MINOR DITERPENE COMPONENTS OF THE DEFENSE SECRETION FROM THE FRONTAL GLAND OF SOLDIERS OF THE SPECIES *Nasutitermes costalis* (HOLMGREN)

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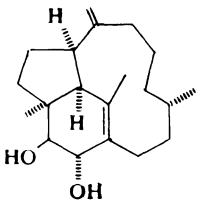
Three minor components of the defense secretion from soldiers of the species *Nasutitermes costalis* (HOLMGREN) have been isolated and characterized by means of spectral methods. The first component was identified as 1(15), 8(19)-trinervitadien-9β-ol (*III*), known to occur with the subfamily *Nasutitermitinae*. For the second component two alternative structures *IVa*, *IVb* have been tentatively suggested, both with the 7,16-secotrinervitane skeleton. The third component was determined as 5β,9β-diacetoxy-2-oxo-11(12)kempen-20-oic acid (*VII*). The kempene skeleton was established on the basis of the ^{13}C NMR spectrum and mass spectral fragmenation of the acid methyl ester. The relative configuration, together with a probable conformation of the tetracyclic framework were determined by ^{1}H NMR. Two major tricyclic components, 1(15),8(19)-trinervitadien-2α,3β-diol (*I*) and 1(15),8(19)-trinervidatien-2α,3α-diol (*II*), were further characterized as mono and diacetates. Different relative rates of acetylation of the hydroxy groups in these diols provided evidence of stable conformation of the six-membered ring within the trinervitane skeleton.

The most evolved species of termites (subfamilies *Termitinae* and *Nasutitermitinae*) use exclusively chemical means for defense[1]. The defense secretion of termite soldiers consists of a solution of oxygenated diterpenes in monoterpene hydrocarbons. The monoterpenes serve both as alarm pheromones for members of the same species, as well as toxic components of the defense system[2,3]. The function of diterpenes has not been fully clarified as yet. Neither monoterpenes nor diterpenes are found in extracts from termite workers. Furthermore, it was found that termite soldiers synthetize the defense compounds themselves and do not take them from food[4,5]. The composition of the defense secretion is specific for a given species and independent of food sources[6]. While diterpenes are characteristic of termites of the above subfamilies, monoterpenes are employed for defense by evolutionally lower termite species, as well[7]. The diterpenes found in the frontal gland of soldiers of the subfamily *Nasutitermitinae* are derived from few basic skeletons, namely, bicyclic 7,16-secotrinervitane, tricyclic trinervitane and tetracyclic kempane and rippertane.

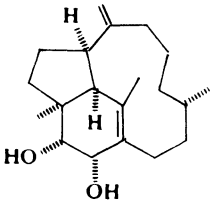
These skeletons are found exclusively with termites. The most frequent substituents are hydroxyl, acetoxyl and oxo groups, combinations of which with the skeletal types make a total of about 40 compounds known up to date[7].

The present paper deals with isolation and identification of minor diterpene components, present in quantities 0·5 – 3%, from the defense secretion of species *Nasutitermes costalis* (HOLMGREN) from Cuba. The composition of the monoterpene fraction[6] as well as the structures of two major diterpene components – 1(15),8(19)--trinervitadien-2α,3β-diol (*I*) and 1(15),8(19)-trinervitadiene-2α,3α-diol (*II*) – have been described earlier[8].

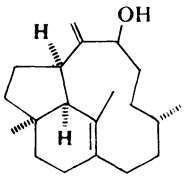
The first minor component isolated from the frontal gland was an alcohol $C_{20}H_{32}O$ ($M^{+\bullet}$, *m/z* 288). The mass spectrum displayed fragment ions at *m/z* 273 $(M-15)^+$, 270 $(M-18)^{+\bullet}$, 175 and 136; the latter two fragments are characteristic of the trinervitane skeleton. The infrared and ^1H NMR spectra confirmed the presence of a secondary hydroxyl (3 390 cm^{-1}, CH—OH, δ 4·10 dd), and exomethylene group (3 065, 1 630, 1 411 and 886 cm^{-1}; δ 4·96 t and 4·82 t) and methyl groups in an arrangement corresponding to the trinervitane skeleton. According to the spectra the alcohol was identified as 1(15),8(19)-trinervitadien-9β-ol (*III*), the most usual component of the subfamily *Nasutitermitinae*[7].



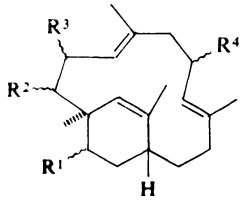
I

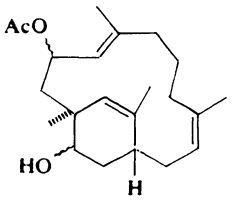


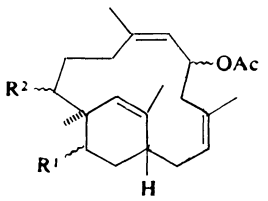
II



III







IVa, R^1 = OH; R^2 = R^4 = H; R^3 = OAc
IVb, R^1 = OH; R^2 = R^3 = H; R^4 = OAc
IVe, R^1 = R^3 = H; R^2 = OH; R^4 = OAc

IVc

IVd, R^1 = OH; R^2 = H
IVf, R^1 = H; R^2 = OH

The second minor component was a hydroxy acetate of an elemental composition $C_{22}H_{34}O_3$ ($M^{+\bullet}$, *m/z* 346). The molecular ion in the electron-impact mass spectrum

loses ketene (*m/z* 304) and acetic acid (*m/z* 286) followed by loss of a methyl group (*m/z* 271) or water (*m/z* 268). The infrared spectrum displayed absorption bands at 3 608 and 3 525 cm^{-1} (hydroxyl), 1 725 and 1 252 cm^{-1} (acetate) and 1 658 cm^{-1} (double bonds). The ^{13}C NMR spectrum (Table I) revealed that the compound contained three trisubstituted double bonds, each carrying a methyl, as also confirmed by the presence of three methyl multiplets and three signals of olefinic protons in the ^{1}H NMR spectrum. As further structural features we have established a tertiary methyl, and secondary hydroxyl (δ 4·20) and acetoxyl (δ 5·71). Proton decoupling experiments confirmed that the hydroxyl was not located at any allylic position and that both oxygenated substituents are not mutually vicinal. The multi-

