Tetrahedron Letters, Vol.22, No.17, pp 1563 - 1566, 1981
Printed in Great Britain

0040-4039/81/171563-04$02.00/0

NEW TRICYCLIC DITERPENE PROPIONATE ESTERS FROM A TERMITE SOLDIER DEFENSE SECRETION

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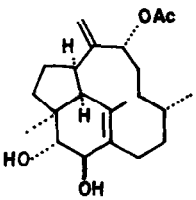
Abstract: The structure of 3α, 9β, 13α-trihydroxy-1β, 8β-trinervita-11(12), 15(17)-diene was established by X-ray crystallography. The naturally-occurring tripropionate and dipropionate monoacetate were isolated from the Malaysian termite Nasutitermes sp.

Chemical defense by the highly evolved nasute termite soldiers (Isoptera: Termitidae: Nasutitermitinae) is effected by the ejection of an irritating viscous glue onto potential predators.[1,2] This defense secretion is composed of monoterpene hydrocarbons (solvents) and polyoxygenated transannular cyclization products of cembrene-A. Three dome-shaped diterpene skeletons have been characterized by X-ray crystallography: the tricyclic trinervitanes (e.g., 1)[3], tetracyclic kempanes (e.g., 2[4] and 3[5]) and the 1,2-methyl-shifted tetracyclic *rippertane* (e.g., 4)[6]. We now report the characterization of two novel tricyclic propionate esters possessing a modified trinervitane skeleton which provides additional insight into the potential biogenesis of the termite diterpenes. This is the first report of a propionate ester as the defensive compound of a termite.[7]



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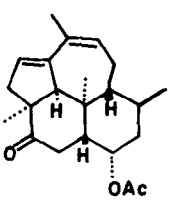

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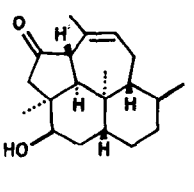

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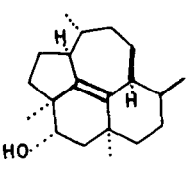

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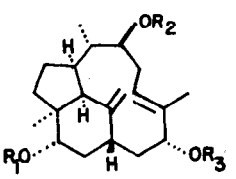


5, $R_1 = R_2 = R_3 = C_2H_5CO$
6, $R_1 = R_3 = C_2H_5CO$, $R_2 = CH_3CO$
7, $R_1 = R_2 = R_3 = H$

The new trinervitanes were isolated from the soldier defense secretion of Nasutitermes sp. by chromatography over Florisil.[8] Compound 5 appeared to be a diterpene tripropionate: HRMS, m/z 488.311 (M^+, 1.8%, $C_{29}H_{44}O_6$), 414.295 ($M^+ - CH_3CH_2CO_2H$, 5, $C_{26}H_{38}O_4$), 340.241 ($M^+ - 2CH_3CH_2CO_2H$,

67, $C_{23}H_{32}O_2$), 266 (M^+ - $3CH_3CH_2CO_2H$, 63, $C_{20}H_{26}$), 57 ($CH_3CH_2C{\equiv}O^+$, 100); IR (CCl_4), 1732 cm^{-1} (ester C=O). Compound 6 showed an analogous fragmentation pattern but with $m/_z$ 474.308 (M^+, $C_{28}H_{42}O_6$) suggesting a dipropionate monoacetate.[9] The 360 MHz ^{1}H-NMR of 5($CDCl_3$) exhibited the following significant resonances: δ5.39 (t, 8.3Hz, H-11), 5.14 (dd, 4.4, 11.7 Hz, H-3), 5.07 (brdd, 6.3, 10.7 Hz, H-9), 5.06, 5.07 (s,s,H-17), 4.91 (dd,4.4,11.7 Hz,H-13), 2.60(brd, 11.2 Hz,H-16), 2.3 (q, 7.3 Hz,$CH_3\underline{CH_2}CO_2$-) 2.30 (two overlapping q, 7.3,Hz,$CH_3\underline{CH_2}CO_2$-), 1.65 (brs, H-20), 1.12 (three overlapping t, 7.3Hz, $\underline{CH_3}CH_2CO_2$-), 0.91 (s, 3H,H-18), 0.86 (d,6.4Hz, H-19). The 20MHz ^{13}C-NMR($CDCl_3$) of 5 showed diagnostic resonances at δ174.51, 174.41, 173.65 (s,s,s, ester carbonyls), 147.04 (s,C-15), 136.07(s,C-12),124.03(d,C-11), 114.09(t,C-17), 79.60, 74.27, 73.39 (d,d,d, C-3,9,13), 59.56 (d,C-16), 48.27 (s,C-4), 44.35(d,C-8). Unassigned alipnatic signals occurred at 38.54, 37.54, 36.51, 36.28, 33.63, 30.15, 28.41, 28.01 and 27.89 ppm, skeletal methyls at 19.81, 11.90, and 11.70 ppm and propionyl methyls at 9.38 and 9.23 ppm.

