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CHEMICAL DEFENSE AND SELF-DEFENSE

BIOCHEMICAL TRANSFORMATIONS OF CONTACT INSECTICIDES PRODUCED BY SOLDIER TERMITES

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Abstract—Soldiers of the advanced genera of rhinotermitids (Isoptera: Rhinotermitidae) produce lipophilic contact insecticides formally derived from fatty acids. Each of these defense substances possesses a reactive electrophilic center (vinyl ketone, nitroalkene, or β-ketoaldehyde) responsible for toxicity. We describe the synthesis of radioactively-labelled defense secretions of two of these species and their use in studying the substrate-selective detoxication by worker termites. Preliminary studies on biosynthesis of defense secretions by termite soldiers are also discussed.

INTRODUCTION

Chemical defense has evolved numerous times in the termite families Rhinotermitidae and Termitidae.[1] Three defensive strategies are employed by chemically-armed soldier termites:[2] (1) biting, with the addition of an oily, toxic or irritating secretion from the frontal gland reservoir (Termitinae), (2) brushing a copious amount of a hydrophobic contact poison onto the cuticle of an attacker (Rhinotermitidae), or (3) ejection of an irritating glue-like secretion (Nasutitermitinae).[1,3] Inherent in each of these modes of chemical defense are biochemical adaptations to allow synthesis[4] and storage of the defensive material and to avoid intoxication by this material. The ability to carry out chemical self-defense[5] has been documented in a preliminary form for two rhinotermitids[6] and in this paper we will describe the experimental details of our synthesis and our studies on the biotransformations of these defense secretions *in vivo* and *in vitro*. First, however, we will provide a brief over-view of the chemistry of these termite subfamilies.

The monomorphic soldiers of *Prorhinotermes simplex* (subfamily Prorhinotermitinae)[7] produce a cephalic defense secretion[8] which consists of >90% of a single nitroalkene, **1**. The remaining 10% is a sesquiterpene hydrocarbon recently identified as (Z)-α-farnesene by Dr. Naya and coworkers (personal communication). Major and minor soldiers of the termites *Schedorhinotermes putorius*[9a] and *S. lamanianus*[9b] produce up to 35% of their dry weight as a mixture of 3-alkanones, 1-alken-3-ones (**2**) and α,ω-alkadien-3-ones (**3**) which act as contact poisons to attacking ants. Recently, we found that the most advanced genera of this subfamily, *Rhinotermes* and *Acorhinotermes* produce β-ketoaldehydes (**4–6**).[10–12] These contact poisons—the nitroalkenes, the vinyl ketones, and the β-ketoaldehydes—are all highly reactive agents and are capable of reaction with biologically important nucleophilic sites containing hydroxyl, sulfhydryl, or amino groups. Rhinotermitine defense chemicals are shown in Fig. 1.

The C_{14} β-ketoaldehydes **4** and **5** were isolated as >80% of the crude hexane extract of the "nasutoid" minor soldiers[7] of the Neotropical species *Rhinotermes hispidus* and *R. marginalis*.[11] The corresponding C_{13} ketones 2-tridecanone and 12-tridecen-2-one were also isolated, suggesting that the β-ketoaldehydes arise from reductive cleavage of β-ketofattyacyl-coenzyme A derivatives.[10] In the major soldiers, however, these compounds were completely absent. This contrasts with the more primitive *Schedorhinotermes* species, in which both major and minor soldiers have copious amounts of chemically identical secretions.