TABLE I
The ^{13}C NMR spectra of *IV*

Carbon type	$\delta^{13}C^a$	$\delta^{13}C^b$	APT amplitude	SFORD multipl.
C=O	169·63	170·09	(−)	s
>C=	140·23	140·68	(−)	s
>C=	137·60	138·08	(−)	s
—CH=	131·88	132·50	(+)	d
—CH=	131·46	132·14	(+)	d
>C=	129·05	129·05	(−)	s
—CH=	127·36	127·51	(+)	d
—CH—O	72·31	71·84	(+)	d
—CH—O	69·39	69·58	(+)	d
—CH_2—	45·29	45·52	(−)	t
—C—	42·58	43·09	(−)	s
—CH_2—	38·70	39·03	(−)	t
—CH_2—	37·59	37·79	(−)	t
—CH_2—	35·99	36·19	(−)	t
—CH—	33·31	33·86	(+)	d
—CH_2—	26·11	26·39	(−)	t
—CH_2—	25·80	26·12	(−)	t
CH_3—	24·63	24·93	(+)	q
CH_3—	21·09	21·15	(+)	q
CH_3—	20·68	20·68	(+)	q
CH_3—	16·34	16·38	(+)	q
CH_3—	14·82	14·93	(+)	q

[a] In $C_6{}^2H_6$, the central solvent line used as reference (δ 128·0); [b] in $(C^2H_3)_2CO$, the central solvent line used as reference (δ 29·8).

plicity of the CH—OH proton signal (dd, J = 11·6 and 4·3 Hz) indicates a subsystem —CH_2—CH(OH)—C—. The proton of the CH—$OCOCH_3$ group appears as a multiplet at δ 5·71 (J = 10·9, 10·5 and 5·1 Hz). Irradiation of the latter leads to a partial collaps of the olefinic proton signal at δ 4·92, which gives evidence of a molecular fragment —CH_2—CH($OCOCH_3$)—CH=C< with an allylic acetoxy group. These findings, however, did not answer the question of the underlying carbon framework, although it was clear that the hydroxy acetate must have a bicyclic diterpene skeleton incorporating three double bonds. Hitherto only a single bicyclic skeleton, a 7,16--secotrinervitane, has been reported for compounds isolated from termites[9]. The spectral data alone of our hydroxy acetate do not constitute a positive proof, but they are in a general agreement with this skeleton and therefore we assume its presence in this case, too. Under this assumption, the trisubstituted double bonds should be localized in positions bearing the methyl groups, *i.e.* 7(8) or 8(9), 11(12) or 12(13) and 15(16), which gives six possible structures *IVa–f* for our hydroxy acetate. To decide among these, we have examined the ν(OH) bands in the infrared spectrum (intramolecular hydrogen bond) and the effect of the shift reagent in the ^{1}H NMR spectrum, but neither of these measurements led to definite conclusions. However, the position of the hydroxyl group could be specified on the basis of the ^{1}H NMR spectrum. The vicinal coupling constants of the CH—OH proton suggest a molecular fragment —CH_2—CH(OH)—C— with dihedral angles and coupling constants shown in Fig. 1. Such a clear-cut, synclinal conformation appears to be consistent with a hydroxyl group located rather in the six-membered ring, than in the more flexible fourteen-membered ring. If the hydroxy acetate possesses one of the structures *IVa–d*, the hydroxyl at $C_{(3)}$ should be equatorial to conform to the observed couplings of the $H_{(3)}$ proton. Unfortunately, this fact does not give sufficient evidence of the hydroxyl configuration, as the actual conformation of the six-membered ring is also unknown and may not be trivial due to the 1,4-bridging. Taking into account the biosynthetic pathway to the 7,16-secotrinervitane skeleton, the structures *IVa,b* appear more probable, for the location of the double bonds resembles that of cem-

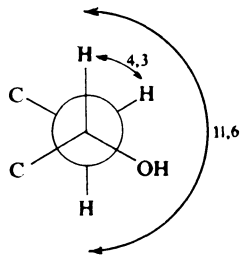
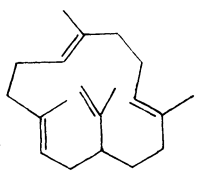


FIG. 1

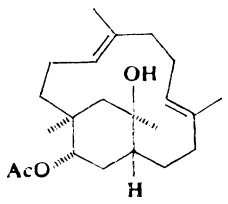
Newman projection of dihedral angles of the C–H bonds in the hydroxy acetate *IV*

brene A (*V*). The latter is considered as an intermediate in the biosynthesis of all diterpene skeletons found in termites[4,5]. The structures *IVa,b* are similar to that of hydroxyacetate *VI*, the only 7,16-secotrinervitane of known structure, established by X-ray diffraction[9]. Our hydroxy acetate for which we suggest alternative structures *IVa* or *IVb* thus represents another bicyclic diterpene from the series of termite defense substances.

