EVOLUTION OF CHEMICAL DEFENSES IN TERMITES

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SUMMARY

The discovery of novel diterpenes in the soldier frontal gland secretions of higher termites has led us to detailed biochemical, and ecological investigations into the evolutionary and biosynthetic origins of defense compounds in termite societies. (1) Termitine soldiers often possess both mechanical and chemical defenses (e.g., *Cubitermes*) based on greasy diterpene hydrocarbons of both irregular and regular terpenoid structures. (2) Nasute termites (e.g., *Nasutitermes*) squirt a terpenoid glue, containing unique tetracyclic and tricyclic diterpenes. New structures, a physiocochemical model for the glue's stickiness, biosynthetic studies, and the use of secretion compositions as clues to termite phylogeny are discussed. (3) Advanced rhinotermitids (e.g., *Prorhinotermes, Schedorhinotermes* and *Rhinotermes*) employ electrophilic lipids (vinyl ketones, nitroolefins, β-ketoaldehydes) as contact poisons. Prevention of auto-intoxication may operate in this subfamily, furnishing evidence for a ancestral insecticide detoxication system.

RESUMEN

Evolución de las defensas químicas en las termitas

El descubrimiento de diterpenos nuevos en las secreciones de la glándula anterior de los termites soldados avanzados nos ha guido a investigaciones detalladas bioquímicas y ecológicas de los orígenes evolutivos y biosintéticos de compuestos defensivos en las sociedades de termitas. (1) Los termites soldatos frecuentemente tienen defensas mecánicas y químicas (por ejemplo, *Cubitermes*) basadas en hidrocarburos diterpenos grasosos de estructuras irregulares y regulares. (2) Los termites nasutes (por ejemplo, *Nasutitermes*) arrojan una goma terpénica, que contiene diterpenos tetracíclicos y triciclicos únicos. Nuevas estructuras, un modelo fisioquímico para la tenacidad de la goma, estudios biosintéticos, y el uso de compuestos de secreciones como guias hacia la evolucion de los comejenes son examinados. (3) Los Rhinotermitidos avanzados (por ejemplo, *Prorhinotermes, Schedorhinotermes* y *Rhinotermes*) usan lípidos electrofílicos como venenos de contacto. La prevención de auto-toxicación puede operar en esta subfamilia, mostrando evidencia para un sistema ancestral de desintoxicación contra insecticidas.

INTRODUCTION

We have been involved for the past five years in the detailed study of chemical defense by termite soldiers, in particular with respect to (1) structure determinations of new chemical substances, (2) elucidation of biosynthetic pathways, (3) biochemical mechanisms of detoxication, (4) inter- and intra-specific chemical variation, and (5) application of chemosystematic characters to concepts of termite evolution. In addition, we are involved in the design of metabolism-activated, delayed-action toxins for selective control of social insects which have been designated as pests. In this paper, I will present first an overview of our work with termite chemical defense by highlighting some of our recent results. Chemical defense in Isoptera has independently evolved numerous times in the families Rhinotermitidae and in the Termitidae (Fig. 1). Quennedy (1975) has classified these mechanisms as (1) biting, with the injection of an irritant as in the Termitinae and Macrotermitinae (or antihealant, cf. Prestwich, 1979a), (2) labral brushing, with the topical application of a contact insecticide as in Rhinotermitinae, (3) squirting, with the ejection of a viscous terpenoid glue, as in the Nasutiterminitinae. The chemistry of these techniques has been reviewed (Prestwich 1979a, b, c). This paper will focus on the following recent discoveries in the poison-brushers and glue-squirters :

1 Rhinotermitinae (poison-brushers)
  a. New Chemicals
  b. Biosynthetic Pathways
  c. Detoxication
  d. Phylogenetic Interpretations

2 Nasutitermitinae (glue-squirters)
  a. New Chemicals
  b.Chemosystematic Analyses
  c. Biosynthetic Pathways
  d. Phyletic Interpretation

