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MONO- AND SESQUITERPENOID CONSTITUENTS OF THE DEFENCE SECRETION OF THE TERMITE *AMITERMES EVUNCIFER*

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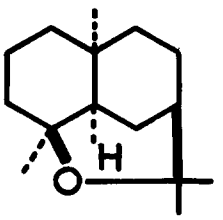
The termite species *Amitermes evuncifer* (Amitermitinae) is native to several areas of West Africa and is particularly prevalent in parts of Nigeria where it is known to reach pest status by inflicting damage on crops and property. A well-defined predator-prey relationship exists between many species of ants and termites[1], and it has been observed that the ant *Odontomachus troglydytes* is an opportunistic predator of *A. evuncifer*. Colony defence in *A. evuncifer* is provided by a soldier caste which relies upon a chemical defence mechanism. The soldier tackles its foe with well-developed mandibles causing an incision into the cuticle, followed by secretion of a toxic frontal gland secretion through a fontanelle into the wound. The ants in turn produce a series of 2,6-dimethyl-3-alkylpyrazines as repellents towards *A. evuncifer* soldiers[2]. We report here a detailed analysis of the constituents of the frontal gland of *A. evuncifer* soldiers.

In earlier studies, we reported that the major component (ca. 90% total) of the defence secretion had structure (1)[3], the absolute configuration having been determined by an enantiomer-specific synthesis[4]. Further analysis of the minor constituents has allowed identification of four further components (2)-(5).

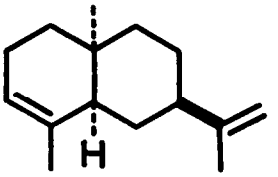
Several samples of soldiers were collected from both Mokwa and Zugurma, Nigeria and were each shown by GC analysis[5] to be essentially identical in the mono- and sesquiterpenoid regions of the chromatogram. A sample of 40 soldiers from Zugurma were used in the studies reported below. Both solvent extracts of crushed soldiers in pure methylene chloride and extracts of 'milked secretion' were used.

The extracts were initially separated into three broad fractions (A-C) by micro-preparative gas chromatography[6] on column (a). Fraction (A) (shortest R_T) consisted largely of the monoterpene (5), whereas fraction (B) contained three sesquiterpenes (2)-(4). The major component (1) was obtained pure as fraction (C). Further GC fractionation of (B) allowed isolation of components (2)-(4) in high purity, and the structure elucidation of these together with (5) is based upon the following evidence:

Component (2) [30 μg]: Combined MS/GC (5% XE - 60) showed[7] m/e 204(34%), 190(14%), 189(91%), 176(50%), 161(39%), 148(45), 147(91), 133(51), 121(40), 109(28), 107(70), 105(60), 95(36), 93(86), 91(75), 81(62), 79(75), 77(49), 69(30), 67(59), 65(24), 55(58), 57(39), 43(23), 41 (100), 39(49), indicative of a sesquiterpene hydrocarbon $C_{15}H_{24}$, possessing an isopropenyl group (m/e 41, base peak) and a cyclohexene moiety (m/e 176 corresponding to a retro-Diels-



(1)



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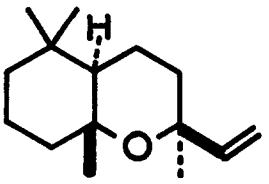

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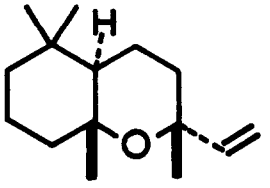
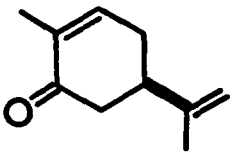


(4)

(5)