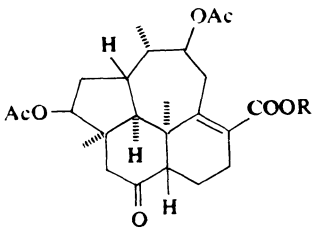
The third minor component reported in the present work is a diterpenic acid *VII* which was isolated as the methyl ester *VIII* after diazomethane esterification of the most polar chromatographic fraction. The mass spectrum of *VIII* showed the mole-



V



VI



VII, R = H
VIII, R = CH_3

cular ion at *m/z* 446 ($C_{25}H_{34}O_7$) and fragments at *m/z* 414 ($C_{24}H_{30}O_6$, $(M-CH_3.OH)^{+\bullet}$), 386 ($C_{23}H_{30}O_5$, $(M-AcOH)^{+\bullet}$), 354 ($C_{22}H_{26}O_4$, $(M-CH_3OH-AcOH)^{+\bullet}$), 326 $(M-2\,AcOH)^{+\bullet}$ and 294 $(M-2\,AcOH-CH_3OH)^{+\bullet}$. A skeletal fragmentation provided ions $C_{16}H_{21}O_4^+$ (*m/z* 277) that further eliminated acetic acid (*m/z* 217 and 157). Hence the compound *VIII* contains two acetoxy groups and one methoxycarbonyl group. The presence of these functions was also evident from the infrared (1 740, 1 250 cm^{-1}) and ^1H NMR spectra (δ 1·99 s and 2·05 s, δ 3·69 s). The signals of the —CH(OAc)— protons appear as a doublet of doublets (δ 4·79) and a quartet (δ 5·05, $J \approx 7{\cdot}8$ Hz) which are not mutually coupled. This points to molecular fragments $-CH_2-CH(OAc)-\underset{|}{C}-$ and $-CH_2-CH(OAc)-\underset{|}{CH}-$, respectively. The ^1H NMR spectrum further showed two singlets of tertiary methyl groups (δ 1·19 and 1·27) and a doublet of a secondary methyl (δ 0·97). The presence of other functional groups, namely, a ketone (1 720 cm^{-1}) and a double bond (1 638 cm^{-1}) was deduced from the infrared spectrum. As the ^1H NMR spectrum does not exhibit olefinic protons, the double bond must be a tetrasubstituted one. Combining the elemental composition ($C_{25}H_{34}O_7$) and the deduced unsaturation, the ester *VIII* must possess a tetracyclic skeleton with two acetoxyls, one methoxycarbonyl and one oxo group. The position of the ketone ν(C=O) band (1 720 cm^{-1}) in the infrared spectrum rules out a cyclopentanone fragment.

Further evidence for the tetracyclic skeleton stemmed from the ^{13}C NMR spectrum summarized in Table II. The spectrum measured in deuteriochloroform displayed 21 carbon signals with a coincidence of two methyls (δ 21·27), two acetate carbonyls

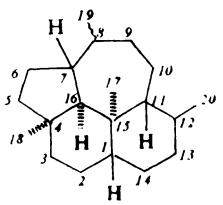
TABLE II
^{13}C NMR spectra of *VIII*

Carbon type	δ^{13}C (APT amplitude)			
	C^2HCl_3		$C^2H_3COC^2H_3$	
C=O	209·10	(−)	[a]	
C=O (OAc)	170·52[b]	(−)	170·62	(−)
C=O (OAc)			169·23	(−)
C=O ($COOCH_3$)	160·70	(−)	159·81	(−)
>C=	121·76	(−)	120·65	(−)
>CH—O	[c]		77·33	(+)
>CH—O	74·38	(+)	75·11	(+)
>CH—	54·12	(+)	55·00	(+)
>CH—	53·22	(+)	54·04	(+)
—C— | —OCH_3	51·99	(~0)[d]	52·93	(−)
			51·97	(+)
—CH_2—	44·30	(−)	44·41[e]	(−)
—CH_2—	43·90	(−)		
—C—	41·04	(−)	41·54	(−)
>CH—	36·16	(+)	36·77	(+)
>CH—	31·78	(+)	32·30	(+)
—CH_2—	30·57	(−)	31·21	(−)
—C—	29·70	(−)	[f]	
—CH_3	26·17	(+)	26·16	(+)
—CH_2—	24·45	(−)	25·20	(−)
—CH_3	23·57	(+)	24·08	(+)
—CH_2—	23.21	(−)	23.89	(−)
—CH_3 (OAc)	21·27[b]	(+)	21·15	(+)
—CH_3 (OAc)			21·00	(+)
—CH_3	19·64	(+)	19·81	(+)

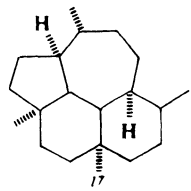
[a] Overlaps with the C=O signal of acetone; [b] two acetate carbonyls overlapped; [c] overlaps with the C^2HCl_3 lines; [d] the overlap of these signals leads to increased intensity in the noise-decoupled spectrum; the intensity in the APT spectrum is practically zero; [e] two signals overlapping; [f] obscured by the C^2H_3 line of the solvent.

(δ 170·52) and other two signals at δ 52·0. Recording the spectrum in hexadeuterioacetone distinguished both acetate groups, the signals at δ 52·0 (now δ 51·97 and 52·93) and also revealed the last missing carbon signal at δ 77·33 which previously coincided with the deuteriochloroform lines. The number of primary, secondary, tertiary and quaternary carbon atoms in *VIII* was deduced from the ^{13}C NMR spectrum, using the "attached proton test" (APT) pulse sequence[11]. An interesting case occurred in the APT spectrum measured in deuteriochloroform in which the carbon signal at 52·0 disappeared due to a superposition of two signals with an opposite amplitude. These signals were discerned by running the APT spectrum in hexadeuterioacetone. Another piece of structural information was obtained from the CD and UV spectra. The values $\Delta\varepsilon_{212} = -17{\cdot}32$ and $\Delta\varepsilon_{252} = +4{\cdot}40$, and the absorption band at 223·5 nm (ε = 9 180) were assigned to an α,β-unsaturated ester. The values pertinent to the keto group ($\Delta\varepsilon_{297} = -6{\cdot}16$ and λ_{max} = 300 nm, ε = 8) ruled out both α,β- and β,γ-enone arrangements, so that the keto group must be located in a saturated ring.