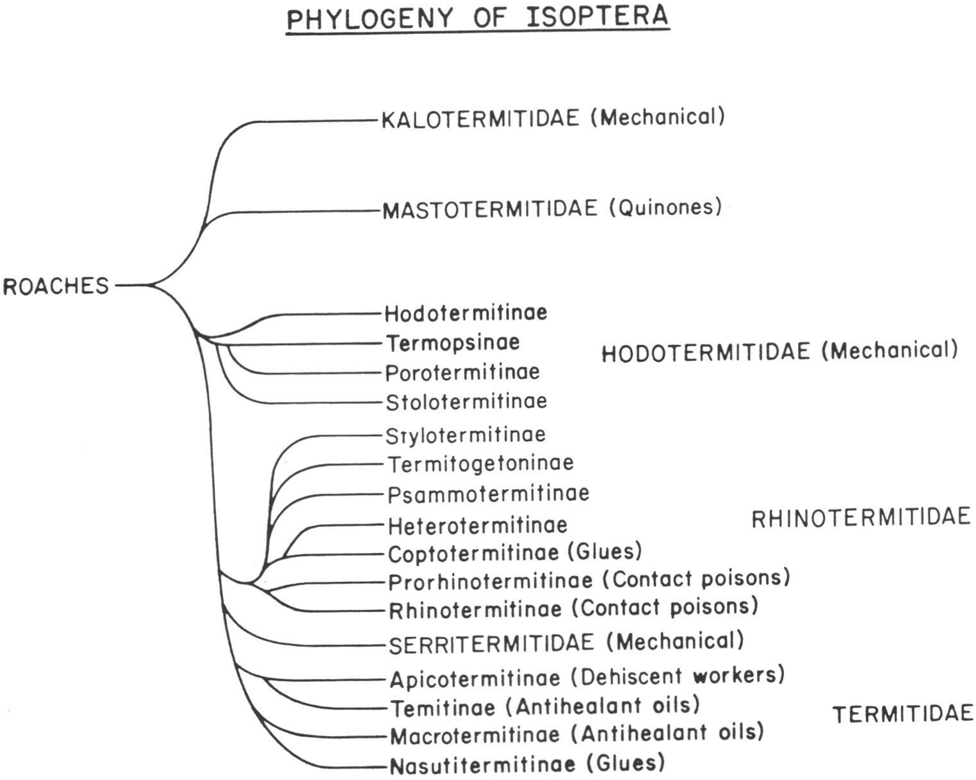


Fig. 1 – Phylogeny of Isoptera, with regard to evolution of chemical defense

Fig. 1 – Filogenia de los Isópteros, con referencia a la evolución de la defensa química

RHINOTERMITINAE

The monomorphic soldiers of *Prorhinotermes simplex* (subfamily Prorhinotermitinae) produce a cephalic defense secretion which is > 90 % of a single nitroolefin, 3, (Vrkoč and Ubik, 1974). Major and minor soldiers of the termites *Schedorhinotermes putorius* (Quennedy et al., 1973) and *S. lamanianus* (Prestwich et al., 1975) produce up to 35 % of their dry weight as a mixture of 3-alkanones, l-alken-3-ones (1) and α, ω-alkadien,3-ones (2) which act as contact poisons to attacking ants. Recently, we have found that the most advanced genera of this subfamily, *Rhinotermes* and *Acorhinotermes* produce β-ketoaldehydes, a hitherto unknown class of naturally-occuring defense chemicals. These contact poisons – the nitroolefins, the vinyl ketones,

