CONSTITUENTS OF FRONTAL GLAND SECRETION OF PERUVIAN TERMITES *Nasutitermes ephratae*

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The defense secretion of *Nasutitermes ephratae* soldiers was analyzed and its constituents were identified. The volatile fraction contains monoterpenic hydrocarbons α-pinene, camphene, sabinene, β-pinene, myrcene, 4-carene, 3-carene, α-terpinene, limonene, γ-terpinene, β-phellandrene, and terpinolene. From the non-volatile fraction four alcohols derived from trinervitane skeleton were isolated: 1(15),8(19)-trinervitadien-3α-ol (*I*), 1(15),8(19)-trinervitadien-2β,3α-diol (*II*) and its 2α,3α- (*III*) and 2α,3β- (*IV*) isomers. 3α-Hydroxy-1(15),8(19)-trinervitadien-2-one (*V*), which was also isolated, is probably an artefact formed during the work-up of the extract. The structure of 3β-hydroxy-1(15),8(9)-trinervitadien-2-one (*VI*) was determined on the basis of mass, IR and ^{1}H NMR spectra, comparison with model compounds and analogy with the literature. The absolute configuration of the trinervitane skeleton was studied by CD spectra of tris(2,2,6,6-tetramethyl-3,5-heptanedionato)praseodymium complexes of the 2,3-diols *II*–*IV* and by the ^{1}H NMR spectra of esters of the alcohol *I* with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid. The results obtained by both methods are identical and confirm the earlier suggested absolute configuration of the trinervitane skeleton.

The chemical defense is very widespread in the highly evolved termites of the *Nasutitermitinae* subfamily. The defense secretion is produced by the frontal gland of the soldiers and represents a mixture of monoterpenes and diterpenes. Only in several cases also sesquiterpenes have been found[1].

The *Nasutitermes ephratae* termites were collected in South American Peru. We studied the soldiers from six different nests at Pucallpa and Iquitos. The *N. ephratae* termites from Central American Panama have been already analyzed by Prestwich[2]. Our present communication concerns qualitative analysis of the defense secretion of this species whereas the varying content of monoterpenes and diterpenes in the secretion of soldiers in the indiviual nests and a comparison with the Panama termites of the same species[2] will be discussed elsewhere.

In the volatile fraction of the secretion we identified 12 monoterpenic hydrocarbons by the GC–MS technique. Their mass spectral data are summarized in Table I.

In the non-volatile fraction we have identified by mass, IR, and ^{1}H NMR spectra the following four known diterpenic alcohols, derived from the tricyclic system

trinervitane: 1(15),8(19)-trinervitadien-3α-ol (*I*) and 1(15),8(19)-trinervitadien-2β, 3α-diol (*II*), found originally in the *Trinervitermes bettonianus* species[3], and further 1(15),8(19)-trinervitadien-2α,3α-diol (*III*) which, together with 1(15),8(19)-trinervitadien-2α,3β-diol (*IV*), had been described for the first time in the *Nasutitermes costalis* species[4]. The originally suggested structure *Ia* has been recently revised by Prestwich in favour of the structure *I* on the basis of a detailed analysis of ^{1}H and ^{13}C NMR spectra (Deuring L. A., Prestwich G. D.: personal communication). The NMR data obtained by us are fully consistent with the revised structure *I*.

TABLE I

Mass spectra of monoterpenic components of the *Nasutitermes ephratae* soldier secretion

