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NMR structure determination of (11*E*)-trinervita-1(14),2,11-triene, a new diterpene from sexual glands of termites

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Abstract—Female alates of *Nasutitermes ephratae* termites from Guadeloupe and *Nasutitermes* sp. from Brazil produce a diterpene hydrocarbon of the molecular formula $C_{20}H_{30}$ as the main component of their tergal gland secretion. Analysis of NMR, IR, and mass spectra of the diterpene led to a structure of (11*E*)-trinervita-1(14),2,11-triene. Based on a comparison with the published oxygenated trinervitane skeleton from termites we prefer the enantiomer with absolute configurations (4*R*,7*S*,8*R*,15*S*,16*S*). The suggested structure is supported by ab initio quantum chemical calculation of ^{1}H and ^{13}C chemical shifts for the optimized geometry of the molecule.

1. Introduction

Diterpenoid compounds often play a role in chemical communication of termites of the subfamily *Nasutitermitinae*. They function as trail pheromones or sex pheromones,[1–5] but most of the diterpenoid compounds were reported as defensive substances of termite soldiers (Refs. 6,7 and references therein). Over 60 different structures have been isolated from the soldiers' frontal gland secretions since the description of first diterpenoid defence substance, a tricyclic trinervitane derivative, from *Trinervitermes gratiosus*.[8] Defence substances usually possess a bicyclic (secotrinervitane), tricyclic (trinervitane), or tetracyclic (kempane, rippertane or longipane) skeleton. A trinervitane skeleton is the most common; the other skeleta show considerably less structural diversity. These skeleta (Scheme 1) are unique to termites and they are likely to be formed via cyclisation of cembrane precursors.[9] Except for neocembrene, all other skeleta shown in Scheme 1 only occur with oxygen substituents.

Diterpenic hydrocarbons are relatively rare in termites. Most of them were described in the family *Termitidae*. Cubitene (**8**), bifloratriene (**9**), cubugene (**10**), and two cembrene isomers neocembrene A (**1**) and (3*Z*)-cembrene A (**11**) occur in the genus *Cubitermes* (Scheme 2).[10,11] These compounds have a molecular formula $C_{20}H_{32}$ and their structures differ substantially from our compound. Thus, the diterpene reported here, and isolated from two species of *Nasutitermes* is the first example of a naturally occurring trinervitane hydrocarbon.

2. Results and discussion

Trinervita-1(14),2,11-triene (**12**) was the main component of solid phase microextraction (SPME) samples from the surface of tergal glands of female alates of both *Nasutitermes ephratae* (92%) and *Nasutitermes* sp. (52% of all compounds adsorbed on the SPME fibre; figures taken from GC integration areas). To obtain larger amounts of the compound for the structure elucidation, whole females were extracted and the diterpene **12** was purified from the crude extract. Its EI mass spectrum was consistent with that of a hydrocarbon of the molecular formula $C_{20}H_{30}$ ($M^{+\cdot}$ *m/z* 270). The molecular mass was confirmed by chemical

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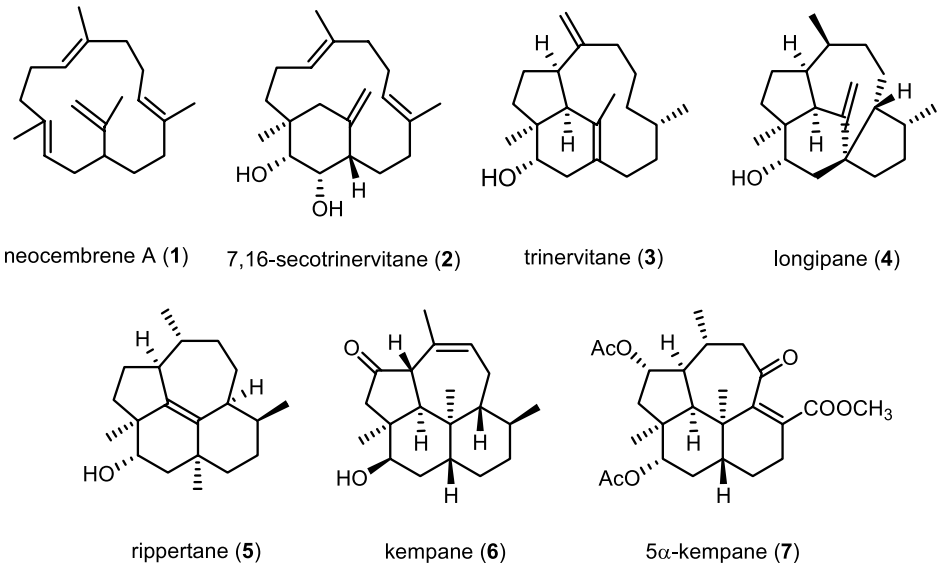
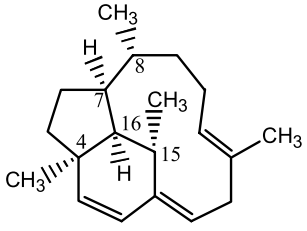