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 these soldiers gave the sixteen-carbon β-ketoaldehyde **6** as the major product and *ca.* 5% of (*Z*)-8-pentadecen-2-one as the minor product.[12] These compounds can be formally derived from reductive cleavage of the β-ketopalmitoleoyl-CoA in a highly parsimonious fashion. That is, defense substance production may result from the harnessing of the fatty acid degradation enzymes to provide lipid-like contact poisons.[10]

The morphological trend in this subfamily is the regression of the mandibles with the prolongation of the labrum into a daubing brush[7] (Fig. 2). The importance of the mandibulate major soldiers decreases until the caste is eventually lost in *Acorhinotermes*. The chemical instability and thus the inherent reactivity of the defense chemicals 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 ability of rhinotermitid termite workers of these genera to survive in the presence of toxic nitroalkenes,[8] vinyl ketones,[9] or β-ketoaldehydes[10] secreted by their respective soldiers requires the existence of enzymes capable of detoxifying these reactive compounds. We found that termite workers of *Prorhinotermes simplex* and *Schedorhinotermes lamanianus* possess substrate-specific alkene reductases which, in the presence of reduced nucleotide cofactors, catalyze the reduction of the electron-deficient double bonds of the unsaturated electrophilic groups.[6] The saturated compounds are subsequently recycled *in vivo* via catabolism to acetate.

†Fellow of the Alfred P. Sloan Foundation (1981–83).

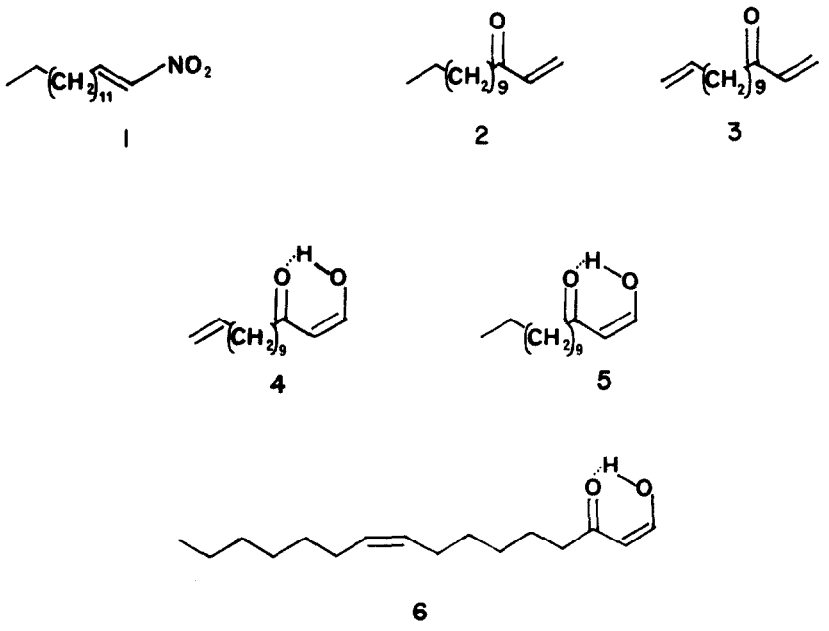


Fig. 1. Rhinotermitine defense substances possessing lipophilic side chains and electrophilic head groups.

RESULTS

Synthesis

In order to study the biosynthesis and detoxication of termite defense secretions, we required synthetic routes to them which allowed the incorporation of non-exchangeable radioactive labels. This was accomplished by homologation reactions with [^{14}C]-sodium cyanide as described below and in the experimental. Unlabelled secretions for toxicity tests were prepared by analogous procedures.[6,8,9] We also required synthetic routes to the detoxication products, which we suspected to be glutathione, cysteine, or N-acetylcysteine conjugates but which were found to be the ethyl ketone **12** and nitroalkane **9**.