Compound	MS fragmentation, *m/z* (%)				
α-Pinene	93 (100)	92 (36)	91 (47)	79 (28)	77 (36)
Camphene	121 (55)	93 (100)	79 (42)	77 (36)	67 (30)
Sabinene	119 (19)	93 (100)	91 (48)	79 (22)	77 (34)
β-Pinene	93 (100)	91 (37)	79 (27)	77 (32)	41 (45)
Myrcene	93 (100)	91 (22)	77 (17)	69 (75)	41 (50)
4-Carene	121 (64)	93 (100)	91 (55)	79 (31)	77 (37)
α-Terpinene	136 (43)	121 (96)	93 (100)	91 (52)	77 (36)
3-Carene	93 (100)	92 (24)	91 (47)	79 (27)	77 (31)
Limonene	93 (100)	91 (40)	79 (49)	68 (92)	67 (89)
γ-Terpinene	136 (22)	93 (100)	91 (50)	79 (23)	77 (36)
β-Phellandrene	136 (22)	93 (100)	91 (42)	77 (30)	44 (33)
Terpinolene	136 (65)	121 (79)	93 (100)	91 (51)	79 (39)

These four trinervitane derivatives represent the principal constituents of the soldiers' secretion from all the six colonies studied. The by far most abundant constituent in all the secretion samples is the diol *IV* which is one of the most common trinervitane derivatives described for the *Nasutitermitinae* subfamily[1]. It is interesting that the diterpenes in *N. ephratae* appear only in the form of free alcohols or ketones and that, unlike in other species, no acetate has been detected.

As a trace constituent we identified the hydroxy ketone *V*, first described in *Trinervitermes bettonianus*[5]. It has been found that this compound is easily formed from the 2β,3α-diol *II* by oxidation with air or traces of peroxides in ether[4]. Small amounts of *V* often accompanied the diol *II* in our extracts. In accord with the literature[4] we assume that *V* is probably an artefact arising during the work-up of the extract.

Further we isolated the hitherto undescribed hydroxy ketone *VI*. Its IR spectrum exhibits bands due to a hydroxyl (3 470 cm^{-1}), a conjugated ketone (1 653 cm^{-1}) and a double bond (1 620 cm^{-1}) but no exomethylene band. The structural similarity of the hydroxy ketones *V* and *VI* follows from their MS fragmentation. The mass spectra of both compounds show characteristic fragments at *m/z* 231 and 151. The ion *m/z* 231 arises probably by allylic cleavage between the $C_{(13)}$ and $C_{(14)}$ atoms followed by cleavage in the other allylic position, between $C_{(9)}$ and $C_{(10)}$. The loss of the C_5H_{11} fragment, containing the atoms $C_{(10)}$, $C_{(11)}$, $C_{(12)}$, $C_{(13)}$, and $C_{(20)}$, gives rise to the ion *m/z* 231 ($C_{15}H_{19}O_2$). Then the five-membered ring is cleaved, losing a C_6H_8 fragment, with simultaneous transfer of one hydrogen atom to the charged fragment. The whole process leads to the ion *m/z* 151 of composition $C_9H_{11}O_2$. For compound *V* this is the main fragmentation pattern. In the case of the hydroxy ketone *VI*, this pathway also operates but the prevalent fragmentation course involves the loss of methyl from the molecular ion. Both these concurrent fragmentations are compared in Table II. The Table contains also data for the dihydroxy ketone *VII* isolated from *Nasutitermes hubbardii*[6]. The hydroxyl in position 13 apparently enhances fission of the eleven-membered ring and fragmentation to the ion *m/z* 151. The loss of methyl in the spectrum of *VII* is insignificant.

Although the mass spectra confirm the same skeleton as well as the same arrangement on the six-membered ring in the hydroxy ketones *V* and *VI*, the compounds obviously differ in the position of the double bond. The ^1H NMR spectrum of *VI* displays two signals of methyl groups at a double bond (δ 1·42 bs and 1·43 d) and a signal of one olefinic proton (δ 4·80 bdd). Thus, in addition to the tetrasubstituted 1(15) double bond, the molecule contains also a trisubstituted 8(9) double bond. Several trinervitane derivatives with double bond in this position are known[1].