Hydrolysis of 5 (C_2H_5OH,15% aq.KOH, 6 hr. 20°C) afforded a 72:28 mixture (GLC OV-17) of the triol 7 and its 3-monopropionate 11 (R_1= C_2H_5CO,R_2=R_3=H). Complete hydrolysis was effected after 2 hour reflux; conversely, selective removal of the allylic 13-propionate (K_2CO_3,C_2H_5OH, 1 hour 40°C, 4 hr 20°) gave >80% of the 13-hydroxy-3, 9- dipropionate derivative 8(R_1=R_2= C_2H_5CO-, R_3=H). Complete hydrolysis of the dipropionate monoacetate 6 gave triol 7 and some of the 3-monopropionate; the attempted selective hydrolysis of the allylic propionate resulted in the partial removal of both the acetate and propionate moieties to give a mixture of the 13-hydroxy-3-propionate-9-acetate 9 (R_1=C_2H_5CO-, R_2=CH_3CO-,R_3=H) and the 9-hydroxy-3,13-dipropionate 10 (R_1=R_3=C_2H_5CO, R_2=H).[9] The triol 7 was chromatographed over Florisil (80% EtOAc in hexane) and was then crystallized by slow evaporation (20°C) from ethanol containing traces of water to give clear prisms, mp 277-278°C.

The structure of 7 was established by single-crystal X-ray experiments. The data crystal of 7 was mounted on an Enraf-Nonius CAD-4A diffractometer under the control of a PDP 11/45 computer system and subjected to Mo X-radiation (λ=0.71073 Å). The space group is $P2_12_12_1$ with a=9.439(4) Å, b=13.506(5) Å, c=14.677(5) Å, Z=4 and ρ_{calc}= 1.14. The data were reduced (p = 0.04) and the structure solved using the MULTAN direct methods series.[10] Of the 1507 reflections measured (0<2θ<46°), 1099 with $F_o^2 > 3\sigma(F_o)^2$ were used in the subsequent refinement. Residuals could not be reduced below 20% for the proposed $C_{20}H_{32}O_3$ molecular formula; in addition, a Fourier difference map indicated the presence of one additional carbon-size atom per molecule in the unit cell. Inclusion of a water of hydration allowed the full-matrix least squares refinement (anisotropic carbons and oxygens, isotropic hydrogens) to converge to values of 0.047 and 0.066 for R and R_w respectively. The absolute stereochemistry shown in 7 and in Fig. 1 (ORTEP drawing) is assigned on the basis of biogenetic analogy to other related termite diterpenes. Extensive intermolecular hydrogen-bonding is evidenced by the following interatomic distances: O(1) - O(2), 2.709 Å; O(1)-O(4), 2.734 Å; O(2)-O(3), 2.722 Å; O(3)-O(4), 2.692 Å.

