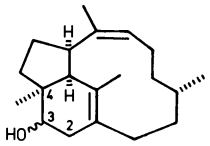
^{1}H NMR data of compounds *IIa*—*IId*

Proton	*IIa*	$\Delta\delta^a$	*IIc*[b]	*IId*[b]
H-2α	1·79 bdd $J(2\alpha, 2\beta) = 16{\cdot}5$ $J(2\alpha, 3) = 10{\cdot}6$ $J(2\alpha, 17) = 0$	0·14	1·805 bdd	1·94 bdd
H-2β	2·34 bdd $J(2\beta, 2\alpha) = 16{\cdot}5$ $J(2\beta, 3) = 6{\cdot}3$	0·21	2·47 bdd	2·51 bdd
H-3	3·93 dd $J(3, 2\alpha) = 6{\cdot}3$ $J(3, 2\beta) = 10{\cdot}6$	1·30	5·33 dd	5·36 dd
H-9	5·13 ddq $J(9, 10) = 10{\cdot}5$ $J(9, 10) = 6{\cdot}0$ $J(9, 19) = 1{\cdot}5$ (3×)	0·07	5·22 ddq	5·23 ddq
H-17	1·58 bs	0·02	1·59 bs	1·59 bs
H-18	0·92 s	0·12	0·95 s	0·89 s
H-19	1·64 d $J(19, 9) = 1{\cdot}5$	0·00	1·64 d	1·64 d
H-20	0·82 d $J(20, 12) = 7{\cdot}0$	0·03	0·86 d	0·86 d

[a] Difference of chemical shifts of alcohol *IIa* and corresponding TAC derivative *IIb* ($\Delta\delta = \delta(IIb) - \delta(IIa)$); [b] all the coupling constants in *IIb*, *IIc* and *IId* are very similar as in *IIa*.

on the protons in the neighbourhood of the esterified hydroxyl – in our case C(4) -methyl and C(2)-methylene protons. Four possible situations – for 3α- resp. 3β-OH and (*R*)- resp. (*S*)-Mosher acid esters are visualized in Scheme 1. As the scheme demonstrates in the case of 3α-OH configuration the C(4)-methyl should

ESTERIFICATION:

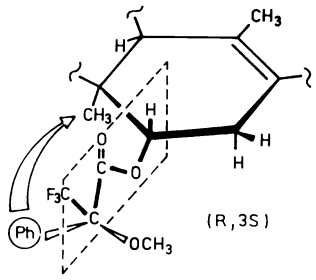


(R)-MTPA → (R,3X)-ESTER

(S)-MTPA → (S,3X)-ESTER

^{1}H NMR THEORETICAL CONSIDERATION:

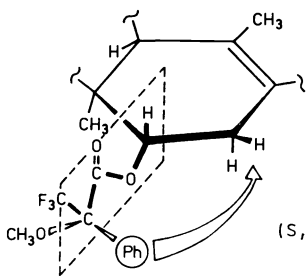
3α-OH (3S)



(R,3S)

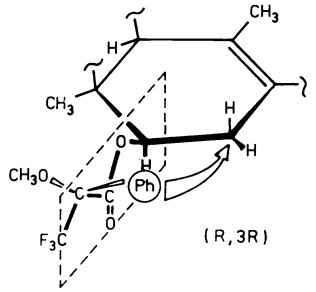
$\delta_{CH_3}(R,3S) < \delta_{CH_3}(S,3S)$

$\delta_{CH_2}(R,3S) > \delta_{CH_2}(S,3S)$



(S,3S)

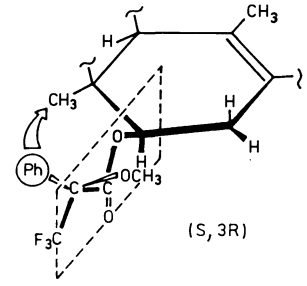
3β-OH (3R)



(R,3R)

$\delta_{CH_3}(R,3R) > \delta_{CH_3}(S,3R)$

$\delta_{CH_2}(R,3R) < \delta_{CH_2}(S,3R)$



(S,3R)