^1H NMR data for compounds *V* and *VI* in hexadeuteriobenzene are given in Table III. The spectra of both compounds exhibit signals of CH—O protons in position 3 (δ 4·50 for *V* and δ 4·28 for *VI*) and of hydroxyl protons (δ 4·01 and 4·00 for *V* and *VI*, respectively). Spectrum of *V* shows also the vicinal coupling between these

protons (^3J(CH, OH) = 2·4 Hz). The position and shape of the mentioned signals indicate a similar arrangement of the keto and hydroxy groups in *V* and *VI*. Configuration of the hydroxyl in position 3 in compound *VI* was determined by the ***in situ*** acylations of *V* and *VI* with trichloroacetyl isocyanate (TAI); the pertinent data are also given in Table III. Upon TAI-acylation, the hydroxyl proton signal disappears in the spectra of both compounds and the H-3 signal is shifted markedly downfield (1·54 and 1·56 ppm for *V* and *VI*, respectively). Acylation of *VI* results, moreover, in a marked downfield shift of signals due to the H-9 and H-20 protons (0·36 and 0·14 ppm, respectively), separated by six or seven bonds from the acylated hydroxyl. Compound *V* has no proton in the position 9, however, the H-20 proton signal is much less affected (0·05 ppm). We can thus conclude that the acyl group in *VI* is probably sufficiently close to the H-9 and H-20 atoms to influence them. Therefore, the acylated hydroxy group has the 3β-configuration, since analysis of models shows that only an axial 3β-O-acyl meets the above-discussed requirements of steric proximity.

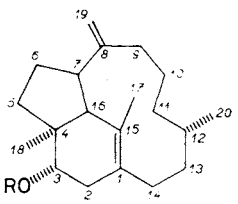
This conclusion is supported also by the IR spectral studies of intramolecular hydrogen bond in the hydroxy ketones *V* and *VI* and the dihydroxy ketone *VII*. We compared the spectra of these compounds with those of 5-hydroxy-5α-cholest-2--en-6-one (*VIII*)[7] (Table IV). The well-defined geometry of axial hydroxyl in this model compound (see formula *A*) does not permit any intramolecular hydrogen bond to the ketone or the double bond (the spectrum shows only a free hydroxyl band at 3 602 cm^{-1} and an unaffected carbonyl band at 1 713 cm^{-1}). On the other hand, the hydroxyl in *V*, *VI*, and *VII* is completely hydrogen-bonded (band at 3 480, 3 490 and 3 506 cm^{-1}, respectively) and no free hydroxyl band appears in their

TABLE II

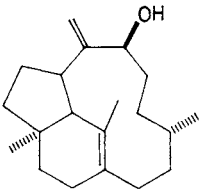
Mass spectral fragmentation of 3-hydroxy-2-trinervitanones

Compound	MS fragmentation, *m/z* (%)				
	M^+	$[M-15]^+$	$[M-33]^+$	$[M-C_5H_{10}R]^+$	$[M-C_5H_{10}R-C_6H_8]^+$
V[a]	302 (100)	287 (22)	269 (3)	231 (21)	151 (85)
VI[a]	302 (73)	287 (100)	269 (40)	231 (5)	151 (36)
VII[b]	318 (17)	303 (5)	285 (6)	231 (3)	151 (100)

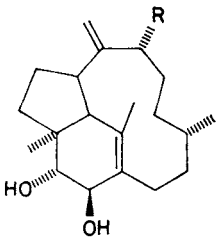
[a] R H; [b] R = OH.



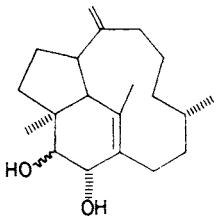
I, R = H
XII, R = (*R*)-MTPA
XIII, R = (*S*)-MTPA