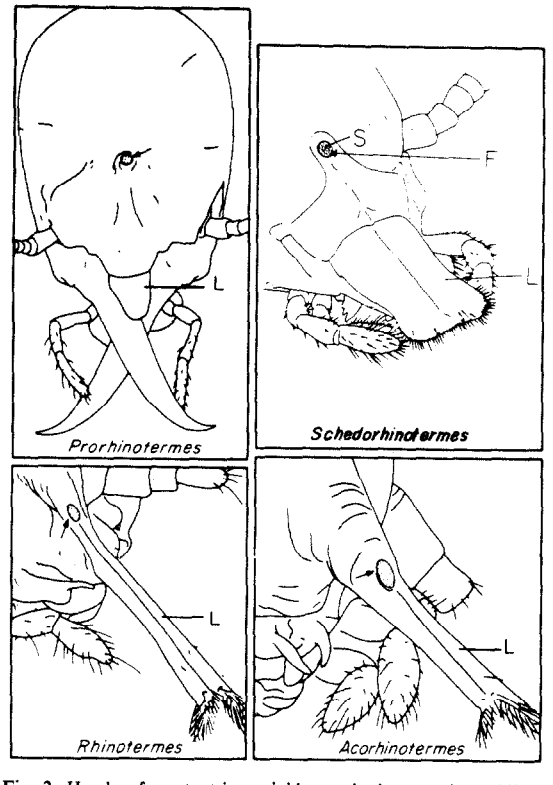
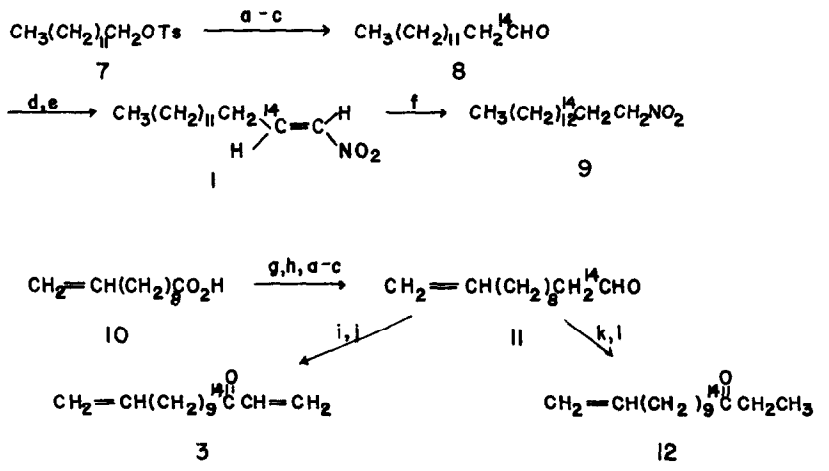


Fig. 2. Heads of contact-insecticide producing termite soldiers, redrawn (by J. Schirmer) from scanning electron microscope photographs (by M. Kaib, C. Bleecher, R. W. Jones, and Ref. 7). Key to symbols: S, secretion; L, labral brush; F (or arrow), fontanelle, the opening of the frontal gland.

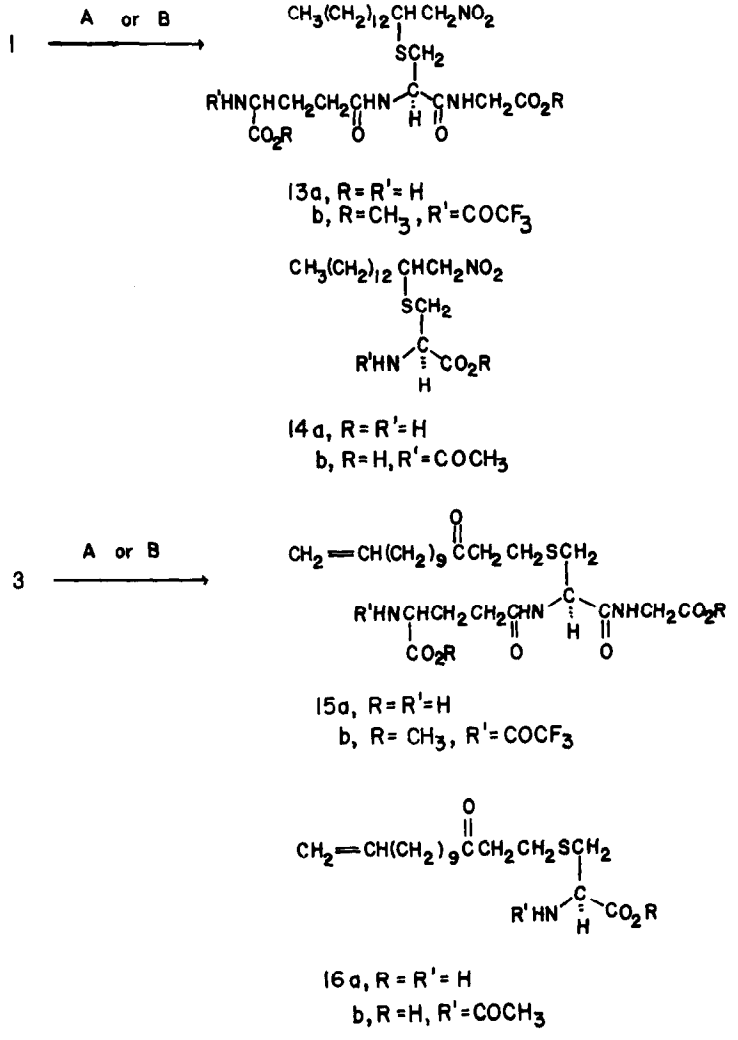
Radiolabelled defense secretion compounds were synthesized as summarized in Scheme 1. Reaction of 500 μCi (4.5 mg) of Na^{14}CN with 1-tosyloxytridecane (**7**) in dimethylsulfoxide, followed by DIBAL-H reduction of the nitrile and hydrolysis of the intermediate imine gave 1-[^{14}C]-tetradecanal (**8**). Condensation of **8** with nitromethane in methanolic sodium hydroxide followed by stirring the resulting nitroalcohol with acetic anhydride-pyridine[8] gave 2-[^{14}C]-(E)-1-nitropentadecene (**1**, 0.05 mCi/mmol) in 48% chemical yield after flash chromatography and recrystallization from hexane at $-10°$. The saturated nitroalkane **9** was prepared from unlabelled **1** via sodium borohydride reduction of **1**.