All the spectral data appeared to be compatible with a kempane (*A*) or rippertane (*B*) type of skeleton. For these, 34 possible structures could be suggested which



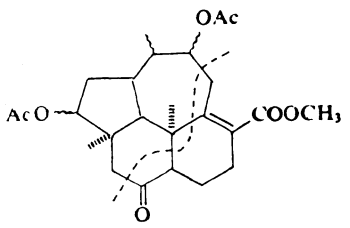
A



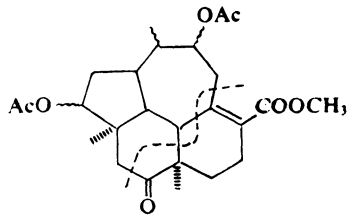
B

essentially agreed with the IR, NMR and UV data. A large part of the structural possibilities could be eliminated on the basis of the mass spectral fragmentation of the tetracyclic skeleton. The fragmentation map, constructed from the observed decompositions of metastable ions, showed a sequence $M^{+\bullet} \rightarrow m/z$ 277 ($C_{16}H_{21}O_4^+$) $\rightarrow m/z$ 217 ($C_{14}H_{17}O_2^+$) $\rightarrow m/z$ 157 ($C_{12}H_{13}^+$). The twofold loss of the $C_2H_4O_2$ molecule undoubtedly corresponds to a stepwise elimination of acetic acid. An alternative loss of methyl formate could be ruled out, for these skeletal fragments did not lose methanol as could have been anticipated for even-electron ions containing the methoxycarbonyl group. It follows that the fragment ion at m/z 277 contains both acetoxy groups and its formation is due to loss of a $C_{(8)}$ neutral part carrying the keto and methoxycarbonyl functions. The skeletal fragmentation is accompanied by transfer of three hydrogen atoms onto the neutral fragment. If we exclude fragmentation between the carbons of the double bond and a simultaneous formation of more than two fragments, the observed scission of the skeleton would be consistent with

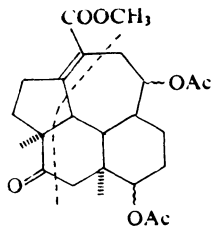
four possible structures *VIIIa–d*, two with kempane and two with rippertane skeletons. Of these, the structures *VIIIb* and *VIIId* appear less probable, for the skeletal fragmentation would neccessitate cutting out one sp^2 carbon.



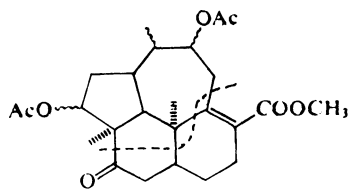
VIIIa



VIIIb

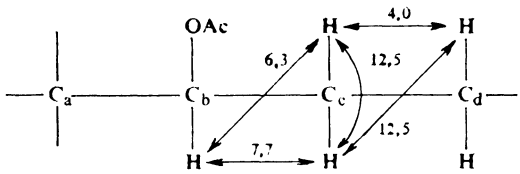


VIIIc



VIIId

The analysis of the ^{1}H NMR data yielded further useful information. The —CH—OAc proton at δ 4·79 has two vicinal couplings, J = 7·7 and 6·3 Hz. An arrangement —CH—CH(OAc)—CH— can be excluded, since this can be realized on neither of the two skeletons. This favors the molecular fragment —CH_2—CH(OAc)—C—, which is corroborated by decoupling experiments. Irradiation of the proton at δ 4·79 made it possible to distinguish the multiplet of the adjacent methylene group at δ 1·44 (J = 12·5, 12·5 and 7·7 Hz) and δ 1·98 (J = 12·5, 6·3 and 4·0 Hz). The next carbon atom (C_d Scheme 1) bears one proton. Therefore,



SCHEME 1

C_d is either in a bridgehead position or it carries the secondary methyl group. The latter case was ruled out by proton decoupling: irradiation of the proton at δ 2·20

led to a collaps of the methyl doublet, while the signals of the C_c methylene group were unaffected. These results clearly localized the acetoxy groups at positions $C_{(5)}$ and $C_{(9)}$. The surroundings of the ketone moiety were also examined through the ^1H NMR spectrum. One of the two protons of the methylene adjacent to the keto group appears as a doublet at δ 2·89 (J = 13·2 Hz). The signal of the second methylene proton falls within the group at δ 2·20, but it could be discerned by decoupling. The methylene protons form an isolated AB system, which in turn localizes the ketone function at $C_{(2)}$. This would favor the alternative structures *VIIIa* and *VIIIb*, the second of which, however, appears very unlikely from the mass spectral point of view. We conclude that the structure *VIIIa* is the only one compatible with all spectral data.