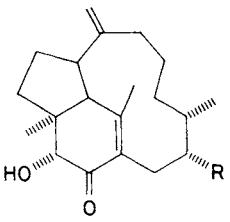
Ia



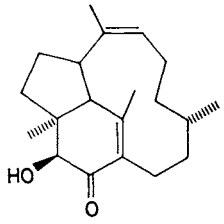
II, R = H
XI, R = OAc



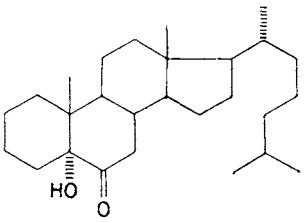
III, 3α-OH
IV, 3β-OH



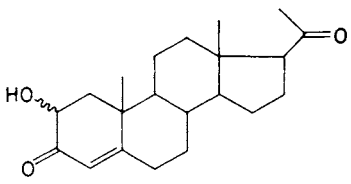
V, R = H
VII, R = OH



VI



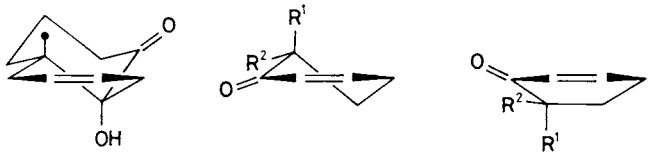
VIII



IX, 2α-OH
X, 2β-OH

spectra. Thus, neither of the trinervitane hydroxy ketones *V*, *VI*, and *VII* has the 3-hydroxy group axial on a half-chair six-membered ring (formula *B*) or pseudoaxial on a half-boat form (formula *D*), in which the hydroxyl would have about the same spatial relation to both the carbonyl and the double bond as in the model compound *VIII* (formula *A*). The 3-hydroxy group in *V*, *VI*, and *VII* is therefore equatorial or pseudoequatorial and roughly eclipsed with the carbonyl group (formulae *C* and *E*). As seen from the models, the equatorial hydroxyl is bonded to the carbonyl and not to the double bond. This is confirmed by lower carbonyl stretching vibrations in the hydroxy ketones *V*, *VI*, and *VII* (1 663, 1 653, and 1 660 cm^{-1}, respectively) as compared with the usual values for α,β-unsaturated cyclohexanones (1 674–1 684 cm^{-1}). If the hydroxyl is equatorial in all the three trinervitane hy-

droxy ketones, then its different configuration in compounds *V* and *VII* and compound *VI* means also a different conformation of the six-membered ring: the 3α--hydroxy ketones *V* and *VII* exist in a half-chair conformation (formula *C*) whereas the 3β-hydroxy ketone *VI* in a half-boat form (formula *E*). In the latter compound,



A *B* R^1= OH ; R^2= H *D*
C R^1= H ; R^2= OH *E*

TABLE III

^{1}H NMR data for 3-hydroxy-2-trinervitanones and their TAI-acylation products (in $C_6{}^2H_6$)

Proton	Compound, ppm (*J*, Hz)			
	V	*V* + TAI	*VI*	*VI* + TAI
H-3	4·50 d (2·4)	6·04 s	4·28 s	5·84 s
H-7	2·93 dt (9·0; 11·0; 11·0)	2·97 dt (9·0; 11·0; 11·0)	*a*	*a*
H-9	*a*	*a*	4·80 bdd (10·6; 6·0)	5·16 bdd (10·0; 6·0)
H-16	2·21 bd (12·4)	2·22 bd (12·0)	3·01 bd (12·0)	2·95 bd (12·0)
H-17	1·60 bs	1·60 bs	1·42 bs	1·45 bs
H-18	1·00 s	1·09 s	1·02 s	1·11 s
H-19	4·84 dt (2·2; 2·0; 0·5)	4·87 m	1·43 d (1·5)	1·44 d (1·5)
H′-19	4·79 dt (2·0; 2·0; 0·9)	4·87 m		
H-20	0·86 bs	0·91 d (6·0)	0·67 d (6·6)	0·81 d (6·0)
H-O	4·01 d (2·4)	—	4·00 bs	
H—N		8·41 s	—	8·64 s

a Unresolved.