Alder fragmentation and expulsion of $CH_2=CH_2$). The FT-NMR spectrum was complex but *inter alia* showed τ ($CDCl_3$) 9.14(3H singlet), 8.23(3H, broad singlet), 5.32(2H, broad singlet) and 4.75(1H, broad singlet) in keeping with a bicyclic diene. The presence of unsaturation was confirmed by reaction with bromine (GC syringe reaction), whereas the absence of reaction with $NaBH_4$/EtOH or silylating reagents was consistent with structure (2). Microscale hydrogenation gave a single product, the mass spectrum of which [m/e 208(69%), 193(21) 166(14), 165(32), 109(58), 96(48), 93(40), 83(50), 81(35), 69(58), 55(90), 43(42), 41(100)] was similar to that of published mass spectra of selinanes[8]. Detailed analysis of the foregoing spectroscopic data taken together with biogenetic considerations were consistent with structure (2) as a working hypothesis. This structure was confirmed by a rational synthesis from (-)-carvone (6), proceeding via α-epicarrisone (7)[4,9]. The latter was reduced by hydrogenation over 10% Pd/C to a mixture of the *cis*- and *trans*-dihydroepicarrisones from which (8) was separated in a pure state[10]. Dihydrooccidentalol (9) was produced by a Bamford-Stevens rearrangement of the p-toluenesulphonylhydrazone of (8) using sodium in ethylene glycol at 180°. Elimination of the tertiary alcohol group ($SOCl_2$, dry pyridine) afforded (2) which was shown to be identical to the natural product by comparative GC on five columns, and by direct comparison of spectroscopic data. The small amount of natural (2) available precluded the determination of chiroptical measurements and thus the relative configuration only is known.

Component (3) [45 μg] exhibited a base peak at m/e 109 and parent ion at m/e 222 in the mass spectrum which was consistent with a bicyclic sesquiterpene of formula $C_{15}H_{26}O$. The presence of one or more double bonds was indicated by GC syringe reactions with bromine, whereas no reaction was found with $NaBH_4$ or BSA. Hydrogenation over 10% Pd/C provided a single product,



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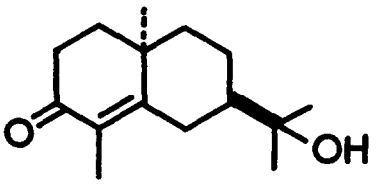

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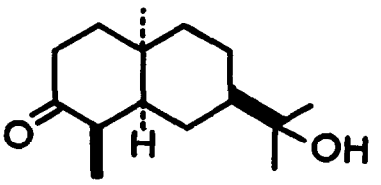
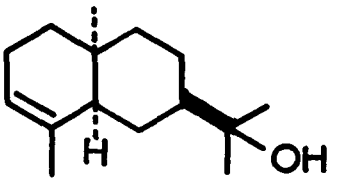


(8)



(9)

the mass spectrum of which (molecular ion at m/e 224) suggested the presence of one double bond in the parent compound. This was supported by FT-NMR which showed inter alia a gem-dimethyl group, (τ $CDCl_3$, 9.28, 9.12 both 3H singlets), two further methyl groups situated α- to an oxygen atom (τ 8.87, 8.79, 3H singlets), and a vinyl moiety (τ 5.08 (2H), 3.95(1H), both doublets of doublets). The combination of the foregoing information suggested the known structure (3) *viz* 8-epi-caparrapi oxide for this component. An authentic sample of (3) was shown to be identical to the natural product by GC coinjection on five phases and by detailed comparison of physical data[11].

Component (4) [15 μg] was shown to be the related caparrapi oxide. The physical and spectroscopic data obtained for this component were broadly similar to that obtained for (3) suggesting the presence of an isomer. In spite of the small amounts available, comparison with an authentic sample of (4)[11] allowed confirmation of structure.

Component (5) [30 μg] was demonstrated to be 5-(Z)-2,6-dimethylocta-2,5,7-triene (*cis*-β-ocimene) on the basis of NMR and mass spectra[12,13]. Further evidence for the specific geometry was obtained from the ultra-violet spectrum. An ethanolic solution of the ocimene was prepared by micropreparative GC using spectroscopic ethanol as the trapping solvent, and the solution made up to a known volume. The concentration of this sample was determined by several replicate comparisons of GC peak area with standard solutions. This provided values of λ_{max} 238, ε_{max} 20700 ± 1000 for the natural product which whilst consistent for those reported for the (Z)-isomer (λ_{max} 237.5, ε_{max} 21000), differed considerably from the reported data of the (E)-isomer (λ_{max} 232, ε_{max} 27600)[12].

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