The labelled vinyl ketone synthesis began with 1-[^{14}C]-11-dodecenal (**11**), obtained from undecylenic acid **10** by hydride reduction, tosylation, [^{14}C]-sodium cyanide displacement, DIBAL-H reduction, and imine hydrolysis as described above for **8**. Condensation of **11** which excess vinyl magnesium bromide followed by oxidation of the allylic alcohol with MnO_2 gave 3 - [^{14}C] - 1,13 - tetradecadien - 3 - one (**3**, 0.32 mCi/mmol) in 53% yield after flash chromatography. The ethyl ketone **12** was prepared from unlabelled **11** by ethylmagnesium bromide addition followed by pyridinium chlorochromate oxidation.

Glutathione, cysteine, and N-acetylcysteine conjugates of the vinyl ketone **3** and nitroalkene **1** were prepared by modifications of the thiosilylether method[13] and were fully characterized by spectroscopic methods (Scheme 2 and Tables 1 and 2) For example, three equivalents of



Scheme 1. Synthesis of defense secretions. Reagents: (a) $Na^{14}CN$, DMSO; (b) DIBAL-H, hexane; (c) H_2SO_4, H_2O, dioxane; (d) CH_3NO_2, NaOH, CH_3OH; (e) Ac_2O, pyridine; (f) $NaBH_4$, C_2H_5OH; (g) $NaAlH_2(OCH_2CH_2OCH_3)_2$, THF; then H_3^+O; (h) TsCl, pyridine; (i) CH_2=$CHMgBr$, THF; H_3^+O; (j) MnO_2, CH_2Cl_2; (k) C_2H_5MgBr, THF; H_3^+O; (l) PCC, CH_2Cl_2.



Scheme 2. Synthesis of glutathione and cysteine conjugates of nitroalkene 1 and vinyl ketone 3. Method A: direct addition in CH_3OH with $(C_2H_5)_3N$ as base; Method B: trimethylsilylthioether method; see experimental section for details.