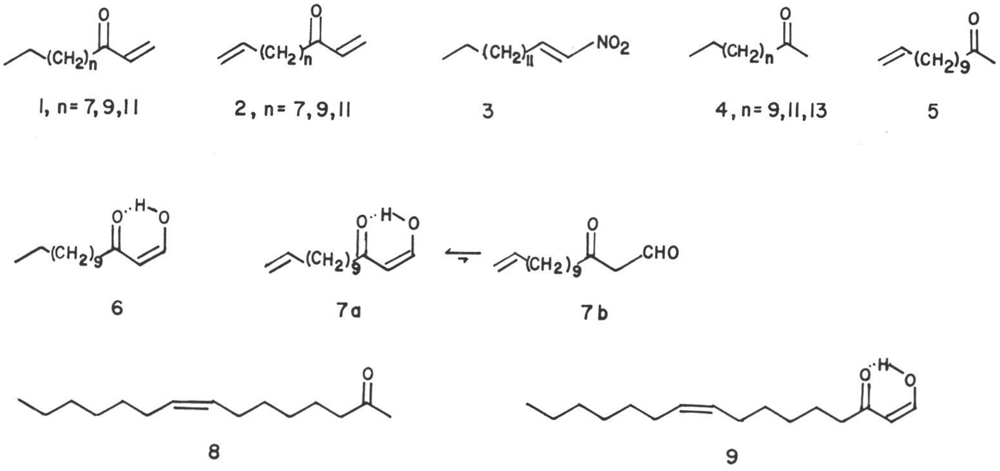


Fig. 2 – Rhinotermitine defense secretions

Fig. 2 – Las secreciones defensivas de los Rhinotermitinos

and the β-ketoaldehydes – are all highly reactive electrophilic agents in terms of their abilities to alkylate biologically important nucleophilic sites (-OH, -SH, $-NH_2$ groups). Rhinotermitine defense chemicals are shown in Fig. 2.

The C_{14} β-ketoaldehydes 6 and 7 were isolated as $> 80\ \%$ of the crude hexane extract of the «nasutoid» minor soldiers of the neotropical species *Rhinotermes hispidus* and *R. marginalis* (Prestwich and Collins, 1980). The corresponding C_{13} 2-alkanones 4 and 5 were also isolated, suggesting that the β-ketoaldehydes arise from reductive cleavage of a β-ketofattyacyl-CoA derivatives which are normally involved in the energy-releasing β-oxidation pathway for fatty acids. In the major soldiers, however, these compounds were completely absent ; this contrasts with the more primitive *Schedorhinotermes* spp., in which major and minor soldiers have identical and abundant chemical defenses. The Guyanese termite *Acorhinotermes subfusciceps* has undergone a secondary loss of the major soldier caste, and thus has only nasutoid minor soldiers. Hexane extracts of the soldiers give the sixteen-carbon β-ketoaldehyde 9 as the major product and *ca.* 5 % of the Z-8-pentadecen-2-one (8) as the minor product (Prestwich and Collins, 1981b). The compounds can be formally derived from reductive cleavage of the β-ketopalmitoleoyl-CoA as described above. This would appear to be an extraordinarily parsimonious biochemical means for defense substance production ; i.e., the