The configuration at tertiary carbon centers 5, 8 and 9 was deduced from the coupling constants in the ^1H NMR spectrum. The angular hydrogen at $C_{(7)}$ (the C_d carbon atom in Scheme 1) of the kempane skeleton has a β-configuration. The corresponding coupling constants with the H—$C_{(6)}$—H protons amount to 12·5 and 4·0 Hz, which gives evidence of a *trans* and *gauche* arrangement with dihedral angles approaching 180° and 60°, respectively (Fig. 2*a*). This geometry requires an envelope conformation (E_{16}) of the five-membered ring. The $J_{5,6}$ coupling constant is 7·7 Hz (near synperiplanarity of $H_{(5)}$ and $H_{(6)}$), while $J_{5,6'}$ = 6·3 Hz due to nearly anticlinal arrangement of $H_{(5)}$ and $H_{(6')}$. The $H_{(6)}$ and $H_{(7)}$ protons are antiperiplanar ($J_{6,7}$ = 12·5 Hz). This combination of coupling constants is possible only for β configuration of the 5-acetoxy group (Fig. 2*b*), in which both the coupling constants can be similar, corresponding to dihedral angles *c.* 20° and 140° in accordance with the Karplus equation. An opposite configuration of the 5-acetoxy group is incompatible with the vicinal coupling constants: in Newman projection the $H_{(5)}$ proton would fall between $H_{(6)}$ and $H_{(6')}$ so that one $J_{5,6}$ constant as well as the sum $J_{5,6} + J_{5,6'}$ should be substantially smaller. The conformation of the five-membered ring is unequivocally determined by conformation of the adjacent six- and seven-membered rings. To conform to the vicinal couplings of $H_{(5)}$, $H_{(6)}$, $H_{(6')}$ and $H_{(7)}$, the molecule of *VIII* should assume the conformation shown in Fig. 3, in which the six-membered ring is in a chair form while the seven-membered ring assumes a boat form. The latter is supported by vicinal coupling constant of the

FIG. 2

Newman projection of dihedral angles of the C–H bond in *VIII*: *a* Projection along the $C_{(6)}$—$C_{(7)}$ bond; *b* Projection along the $C_{(5)}$—$C_{(6)}$ bond

$H_{(9)}$ proton (δ 5·05 q, J = 7·8 Hz). Irradiation of this proton causes observable changes in the shape of the multiplet at δ 2·20 in which region there are located all three vicinal protons. The apparent coupling of the $H_{(9)}$ proton may not be due to true coupling constants because of second-order effects. It appeared more reliable to take into account the sum of coupling constants given by the multiplet width (23·5 Hz). It follows from this value that $H_{(9)}$ should be antiperiplanar to two adjacent protons, at least. For the boat conformation of the seven-membered ring we then obtain equatorial configuration of both the 9β-acetoxyl and 8α-methyl group. Such a diequatorial substitution may be the cause for the stability of the cycloheptane boat conformation in this case.

On the basis of the above arguments we suggest the kempane structure *VII* for the acid isolated from the species *N. costalis*. The acid *VII* appears rather unusual in comparison with other components of the termite defense arsenal. Hitherto, no other carboxylic acid has been isolated that would have been derived from the diterpene skeletons found in termites.

Derivatives of the Isolated Diols I *and* II

Diols *I* and *II*, isolated as major components of the defense secretion, were acetylated with acetic anhydride in pyridine as usual. Diol *I* afforded both monoacetates *IX* and *X* in approximately 1 : 1 ratio (Scheme 2). The monoacetates *IX* and *X* have different R_F values and can be separated by column chromatography. On prolonged reaction time we obtained the diacetate *XI*. The acetylation proceeded cleanly, and the reverse hydrolysis of *IX* afforded again the diol *I*. The monoacetates *IX* and *X* were distinguished through their ^{1}H NMR (Table III) and mass spectra. With the diols *I* and *II*, the signals of the $H_{(2)}$ and $H_{(3)}$ protons differ in both the chemical shift and the shape of multiplets[8]. The $H_{(3)}$ proton appears as a doublet at δ 3·2–3·9, whereas the $H_{(2)}$ proton shows a doublet broadened by allylic interaction with the 15-methyl. The $H_{(2)}$ signal is shifted to δ 4·1 due to the effect of the adjacent double bond. Acetylation of the 3-hydroxyl, besides shifting the signal

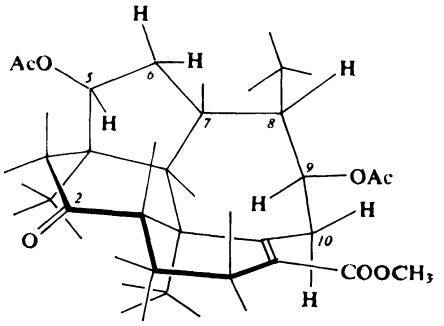
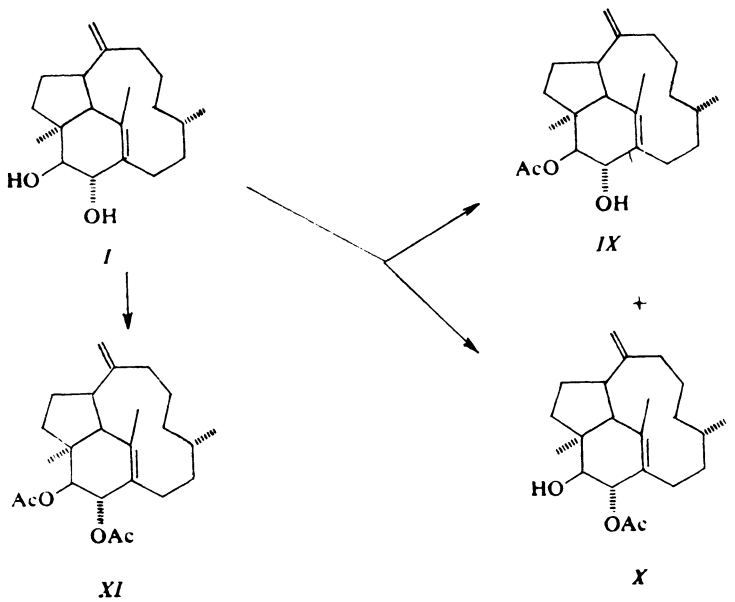


FIG. 3
Model conformation of the skeleton in *VIII*

of the $H_{(3)}$ proton downfield by *c.* 1·5 ppm, also affects the shift of the protons of the 4-methyl group. In contrast, the shift of the methyl group appears unaffected by acetalytion of the 2-hydroxyl in *I* and *II* (Table III).