the half-boat conformation is obviously stabilized by the intramolecular hydrogen bond between the hydroxyl and the carbonyl group. The different conformation of the six-membered ring in isomers *V* and *VI* causes probably also the observed difference in carbonyl vibrations (Table IV). The half-boat conformation is known[8] to be stabilized by hydrogen bond in the steroid model compound *X*. Unlike the isomeric 2α-hydroxy ketone *IX* in which the half-chair conformation is suitable for an intramolecular hydrogen bond, the 2β-hydroxy ketone *X* in the same conformation cannot be hydrogen bonded. In spite of this, both the compounds show strong intramolecular hydrogen bond (Table IV), proving the existence of a half-boat conformation in compound *X*. This situation exactly corresponds to the case of our trinervitane derivatives *V* and *VI*. After the reaction with TAI, hydrogen bond formation is no longer possible and therefore there is no stabilization of the half-boat conformation in the acylated ketone *VI* which apparently adopts the now more favourable half-chair conformation with axial 3β-substituent. This brings the acyl close to the H-20 methyl protons and the H-9 olefinic proton and can bring about the observed acylation-induced changes in their chemical shifts. The TAI-acylation of the 3α-hydroxy ketone cannot substantially affect chemical shifts of the mentioned protons, irrespective of the equatorial or axial position of the 3α-substituent. We cannot exclude that also 3β-hydroxy-1(15),8(9)-trinervitadien-2-one (*VI*) is an artefact formed during the processing of the extract; on the other hand, not even traces of the corresponding diol have been found in the extract.

We detected three diterpenic compounds whose struture cannot be determined because of too minute quantity. According to the mass spectral fragmentation, two of them were isomeric monohydroxy trinervitane derivatives of composition $C_{20}H_{32}O$ (M^+ 288) with the hydroxyl attached to the five- or six-membered ring. Neither their mass nor infrared spectra were identical with those of any described alcohols

TABLE IV

Hydroxyl and carbonyl stretching vibrations in IR spectra of α-hydroxy ketones *V*—*X*

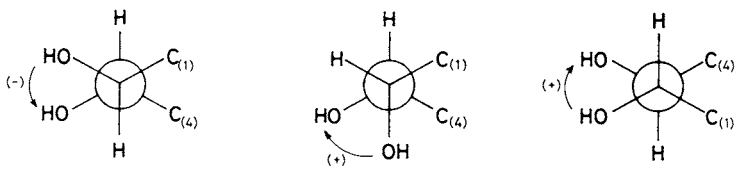
Compound[a]	ν(OH) cm^{-1}	ν(CO) cm^{-1}
V	3 480	1 663
VI	3 490	1 653
VII	3 506	1 660
VIII[b]	3 602	1 713
IX[c]	3 496	[d]
X[c]	3 498	[d]

[a] Measured on PE 621 and UR 20 instruments in CCl_4, concentration $1 \cdot 10^{-3}$ mol l^{-1}; [b] compound described in ref.[7]; [c] values taken from ref.[8]; [d] value not given.

of the same composition. The third diterpenic compound was a tetracyclic ketone of composition $C_{20}H_{30}O$ (M^+ 286).

The extract contained also a non-diterpenic compound. Its molecular ion was M^+ 436 and the fragmentation indicated an unbranched aliphatic chain. According to the IR spectrum the compound was a primary alcohol (3 633 cm^{-1}) with a long chain (731, 721 cm^{-1}). Also the ^1H NMR spectrum confirmed the presence of an unbranched chain and revealed a double bond of *Z*-configuration (δ 5·35 m, J = = 10·5 Hz). The position of the double bond was determined by oxidation with osmium tetroxide in pyridine to a triol whose mass spectrum afforded the required information. The principal ion peak at m/z 327 ($C_{21}H_{43}O_2$) arose by fission between the carbon atoms, bearing the vicinal hydroxy groups. Hence, the double bond is situated between the carbon atoms $C_{(21)}$ and $C_{(22)}$ and the compound is (Z)-21--triaconten-1-ol.