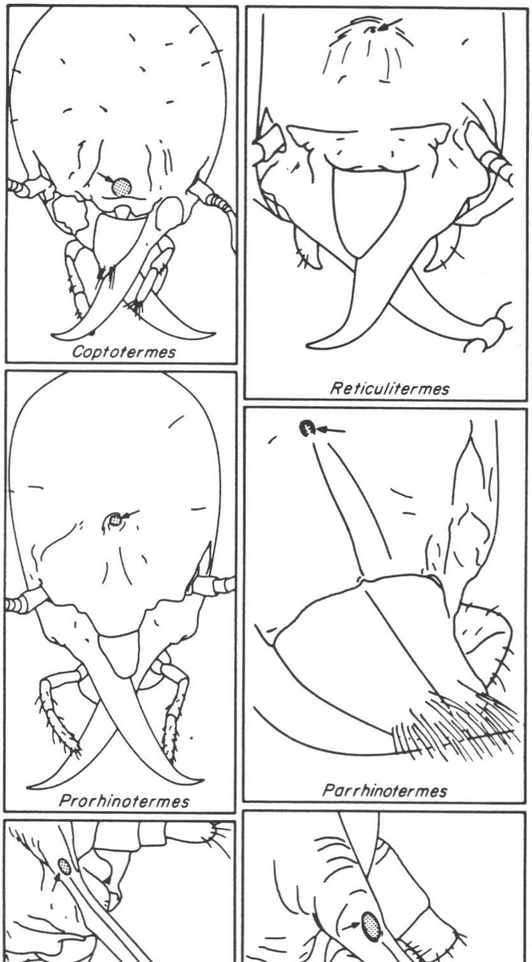


Fig. 3 – Morphological trends in the evolution of the frontal weapon of Rhinotermitidae (redrawn from Quennedey and Deligne, 1975)

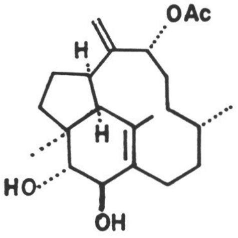
Fig. 3 – Tendencias morfológicas en la evolución del arma frontal de los Rhinotermitidae

fatty acid degradation enzymes have been harnessed to provide lipid-like contact poisons. The morphological trend in this subfamily is the regression of the mandibles with the prolongation of the labrum into a daubing brush (Quennedey and Deligne, 1975). (Fig. 3). The mandibulate major soldier caste decreases in numbers and chemical weaponry until it is eventually lost in *Acorhinotermes*. The chemical instability and thus inherent reactivity of the defense chemical which the nasutoid minor soldiers deploy increases with evolutionary advancement. Finally, it appears that selection favors the minimum amount of additional enzymic machinery to carry out the preparation of the defensive chemicals. As a result, the more advanced termites employ chemicals which are structurally more similar to normal intermediates in fat metabolism.

The increasing toxicity of chemical weapons also demands increased ability to autodetoxify these metabolic poisons. Using ^{14}C-labelled defense secretions of *P. simplex* and *S. lamanianus*, we have now established that workers of these termite species possess substrate specific detoxication pathways which convert the lipid-soluble defense compounds of conspecific soldiers into less reactive, double bond reduced forms (Spanton and Prestwich, 1981). One of these detoxication systems, that of *P. simplex* may also involve a glutathione S-transferase enzyme which converts the nitro-olefin 3 by a Michael-type addition reaction to the glutathione conjugate. Simple log dose-survival experiments have been performed to compare mortality in *P. simplex, S. lamanianus*, and the non-adapted species *Reticulitermes flavipes*. Worker survival for the *S. lamanianus* soldier vinyl ketone is *S. lamanianus* > *P. simplex* > *R. flavipes* ; however, worker survival in the presence of the *P. simplex* nitro-olefin decreases in the order *P. simplex* > *S. lamanianus* > *R. flavipes* (Spanton and Prestwich, 1981).

NASUTITERMINAE

The soldiers of the advanced genera of this subfamily possess an elongate rostra, called the nasus, which is used to eject a viscous and sticky terpenoid secretion. These secretions are sticky because they contain high concentrations of hydrogen-bonded, dome-shaped diterpenes in a monoterpene solvent (Prestwich, 1979b). The structures of the tricyclic trinervitanes (10) (Prestwich et al., 1976), tetracyclic kempanes (11) (Prestwich et al., 1977) inspired further investigations into their biosynthetic origins and further searches for biosynthetic key intermediates (Prestwich, 1979a, c). Recent discovery of bicyclic secotrinervitanes in *Nasutitermes* (Braekman et al., 1980) and *Longipeditermes* (Prestwich, Goh, and Tho, unpublished) provide



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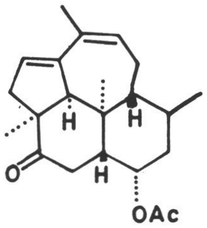