^{1}H NMR EXPERIMENT:

$$\delta_{CH_3}(R,3X) = 0.895 < \delta_{CH_3}(S,3X) = 0.95$$

$$\delta_{CH_2}(R,3X) = \begin{matrix}2.51\\1.94\end{matrix} > \delta_{CH_2}(S,3X) = \begin{matrix}2.47\\1.80\end{matrix}$$

⇓

3α-OH (3S)

be more shielded in the (*R*, 3α)-ester *IId* by the phenyl ring while the C(2)-methylene protons should be more shielded in the (*S*, 3α)-ester *IIc*. In the case of 3β-OH configuration the shielding effects should be reversed. Our experimental data clearly fit the former shielding relations and therefore the configuration 3α (*S*) can be assigned to our alcohol.

Until now, *Z*-configuration of the double bond C(8)=C(9) has been postulated without experimental evidences. However, the molecular models show considerable steric interactions and the limited flexibility of the macrocycle for 8*Z*- in comparison with 8*E*-arrangement. therefore we made an attempt on the experimental verification of the C(8)=C(9) double bond geometry by means of the selective proton–proton NOE measurement. At irradiation of the C(8)-methyl protons (δ 1·64) we have observed the intensity enhancement for signal of C(9)-proton by 9·3%. This NOE proves the 8*Z*-configuration on C(8)=C(9) unambiguously.

EXPERIMENTAL

Soldiers of termites of the species *Nasutitermes nigriceps* originated from Peru, Iquitos locality. The defense secretion was extracted with ethanol and the extract was treated as described in our previous paper[8]. Mosher esters *IIc* and *IId* were prepared according to the procedure described in the ref.[6]. ^{1}H and ^{13}C NMR spectra of the isolated compounds were measured on FT-NMR spectrometer Varian XL-200 at 200 MHz resp. 50·3 MHz frequencies in deuterochloroform solution with tetramethylsilane as internal reference. The NOE on compound *IIa* was determined as the signal intensity enhancement of H(9) signal comparing two spectra differing in the irradiation frequency — "on" resp. "off" (200 Hz upfield) resonance position of

TABLE II

^{13}C NMR chemical shifts of the compound *IIa*

δ	Type	Assignment[a]	δ	Type	Assignment[a]
131·78	>C=	C-8	36·65	$-CH_2-$	
128·10	-CH=	C-9	34·86	$-CH_2-$	
127·87	>C=	C-15	30·39	>CH-	C-12
127·61	>C=	C-1	30·06	$-CH_2-$	
73·39	>CH-O	C-3	28·36	$-CH_2-$	
62·19	>CH-	C-16	24·74	$-CH_2-$	
48·91	>CH-	C-7	20·97	$-CH_3$	
43·67	>C<	C-4	20·38	$-CH_3$	
39·94	$-CH_2-$		18·18	$-CH_3$	
37·52	$-CH_2-$		16·07	$-CH_3$	

[a] The structural assignment of $-CH_2-$ and $-CH_3$ signals could not be done.

C(8)-methyl signal. All other experimental conditions were same for both spectra; the decoupler was always switched on during time interval 16 s before the acquisition to built-up NOE. Trichloroacetylcarbamoyl derivative *IIb* was prepared by in situ acylation with trichloroacetyl isocyanate (a small excess of the reagent was added to the solution of *IIa* in sample tube). ^{13}C-chemical shifts and the number of directly bonded protons were obtained from the „attached proton test" spectrum[9].

Mass spectrum of *IIa* was measured on AEI MS 902 instrument. MS, *m/z* (%): 288 (34), 273 (31), 270 (7), 255 (21), 204 (15), 189 (13), 177 (19), 175 (17), 159 (19), 136 (44), 135 (45), 41 (100). Infrared spectrum was recorded on a Perkin–Elmer 621 spectrophotometer in KBr micropellet (1·5 diameter). IR: 3 370 cm^{-1} (OH).

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