Prestwich measured the CD curve of a complex of the dihydroxy acetate *XI* with tris(2,2,6,6-tetramethyl-3,5-heptanedionato)praseodymium[9] ($Pr(dpm)_3$) and from the known relative configuration of the hydroxy groups and conformation of the six-membered ring, together with the negative Cotton effect, he derived the absolute configuration of the whole skeleton[3]. In the present study we applied the same method to three trinervitane diols and confirmed the absolute configuration by another, independent method. We investigated the CD curves of the complexes of diols *II*, *III*, and *IV* with $Pr(dpm)_3$. The relative configuration of the hydroxy groups and conformation of the six-membered ring was determined from the ^1H NMR spectra[4,10]. The helicity of the segment under consideration in the diols is depicted in Fig. 1 and the mean $\Delta\varepsilon$ values from repeated measurements are given in Table V. In accord with the literature[9] we observed a considerable sensitivity of the method to moisture manifested by somewhat scattered $\Delta\varepsilon$ values. Nevertheless, the sign of the Cotton effects invariably corresponded to the assumed helicity. As shown on a series of steroid models, substitution with further oxygen functionality in another part



II, *XI* *III* *IV*

FIG. 1

Projection of trinervitane-2,3-diols along the $C_{(2)}$—$C_{(3)}$ bond

of the molecule can affect the mangitude of $\Delta\varepsilon$ but not the sense of the Cotton effect. The CD results agree with the expected situation. Whereas the negative Cotton effect confirms the *M*-helicity of diol *II*, the positive effect proves the *P*-helicity of diols *III* and *IV*. The same compounds from different termite species had the same sign of Cotton effect, confirming thus that the trinervitane system arises by the same biosynthetic pathway in all the termite species.

TABLE V

Values of CD maxima for complexes of trinervitane diols with $Pr(dpm)_3$ at 316 nm

Compound[a]	$\Delta\varepsilon$, l mol^{-1} cm^{-1}
XI (ref.[3])	−14·35
II	− 3·5
III	+ 2·0
IV	+10·8

[a] Measured immediately after mixing with the reagent; concentration of the complex 2 . 10^{-4} mol. . l^{-1}, cell thickness 1 cm.

The absolute configuration of trinervitane skeleton was confirmed by ^{1}H NMR spectra of esters of alcohol *I* with (*R*) and (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA). The diastereoisomeric esters *XII* and *XIII* were prepared by boiling *I* with the acid in dichloromethane in the presence of 2-chloro-1-methylpyridinium iodide and 4-dimethylaminopyridine[11,12]. We studied the effect of the phenyl group of the acid moiety in its known preferred conformation on the H-2 methylene protons of the alcoholic part[13]. The H-2 signals were found by differential decoupling with irradiation frequency on and off resonance position of the H-3 signal. In the spectrum of ester *XII*, prepared from the (R)-acid, the H-2 signals appear at δ 2·35 and 2·09 as compared with δ 2·28 and 1·97 found for *XIII* (from the (S)-acid). This upfield shift in transition from *XII* to *XIII* is due to a greater shielding by the phenyl ring in *XIII* where it is closer to the H-2 atoms than in *XII*. In the case of opposite absolute configuration at $C_{(3)}$ the H-2 chemical shifts would be influenced in an opposite sense. The magnitude of the observed differences in shielding of H-2 protons in esters *XII* and *XIII* corresponds to the values published for analogous diastereoisomeric pairs[13].

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Mass spectra were measured on AEI MS 902 and Jeol D-100 instruments. IR spectra were recorded on a Perkin–Elmer 621 spectrophotometer in KBr micropellets (1·5 mm diameter) or in tetrachloromethane.

^{1}H NMR spectra were obtained on a Varian XL-200 (200 MHz) instrument in deuteriochloroform or hexadeuteriobenzene with tetramethylsilane as internal standard, CD spectra were taken on a Roussel–Jouan II 185 dichrographe in tetrachloromethane. The GC–MS analysis was performed on a Hewlett-Packard 5985 A instrument, using a 25 m SE-54 capillary column (1% vinyl, 5% phenyl, methyl silicone, i.d. 0·3 mm). Column chromatography was carried out on silica gel with 15% water (Herrmann, Köln-Ehrenfeld), preparative thin-layer chromatography (TLC) on silica gel 60 PF_{254} (Merck, Darmstadt). Sample purity was checked by TLC on silica gel 60 G (Merck, Darmstadt) or by gas-liquid 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), length 2·5 m, i.d. 3 mm.