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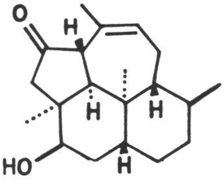

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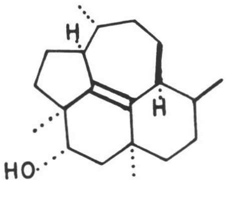

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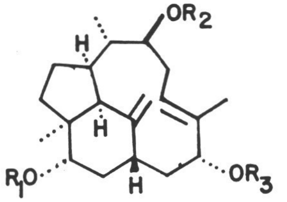

14, $R_1 = R_2 = R_3 = C_2H_5CO$

Fig. 4 – Structures of diterpenes from Nasutitermitinae

Fig. 4 – Estructuras de los diterpenos de Nasutitermitinae

further evidence for a stepwise progression from the monocyclic fourteen-menbered ring cembrene-A to bicyclic, tricyclic, and tetracyclic carbon skeletons. The correct cembrene-like arrangement of carbon atoms in space is preserved in the bicyclic compounds and in a new tricyclic tripropionate (14) (Prestwich et al., 1981a). Furthermore, alternate ring-forming pathways may occur in the tricyclic to tetracyclic conversion, as shown by the existence of the 1,2-methyl shifted skeleton of the rippertanes (13) (Prestwich et al., 1980a). Unique functionalization of the tetracyclic kempane skeleton (12) occurs in *Nasutitermes octopilis* from Guyana (Prestwich et al., 1979), and this may provide an adaptive edge in survival in woody litter relative to sympatric arboreal *Nasutitermes* species (Collins and Prestwich, 1982). These different diterpenes are shown in Fig. 4.

Intraspecific variation of defense chemicals in isolated populations of *Trivervitermes gratiosus* (Prestwich, 1978), and *T. bettonianus* (Prestwich and Chen, 1981) give rise to genetically distinct chemical «races». The chemical

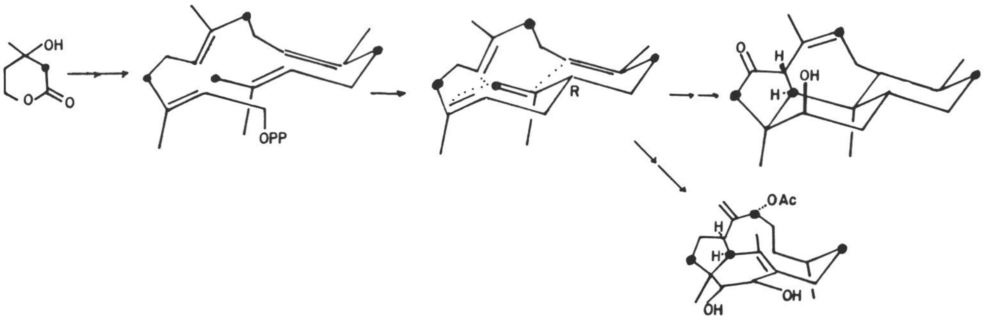

Fig. 5 – Biosynthetic pathway for nasute diterpenes, showing the incorporation of 2-^{14}C-mevalonolactone into geranylgeranyl pyrophosphate, cembrene-A, and then into trinervitanes and kempanes.

Fig. 5 – Via biosintética de los diterpenos de nasutes, mostrando la incorporación de 2-^{14}C mevalonolactona en el geranilgeranil pirofósfato, cembreno-A, y después en los trinervitanos y kempanos

composition of the soldier secretion was shown to be invariant under conditions in which field-collected alates were raised to incipient colony status in the laboratory and fed grass from the region of a different *T. bettonianus* chemotype. This was also taken as further evidence for the *de novo* biosynthetic origin of these secretions in the soldier frontal glands.