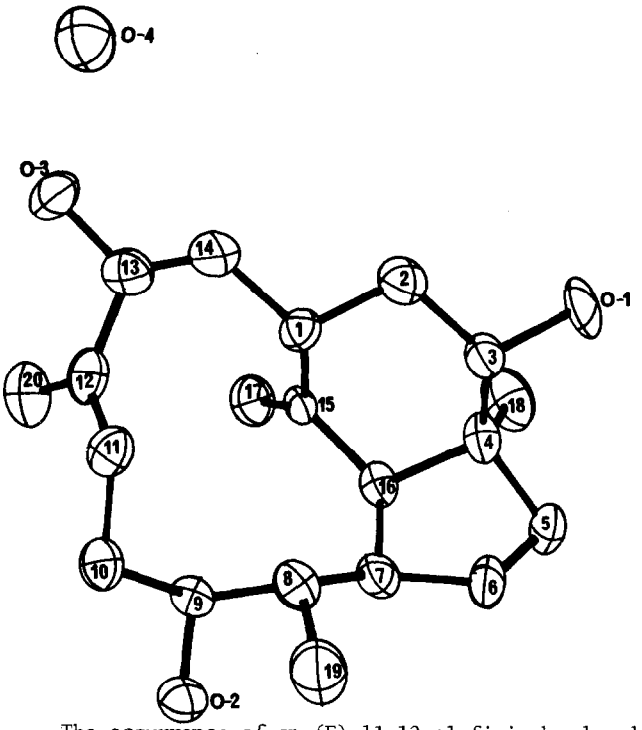
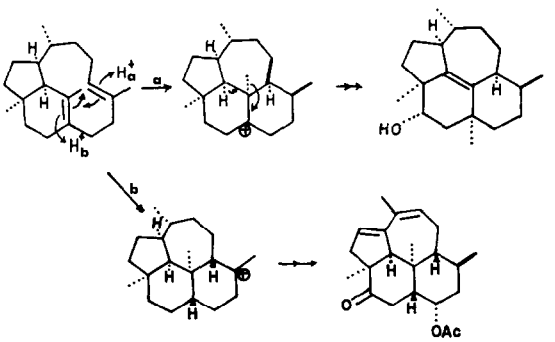


Figure 1. Computer-generated perspective drawing (ORTEP) of 7, in which hydrogens have been omitted for clarity. O-4 is the water of hydration.

The occurrence of an (E)-11,12 olefinic bond and an (R)-1 center in a trinervitane molecule lends further credence to the proposed biosynthetic scheme, since these groups have the correct position and stereochemistry to be derived from analogous positions in cembrene-A.[12] Moreover, we have proposed [1,6] that the tetracyclic compounds may arise from further cyclization of tricyclic intermediates, as shown in Fig. 2. Biosynthetic studies are in progress to test these hypotheses.

Figure 2. Hypothetical origin of tetracyclic kempane and rippertane skeletons from tricyclic trinervitanes.



Acknowledgments: We thank the NSF (DEB-7823257 to G.D.P.) and the University of Malaya (grants to S.H.G.) for financial support, Dr. C. Iden and Mr. P. Chang (Stony Brook) for mass spectra, Dr. A. McLaughlin and Mr. D. Lawler (Brookhaven) for 360 MHz ^{1}H-NMR spectra, and Prof. J. Lauher for assistance with X-ray experiments.

References and Notes:

1. G.D.Prestwich, J. Chem. Ecol. 5, 459 (1979)

2. G.D. Prestwich, Biochem. System. Ecol. 7, 211 (1979); Insect Biochem. 9, 563 (1979).

3. G.D. Prestwich, S.P. Tanis, J.P. Springer, and J. Clardy, J. Am. Chem. Soc. 98, 6062 (1976).

4. G.D. Prestwich, B.A. Solheim, J. Clardy, F.G. Pilkiewicz, S.P. Tanis, and K. Nakanishi, J. Am. Chem. Soc. 99, 8082 (1977).

5. G.D. Prestwich, J. W. Lauher, and M.S. Collins, Tetrahedron Letters, 3827 (1979).

6. G.D. Prestwich, S. G. Spanton, J.W. Lauher, and J. Vrkoc, J. Am. Chem. Soc., submitted (1980)

7. R. Baker(private communication) reports that diterpene propionates have been found in Cortavitermes sp. from Brazil.

8. Nasutitermes havilandi were collected from carton nests on rubber tree stumps in Damansara, Selangor, Malaysia. Soldiers were removed, cooled to -10°C, decapitated, and their heads were crushed in hexane. The crude extract(75 mg from 1000 soldiers) was chromatographed on 100/200 mesh Florisil with 10% ethyl acetate-hexane. Diterpenes 5 and 6 had R_f values of 0.42 and 0.36 respectively (MN Polygram Sil G-UV, 25% EtOAc-hexane) and stained purple-gray with the ethanolic vanillin-sulfuric acid visualization reagent.[2] These two compounds represent 67% and 12% of the total secretion respectively, and each was homogeneous by TLC and by GLC (3% OV-17, 220° + 6°/min to 280°). No other trinervitanes were detected at the 1% levels and traces (<3%) of kempanes[4] NK-1 and NK-2 were detected (GC-MS).