SCHEME 2

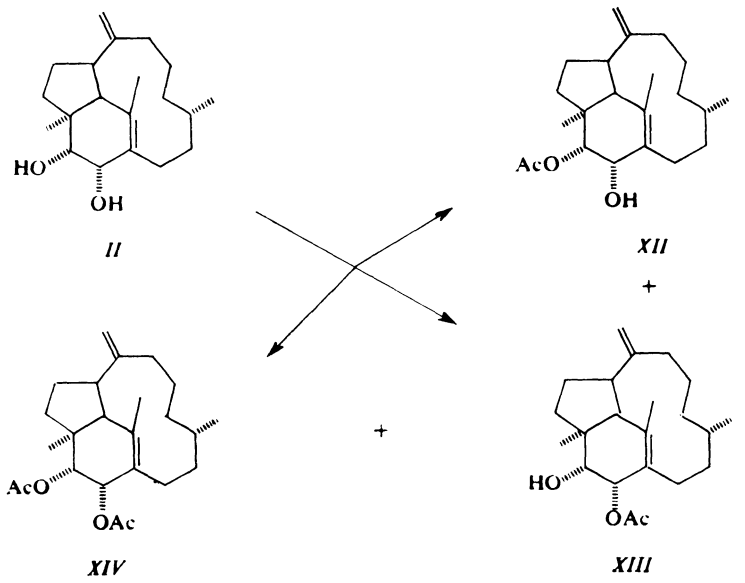
Acetylation of the diol *II* afforded mainly the 3-monoacetate *XII* accompanied by traces of the second isomer *XIII* (Scheme 3). The isomeric acetates turned out to be unseparable by column chromatography and so the characterization of each was achieved through ^{1}H NMR spectra in the same way as done for monoacetates *IX* and *X*. The positional assignment of the acetoxy groups in *IX*, *X* and *XII* is supported by mass spectra. Following ionization, the 3-monoacetates exhibit only insignificant $(M-H_2O)^{+\cdot}$ ions (2% and 1% of the base peak for *IX* and *XII*, respectively), while the 2-acetate *X* eliminates water to a much greater extent (19% of the base peak). A similar, although not so pronounced effect is found for the loss of acetic acid (44, 40 and 96% of the base peak for *IX*, *XII* and *X*, respectively). The enhanced propensity for the elimination of acetic acid from ionized *X* may be due to the allylic position of the acetoxy groups.

It is noteworthy that the acetylation of *I* affords a 1 : 1 mixture of monoacetates, while *II* gives mainly the 3-monoacetate. This may be connected with conformation of the six-membered ring. The hydroxy groups in *I* (2α, 3β-OH) and *XV* (2β, 3α-OH) are known to be oriented equatorially[8], which means that the compounds must differ in ring conformation. The ring conformation in *II* (2α, 3α-OH) is not deducible from the ^{1}H NMR spectrum, for the vicinal coupling constants $J_{2,3}$ should be

TABLE III

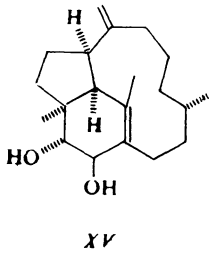
^{1}H NMR spectra of diols *I*, *II* and acetates *IX* – *XIV*

Proton	δ, ppm (*J*, Hz)							
	compound							
	I	*IX*	*X*	*XI*	*II*	*XII*	*XIII*	*XIV*
2-H	4·18 bd (8·5)	4·28 bd (9·0)	5·64 bd (9·0)	5·78 bd (8·9)	4·08 bt (5·0; 5·0)	4·16 bd (4·6)	5·51 nd (5·0)	5·57 bd (4·7)
3-H	3·22 d (8·5)	4·74 d (9·0)	3·38 m	4·93 d (8·9)	3·91 dd (5·0; 8·0)	5·32 d (4·6)	4·14 d (5·0)	5·40 d (4·7)
7-H	3·38 bt (9·0; 9·0)	3·37 dt (9·5; 9·5; 2·5)	3·38 m	3·39 dt (9·8; 9·6; 2·5)	3·25 dt (9·5; 9·5; 11·5)	3·23 m		3·28 dt (9·5; 9·5; 11·5)
16-H	2·60 bd (10·0)	2·66 bd (10·0)	2·63 bd (10·0)	2·69 bd (10·5)	2·53 bd (12·0)	2·55 bd (12·0)		2·58 bd (12·0)
17-H	1·70 d (1·8)	1·71 d (1·5)	1·72 d (1·5)	1·73 bs	1·78 bs	1·79 bs		1·81 bs
18-H	1·17 s	1·04 s	1·17 s	1·04 s	1·10 s	1·23 s	1·11 s	1·18 s
19-H	4·88 m	4·89 t	4·88 t	4·90 m	4·96 m	4·96 m		4·97 m
19-H′	4·80 m	4·82 m	4·81 m	4·82 q	4·84 m	4·85 m		4·89 m
20-H	0·89 d (6·2)	0·88 d (6·0)	0·83 d (6·0)	0·83 d (6·0)	0·89 d (6·0)	0·89 d (6·0)		0·90 d (6·0)
OAc	—	2·13 s	2·16 s	2·04 s 2·05 s	—	2·18 s	2·09 s	2·04 s 2·05 s



SCHEME 3

similar for both forms. The acetylation is known to proceed faster with equatorial hydroxyl groups, compared with axial ones. The preferential acetylation of the $C_{(2)}$



XV

hydroxyl in *II* can be therefore interpreted as being due to an equatorial position, while the $C_{(3)}$ hydroxyl is axial. Hence it follows that the *cis*-diol *II* assumes the same conformation as the *trans*-isomer *XV*.