Collection: The termites were collected at Pucallpa and Iquitos (Peru, South America), 500 km apart, situated at the beginning of the Amazonian lowland. In each locality samples were collected from three nests.

Isolation procedure: *N. ephratae* soldiers were extracted according to the literature[4]. Extracts from soldiers from the individual nests were chromatographed on a column of silica gel and eluted with light petroleum–ether mixtures (10–40%). The crude fractions were further purified either by column chromatography or preparative TLC.

1(15),8(19)-*Trinervitadien*-3α-ol (I): Elution with light petroleum–ether (10%) afforded the alcohol *I*, m.p. 128–130°C (light petroleum–ether); reported[14] m.p. 128–131°C. All spectra of *I* were identical with those of a standard[14].

1(15),8(19)-*Trinervitadien*-2β,3α-*diol* (II): Elution with light petroleum–ether (30%) gave the diol *II* which, upon crystallization from light petroleum–ether, melted at 179–180°C (reported[14] m.p. 174–176°C). All spectral data agreed with those in the literature[14].

1(15),8(19)-*Trinervitadien*-2α,3α-*diol*(III): The diol *III* was eluted with light petroleum–ether (30%) and crystallized from the same solvent mixture; m.p. 73–76°C (reported[4] m.p. 75–77°C). All spectral data were identical with those of a standard[4].

1(15),8(19)-*Trinervitadien*-2α,3β-*diol* (IV): Elution with light petroleum–ether (30%) furnished the diol *IV*, m.p. 88–91°C (ethanol); stated[4] m.p. 93–96°C; identical spectra with those of a standard[4].

3α-*Hydroxy*-1(15),8(19)-*trinervitadien*-2-*one* (V): Light petroleum–ether (10%) eluted the amorphous hydroxy ketone *V* whose spectral data were identical with those reported[4] for a standard sample.

3β-*Hydroxy*-1(15),8(9)-*trinervitadien*-2-*one* (VI): The amorphous hydroxy ketone *VI* was eluted with light petroleum–ether (10%). Mass spectrum, *m/z* (%): M^+ 302 (73; $C_{20}H_{30}O_2$), 287 (100), 284 (8), 274 (7), 273 (8), 269 (40), 259 (5), 256 (4), 251 (8; $C_{19}H_{23}$), 241 (14; $C_{18}H_{25}$ + $C_{17}H_{21}O$, 4 : 1), 231 (5; $C_{15}H_{19}O_2$), 213 (5), 151 (36; $C_9H_{11}O_2$), 135 (15). IR spectrum: 3 470 (OH), 1 658 (CO), 1 618 cm^{-1} (C=C).

(*Z*)-21-*Triaconten*-1-*ol*: Light petroleum–ether (20%) eluted the title compound which upon crystallization from light petroleum–ether melted at 56–57°C. Mass spectrum, *m/z* (%): M^+ 436 (2), 434 (1), 418 (29), 404 (1), 390 (2), 376 (1), 362 (2), 55 (100). IR spectrum: 3 360 (OH), 731, 721 cm^{-1} ($(CH_2)_n$, $n \geq 4$). ^{1}H NMR spectrum (C^2HCl_3): 0·88 t, J = 6·5 Hz (CH_3); 1·26 b ($(CH_2)_n$); 2·01 bq (2 × CH_2—C=); 3·63 t, J = 6·5 Hz (CH_2OH); 5·35 m, J = 10·5 Hz (—CH=CH—).