Scheme 1. Examples of diterpenes with different skeletons found in termites.

ionization (methane); pseudomolecular ions *m/z* 271 ($MH^{+\cdot}$), *m/z* 229 [$(M+C_2H_5)^{+\cdot}$], and *m/z* 311 [$(M+C_3H_5)^{+\cdot}$] were observed. By analogy of the fragment ions *m/z* 159 ($C_{12}H_{15}$) and *m/z* 119 (C_9H_{11}) with those of the previously described trinervitane alcohol **3**,[12] *m/z* 175 ($C_{12}H_{15}O$) and *m/z* 135 ($C_9H_{11}O$) it was indicated that the isolated diterpene may be a trinervitatriene. The infrared spectrum showed a presence of a trisubstituted double bond (850 cm^{-1}) and excluded an exomethylene group (absence of a band at 890 cm^{-1}).

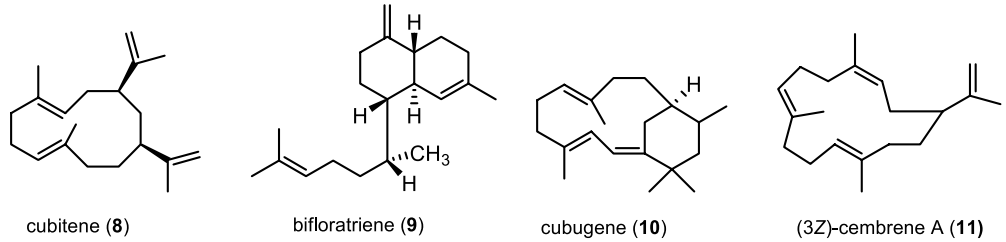


12
(4*R*,7*S*,8*R*,11*E*,15*S*,16*S*)-trinervita-1(14),2,11-triene

The proton 1D-NMR spectrum displayed four methyl signals—two secondary methyl groups (doublets at δ 0.77 and 1.11), one tertiary methyl (singlet at δ 1.22) and one methyl on the double bond (broad singlet at δ 1.62). The low-field region of its ^{1}H NMR spectrum contained multiplets of four olefinic protons (δ 5.89dt, 5.49ddt, 5.36bdd and 5.23dq). The additional four protons gave well separated multiplets at 3.58bq, 2.88bdd, 2.64bdd and 2.17m (their chemical shifts indicated allylic type of protons) while 10 remaining protons appeared in the upfield region δ 0.95–1.95.

The 'attached proton test' ^{13}C NMR spectrum confirmed the presence of 20 carbon atoms in the molecule, consisting of four CH_3, five CH_2, eight CH and three quaternary carbon atoms (see Table 1). Six low-field signals between 145 to 123 ppm clearly indicated the presence of three double bonds that could be (in agreement with ^{1}H NMR data) identified as one disubstituted (–CH=CH–) and two trisubstituted (>C=CH–) double bonds. Three double bonds together with the inferred molecular formula $C_{20}H_{30}$ led to the conclusion that there are three rings in the molecule.

Directly bonded carbon and hydrogen atoms were assigned from the 2D-^{1}H,^{13}C-HSQC spectrum as is indicated in Table 1. The *J*-couplings between protons in the identified CH_3, CH_2 and CH groups were detected in 2D-^{1}H,^{1}H-PFG-COSY and allowed us to determine structural fragments (spin systems) shown in Figure 1A. They had to be connected via three quaternary carbon atoms and such interconnection could be accomplished from the detailed analysis of its 2D-^{1}H,^{13}C-HMBC spectrum using the correlation of carbon and hydrogen via *J*(C,H) over two and three bonds. The topology of the molecule containing the anellated five-, six- and eleven-membered rings was thus established (Fig. 1B).



Scheme 2. Diterpene hydrocarbons previously isolated from termites.[10,11]