EXPERIMENTAL

Melting points (uncorrected) were determined on a Kofler block. The infrared spectra were measured in potassium bromide micropellets (1·5 mm diameter) on a Perkin–Elmer 621 spectrophotometer. The mass spectra were recorded on AEI MS 902 and Jeol D-100 instruments. The NMR spectra were obtained on a Varian XL-200 instrument (200·06 and 50·31 MHz for ^1H and

^{13}C, respectively) in deuteriochloroform, hexadeuteriobenzene and hexadeuterioacetone (Aldrich, Merck) with tetramethylsilane as internal reference. The chemical shift values are given in δ (ppm) scale. CD spectra were obtained on a Roussel-Jouan 185 dichrograph in methanolic solution. UV spectra were taken on a Cary 219 spectrometer in methanol. Column chromatography was performed on silica gel (Herrmann, Koln-Ehrenfeld) containing 15% of water. Preparative thin layer chromatography was performed on Merck (Darmstadt) 60 PF_{254} silica gel with a UV indicator. For analytical purposes we have used 60G silica gel; detection was achieved by spraying with sulfuric acid and thermal charring. The purity of the isolated compounds was checked by gas chromatography (Chrom 4, Laboratory Instruments, Prague), column OV-17, 3% on Gas Chrom Q (100–200 mesh), length 2·5 m, 3 mm i.d.

Isolation Procedure

Soldiers of the species *N. costalis* (collected on Cuba) were extracted as described in ref.[8]. The extract (1·56 g) was chromatographed on a silica gel column (100 g), eluted with a mixture of benzene and ether (0–5%).

1(15),8(19)-*Trinervitadien*-2α,3β-*diol* (I): Elution with benzene–ether (5%) afforded 250 mg of *I* which was crystallized from ethanol. M.p. 86–90°C, all spectral data identical with those of a standard sample[8].

1(15),8(19)-*Trinervitadien*-2α,3α-*diol* (II): Elution with benzene–ether (1%) gave 90 mg of *II* which was further chromatographed in chloroform–ethyl acetate (0·5%) and then crystallized from light petroleum. M.p. 74–77°C, all spectral data identical with those of a standard[8].

1(15),8(19)-*Trinervitadien*-9β-*ol* (III): Elution with benzene–ether (0·5%) gave 45 mg of *III* which was crystallized from light petroleum–ether. M.p. 128–130°C, all spectral data identical with those of a standard[12].

Hydroxy Acetate *IVa* or *IVb*

Elution with benzene–ether (1%) afforded 21 mg of amorphous *IVa* or *IVb*, which was purified by second chromatography (chloroform–ethyl acetate, 0·1%) to yield again an amorphous product. Mass spectrum (*m/z*, rel. int. %) 346 ($M^{+\cdot}$, 18; $C_{22}H_{34}O_3$), 328 (1), 304 (2), 286 (65; $C_{20}H_{30}O$), 271 (26), 268 (7), 253 (11), 243 (7), 187 (20; $C_{13}H_{15}O + C_{14}H_{19}$, 2 : 3), 175 (37), 136 (100), 135 (95). IR spectrum: 3 608, 3 525 (OH), 1 658, 1 602 (C=C), 1 725, 1 252 (OAc) cm^{-1}. ^1H NMR spectrum ($^2H_6$-acetone): 0·87 s (tert-CH_3), 1·41 q, J = 1·5, 1·5 and 1·5 Hz (CH_3—C=), 1·64 t, J = 1·5 and 1·4 Hz (CH_3—C=), 1·77 d, J = 1·5 Hz (CH_3—C=), 1·94 s (Ac), 3·32 d, J = 4·5 Hz (OH), 4·25 dt, J = 11·6, 4·5 and 4·5 Hz (CHOH), 4·90 p, J = 3·0, 1·5, 1·5 and 1·5 Hz (16-H), 4·92 bd, J = 10·2, 1·5, 1·5, 1·5 and 0·7 Hz (H—C=), 5·33 bdt, J = 10·6, 2·4, 2·4, 1·5, 1·5 and 1·5 Hz (H—C=), 5·71 ddd, J = 11·2, 10·3 and 5·1 Hz (CHOAc); ^1H NMR spectrum (^2H-chloroform): 0·91 s (tert-CH_3), 1·39 q, J = 1·2, 1·2 and 1·2 Hz (CH_3—C=), 1·64 t, J = 1·4 and 1·4 Hz (CH_3—C=), 1·77 d, J = 1·3 Hz (CH_3—C=), 2·01 s (Ac), 4·20 dd, J = 11·6, 4·3 Hz (CHOH), 4·90 p. J = 3·0, 1·5, 1·5 and 1·5 Hz, (16-H), 4·92 bd, J = 10·2, 1·5, 1·5, 1·5 and 0·7 Hz (H—C=), 5·30 bd, J = 10·6, 2·4, 1·5, 1·5 and 1·5 Hz (H—C=), 5·71 ddd, J = 10·9, 10·5 and 5·1 Hz (CHOAc); CD spectrum: $\Delta\varepsilon_{208} = +16{\cdot}50$ l mol^{-1} cm^{-1}.