9. The 360 MHz ^{1}H-NMR of 6 possessed the same resonances as 5 with the following exceptions: δ4.90 (m, H-9), 2.30 (q, 7.3Hz, propionate methylenes), 2.03 (s, acetate methyl), 1.21 (t, 7.3Hz, propionate methyls). Dipropionate 8: CI-MS(CH_4): m/z 433 (M+1,3%), 415 (M+1-H_2O,3), 359 (M+1-$CH_3CH_2CO_2H$, 41), 341 (M+1-H_2O-$CH_3CH_2CO_2H$, 45), 285 (M+1-2$CH_3CH_2CO_2H$,100), 267 (M+1-H_2O-2$CH_3CH_2CO_2H$, 85), ^{1}H-NMR(80 MHz,$CDCl_3$, negative numbers indicate downfield shift in ppm after addition of 10 mole percent Eu(fod)$_3$, 5.1-5.3 (m, H-11,H-3, unresolved), 5.06 (brs, H-17, -0.21 and -0.32), 4.92 (apparent dd, 11.2, 4.5, H-9, -0.35), 4.12 (dd, 10.8, 6.5, H-13, -4.7), 1.70 (s, H-20, -0.56), 1.14 (t, 7.3Hz, propionate methyls, -0.08), 0.93 (s, H-18, -0.14), 0.86 (d, 7Hz, H-19, -0.09). Dipropionate 10: same CI-MS as 8. Monoacetate-monopropionate 9, CI-MS(CH_4), m/z 419 (M+1, 3%), 401 (M+1-H_2O, 5), 359 (M+1 - CH_3CO_2H, 25), 327 (M+1 - H_2O - $CH_3CH_2CO_2H$, 60), 285 (M+1 - $CH_3CH_2CO_2H$ - CH_3CO_2H, 100), 267 (M+1 - $CH_3CH_2CO_2H$ - CH_3CO_2H - H_2O, 90); ^{1}H-NMR ($CDCl_3$, 80 MHz), 4.10 (dd, 10, 6 Hz, H-13), 2.03 (s, acetate methyl), 1.69 (s, H-20), 1.13 (t, 7.3 Hz, propionate methyl), 0.92 (s, H-18), 0.88 (d, 7Hz, H-19). Monopropionate 11: CI-MS (CH_4), m/z 377 (M+1, 1%), 359 (M+1 - H_2O, 21), 303 (M+1 - $CH_3CH_2CO_2H$, 15), 285 (M+1 - $CH_3CH_2CO_2H$ - H O, 100), 267 (M+1 - $CH_3CH_2CO_2H$ - 2H_2O); ^{1}H-NMR ($CDCl_3$, 80 MHz), 4.09 (dd, 11, 7Hz, H-13), 3.63 (dd, 11, 4 Hz, H-9). Triol 7: CI-MS (CH_4), m/z 321 (M+1, 1%, 303 (M+1-H_2O, 44), 285 (M+1-2H_2O),100), 267 (M+1 - 3 H_2O, 41).

10. The programs used were those of the Enraf-Nonius Structure Determination Package developed chiefly by Okaya and Frenz.

11. Tables of fractional coordinates, thermal parameters, bond distances, bond angles and their errors and values of 10 x F_{obs} and 10 x F_{calc} have been deposited with the Cambridge Crystallographic Data Center.

12. J. C. Braekman et al. have isolated a bicyclic 7,16-secotrinervitane from an Oriental Nasutitermes. This molecule possesses (E)-8 and (E)-11 olefinic bonds and the (R)-1 center of the proposed cembrene - A precursor, thus providing further support for the cembranoid origin of these diterpenoids. We thank Dr. Braekmann for a preprint of this paper.

(Received in USA 10 June 1980)