Biosynthesis (Fig. 5) was unambiguously demonstrated by injection of ^{14}C-labelled acetate and mevalonate derivatives into the abdomens of *N. octopilis* soldiers using microcapillary techniques (Prestwich, Jones and Collins, 1981b). After 12-24 hr, soldiers were decapitated and 2-5% of the radioisotopic label was recovered from the hexane-soluble material in the soldier's head. Purified mono- and diterpenes showed .05-0.3 % incorporation of label, indicating relatively rapid and efficient incorporation of the precursors.

Chemical investigations of the frontal gland secretions of three *Subulitermes* spp. from Guyana reveal the presence of sesquiterpenes neo-intermediol and T-cadinol and tricyclic diterpenes identical to the trinervitanes found in several neotropical *Nasutitermes* species (Prestwich and Collins, 1981a). Thus, identical diterpenes occur in advanced nasutes at the distal ends of both alleged phlyetic lines as originally proposed by Emerson (1961). In view of the absence of diterpenes in the primitives manidibulate nasutes *Syntermes, Cornitermes, Armitermes*, and *Rhynchotermes*, we have suggested that the diphyletic hypothesis for the origins of Nasutitermitinae is probably no longer tenable. An alternate monophyletic route (Fig. 6) is proposed in which all glue-squirting, non-mandibulate nasutes are derived from a common ancestor which probably evolved prior to the separation of West Gondwana into proto-Africa and proto-South America in the Cretaceous.

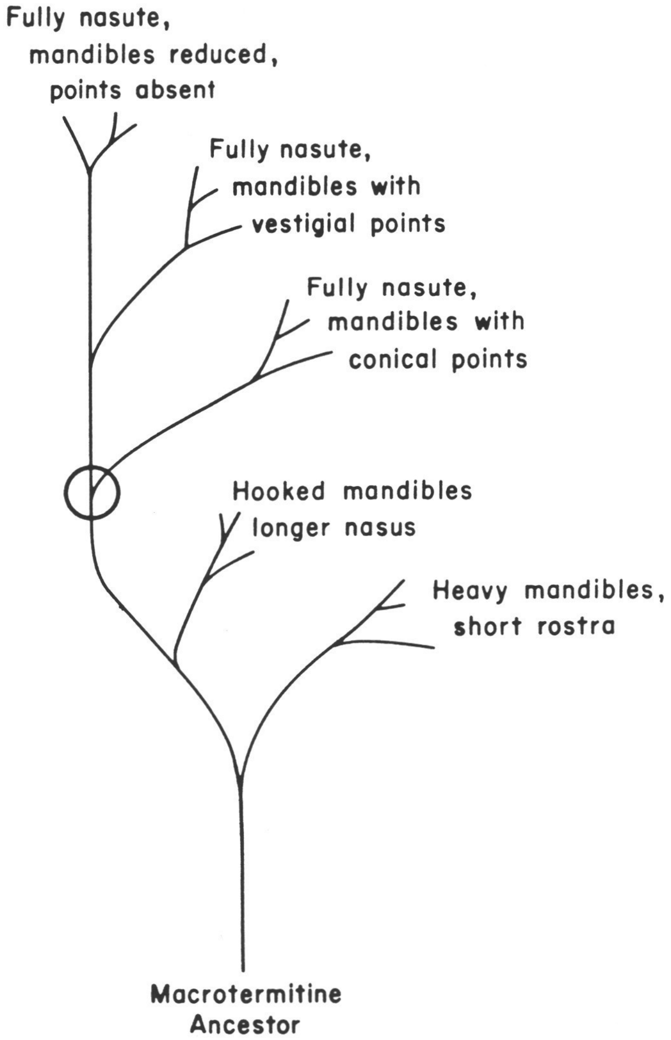

Fig. 6 – Proposed monophyletic origin for glue-squirting nasutes

Fig. 6 – Origen monofiletico propuesto para los nasutes que proyectan goma

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