Methyl 5β,9β-Diacetoxy-2-oxo-11(12)kempen-20-oate (*VIII*)

The fraction, eluted with ether, was further separated by thin layer chromatography (elution with chloroform–ethyl acetate, 8 : 2). The most polar fraction was esterified with diazomethane and the resulting mixture separated on a silica gel plate, giving 6 mg of amorphous ester *VIII*.

Mass spectrum (*m/z*, rel. int. %) 446 ($M^{+\cdot}$, 45; $C_{25}H_{34}O_7$), 414 (16; $C_{24}H_{30}O_6$), 386 (55; $C_{23}H_{30}O_5$), 371 (7), 354 (100; $C_{22}H_{26}O_4$), 339 (15), 326 (45), 311 (26; $C_{20}H_{23}O_3$), 294 (34), 277 (59; $C_{16}H_{21}O_4$), 266 (24; $C_{19}H_{22}O$), 217 (77; $C_{14}H_{17}O_2$), 157 (50; $C_{12}H_{13}$). IR spectrum: 1 740, 1 250 (OAc), 1 720 (C=O), 1 638 (C=C) cm^{-1}. ^1H NMR spectrum (^2H-chloroform): 0·97 d, J = 5·1 Hz (19-H), 1·19 s and 1·27 s (18-H and 17-H), 1·44 dt, J = 7·7, 12·5 and 12·5 Hz (6α-H), 1·98 ddd, J = 12·5, 6·3 and 4·0 (6β-H), 1·99 s (OAc), 2·05 s (OAc), 2·89 d, J = 13·2 Hz (3-H), 3·69 s ($COOCH_3$), 4·79 dd, J = 7·7 and 6·3 Hz (5α-H), 5·05 q, J = 7·8, 7·8 and 7·8 Hz (9α-H). CD spectrum: $\Delta\varepsilon_{297} = -6{\cdot}16$; $\Delta\varepsilon_{268} = 0$, $\Delta\varepsilon_{252} = +4{\cdot}40$, $\Delta\varepsilon_{240} = 0$, $\Delta\varepsilon_{212} = -17{\cdot}32$ l mol^{-1} cm^{-1}. UV spectrum: $\lambda_{sh} = 300$ ($\varepsilon = 8$), $\lambda_{max} = 223{\cdot}5$ ($\varepsilon = 9\,180$) nm.

Acetylation of 1(15),8(19)-Trinervitadien-2α,3β-diol (*I*)

a) Acetic anhydride (0·1 ml) was added to a solution of diol *I* (10 mg) in pyridine (0·4 ml). After 2·5 h the mixture was additioned with ice and worked up as usual. The product was chromatographed on a silica gel column (3 g). Elution with benzene–ether (0·2%) afforded first the 3-monoacetate *IX* (3 mg) and then the 2-monoacetate *X* (4 mg).

1(15),8(19)-*Trinervitadien*-2α,3β-*diol* 3-*acetate* (IX). Mass spectrum (*m/z*, rel. int. %) 346 ($M^{+\cdot}$; 3), 331 (1), 328 (2), 304 (20), 286 (44), 271 (11), 268 (6), 215 (8), 175 (26), 173 (10), 151 (14), 135 (100). IR spectrum: 3 580, 3 490 (OH), 3 080, 1 633, 909 ($C{=}CH_2$), 1 720, 1 251 (OAc) cm^{-1}.

1(15),8(19)-*Trinervitadien*-2α,3β-*diol* 2-*acetate* (X): Crystallized from pentane. m.p. 166 to 167°C (change of modification at 145–155°C). Mass spectrum (*m/z*, rel. int. %) 346 ($M^{+\cdot}$; 4), 328 (19), 304 (41), 286 (96), 271 (11), 268 (15), 215 (9), 175 (36), 173 (13), 135 (100). IR spectrum: 3 625, 3 580 (OH), 3 080, 1 633, 895 ($C{=}CH_2$), 1 726, 1 252 (OAc) cm^{-1}.

b) Acetic anhydride (0·2 ml) was added to a solution of diol *I* (7 mg) in pyridine (0·4 ml). The mixture was left for 3 days at ambient temperature and then worked up as above to yield 5 mg of 1(15),8(19)-trinervitadien-2α,3β-diol diacetate (*XI*). Mass spectrum (*m/z*), rel. int.%) 388 ($M^{+\cdot}$; 3), 346 (8), 328 (21), 304 (13), 286 (79), 271 (8), 268 (26), 253 (5), 175 (26), 151 (13), 135 (92), 43 (100). IR: 3 080, 1 633, 898 ($C{=}CH_2$), 1 740, 1 250 (OAc) cm^{-1}.

Acetylation of 1(15),8(19)-Trinervitadien-2α,3α-diol (*II*)

Diol *II* was acetylated according to the procedure *a*) described for *I*. The crude product was chromatographed on a silica gel column (3 g). Elution with benzene yielded the diacetate *XIV* (1·5 mg), elution with benzene–ether (0·2%) afforded the 3-monoacetate *XII* (3 mg), which contained traces of *XIV*.

1(15),8(19)-*Trinervitadien*-2α,3α-*diol* 3-*acetate* (XII): Mass spectrum (*m/z*, rel. int. %) 346 ($M^{+\cdot}$; 9), 331 (1), 328 (1), 304 (100), 286 (40), 271 (12), 268 (5), 215 (8), 175 (25), 173 (10), 151 (36), 135 (98).

1(15),8(19)-*Trinervitadien*-2α,3α-*diol diacetate* (XIV): Mass spectrum (*m/z*, rel. int. %) 388 ($M^{+\cdot}$, 7), 346 (21), 328 (37), 304 (56), 286 (100), 271 (9), 268 (43), 175 (21), 135 (75). The intensities are related to the highest-abundance peak of *m/z* above 100.

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