1,21,22-*Triacontanetriol*: A solution of osmium tetroxide in pyridine (0·26 ml; 200 mg in 10 ml) was added to (*Z*)-21-triaconten-1-ol (5·3 mg) in pyridine (0·26 ml). After standing at room

temperature overnight, a solution of $K_2S_2O_7$ (0·7 ml; 0·1 g $K_2S_2O_7$ in 2 ml of pyridine and 2 ml of water) was added. The mixture was set aside for 30 min, diluted with water and extracted with ether. The ethereal extract was washed with 2M HCl, water, saturated sodium hydrogen carbonate solution, again water, and dried over anhydrous sodium sulfate. The solvent was evaporated and the product (1 mg) was purified by TLC (7·5 × 10 cm) in chloroform–ethyl acetate (9 : 1). The most polar fraction was identified as 1,21,22-triacontanetriol. Mass spectrum: 452 ($C_{30}H_{60}O_2$; M − H_2O), 327 ($C_{21}H_{43}O_2$; base peak), 309 (327 − H_2O), 291 (309 − H_2O).

Esterification of I *with* (*R*)-α-*methoxy*-α-(*trifluoromethyl*)*phenylacetic acid*: To a solution of the alcohol *I* (10·5 mg) in dichloromethane (1 ml) were added (*R*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (19 mg), 2-chloro-1-methylpyridinium iodide (22 mg) and 4-dimethylamino-pyridine (41·5 mg). After boiling for 3 h, the same amounts of the three last-mentioned reagents were aded. After another hour of refluxing all the alcohol *I* reacted and the solvent was removed *in vacuo*. The residue was partitioned between ether and water, the ethereal extract was dried over sodium sulfate and taken down *in vacuo*. The residue was dissolved in light petroleum (2 ml) and filtered through a Sep-pak silica gel cartridge. Elution with light petroleum–ether (10%) mixture (2 ml) afforded the chromatographically homogeneous product (12 mg). Mass spectrum: M^+ 504, 270 (base peak), 255, 189, 119. IR spectrum: 3 070, 1 631, 892 (>C=CH_2), 1 745 cm^{-1} (CO). ^{1}H NMR spectrum (C^2HCl_3): 0·91 d, 3 H, J = 6·3 Hz (H-20); 0·92 s, 3 H (H-18); 1·71 bs, 3 H (H-17); 2·09 m, 1 H and 2·35 m, 1 H (H-2); 3·20 m, 1 H, J = 11·9; 10·2, 8·8 Hz (H-7); 3·59 q, 3 H, J = 1·3 Hz (OCH_3); 4·86 t, 1 H, J = 2·1 Hz and 4·96 t, 1 H, J = = 2·1 Hz (H-19); 5·55 dd, 1 H, J = 10·7; 6·2 Hz (H-3); 7·38–7·61 m, 5 H (phenyl).

Esterification of I *with* (*S*)-α-*methoxy*-α-(*trifluoromethyl*)*phenylacetic acid*: The alcohol *I* (16 mg) was esterified with (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (35 mg) in the presence of 2-chloro-1-methylpyridinium iodide (31 mg) and 4-dimethylaminopyridine (41 mg) in dichloromethane (1 ml) as described for the preceding esterification; yield 22 mg of a non-crystalline product. Mass spectrum: M^+ 504, 270 (base peak), 255, 189, 119. IR spectrum: 3 075, 1 635, 895 (>C=CH_2), 1 747 cm^{-1} (CO). ^{1}H NMR spectrum (C^2HCl_3): 0·91 d, 3 H, J = 6·3 Hz (H-20); 0·96 s, 3 H (H-18); 1·71 bs, 3 H (H-17); 1·97 m, 1 H and 2·28 m, 1 H (H-2); 3·22 m, 1 H, J = 12·0; 9·8, 9·0 Hz (H-7); 3·55 q, ? H, J = 1·3 Hz (OCH_3); 4·86 t, 1 H, J = 2·1 Hz and 4·96 t, 1 H, J = 2·1 Hz (H-19); 5·52 dd, 1 H, J = 10·6, 6·1 Hz (H-3); 7·38–7·59 m, 5 H, (phenyl).

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