Table 1. Carbon-13 shifts of nitroalkene **1** and derivatives

Assignments	17	1	9	13b	14a
C-1	80.9	142.8	75.8	79.3	79.3
2	68.9	139.7	26.4	44.0	43.9,43.8
3	34.0	32.0	27.5	32.7	33.0,33.2
4	25.4	29-27	29-27	32.6	32.6,31.9
5-13	29-27	29.27	29-27	29-27	29-27
14	22.8	22.8	22.8	22.8	23.0
15	14.2	14.2	14.2	14.1	14.1
Cysteinyl -CH-				52.9	52.8,52.2
$-CO_2H$					170.1
$-CH_2S-$				26.6	26.5,26.3
N-acetyl					170.1,27.7
$-\overset{O}{\overset{\|}{C}}-O-$, $-\overset{O}{\overset{\|}{C}}-NH-$				170.1,170.6,171.0,172.8	
methyl esters				52.6	
Glutamyl $-CH-\underline{CH}_2$				26.7	
$\underline{CH}_2-CO-$				31.9	
$-NH-CO-CF_3$				157(q) ^2J = 38 Hz	
				115(q) ^1J = 288 Hz	
-CH-				52.5	
Glycinyl $-CH_2-$				41.4	

Table 2. Carbon-13 shifts of vinyl ketone **3** and derivatives. Resonances bearing the same superscript may be interchanged

Assignments	18	3	12	16b	15b	13a
C-1	114.0	126.6	7.8	33.8	33.8	33.7
2	141.3	136.9	35.9	43.2[a]	43.0	43.0
3	72.8	193.9	211.0	209.9	209.8	209.8
4	36.9	39.7	42.5	42.5[a]	42.3	42.6
5	25.2	24.2	24.0	23.8	23.7	23.7
6-10	29.0-29.4	29.5-29.8	29.0-29.4	29.2-29.4	29.1-29.5	29.0-30.0
11	28.8	29.3	29.4	28.9	28.9	28.9
12	33.6	34.1	33.8	34.2	34.3	34.2
13	138.8	139.2	139.2	139.2	139.2	139.1
14	114.0	114.4	114.1	114.2	114.2	114.1
Cysteinyl -CH-				52.4	52.3	52.3
$-CO_2H$				171.8[b]		
$-CH_2-S-$				26.6	26.2	26.2
N-acetyl				*173.0[b],22.8*		
$-\overset{O}{\overset{\|}{C}}-O$, $-\overset{O}{\overset{\|}{C}}-NH-$					169.9,170.6,170.8,172.6	169.2,169.4,169.7,170.4
methyl esters					52.5	
Glutamyl $-CH-\underline{CH}_2$					25.9	25.2
$-CH_2-\overset{O}{\overset{\|}{C}}-$					31.7	31.8
$-NH-\overset{O}{\overset{\|}{C}}-CF_3$					157(q) ^2J = 38 Hz	
					115(q) ^1J = 288 Hz	
-CH-					52.8	53.0
Glycinyl $-CH_2-$					41.3	41.3

trimethylsilyl chloride was added to a suspension of 1 equivalent of reduced glutathione in 1,2-dichloroethane containing 3 equiv. triethylamine, and the vinyl ketone **3** (1 equiv.) was then added to the thiosilyl ether solution. After 12 hr, the reaction was quenched with water and the product was precipitated from a pH 2 solution at 4°. This procedure afforded 73% of the glutathione adduct **15a** which gave spectral data consistent with the expected β-keto thioether structure. Both the glutathione and cysteine conjugates of **3** could be prepared by reaction of the appropriate thiol compound in methanol with triethylamine as the base. The adducts prepared in this

fashion were easily analyzed by hplc and NMR but some could not be sufficiently purified for other analytical techniques. Although cysteine conjugates of nitroalkene **1** could be formed in this manner, the attempted formation of the GSH conjugate of **1** in buffered methanol or dioxane gave anomolous products.

Detoxication by workers

Twenty workers of the African termite *Schedorhinotermes lamanianus* were treated with the labelled vinyl ketone (3-[^{14}C]-**3**) by topical application of 100 μg/termite and were held in sealed dishes for 24 hr at 27°. The termites were then homogenized in methanol, the extract was centrifuged, the supernatant was evaporated, and the residue was subjected to reverse-phase hplc. Three radioactive peaks could be observed during the gradient elution from water (pH 5.6 containing 0.1% ammonium acetate) to methanol. Peak I (36% of soluble radioactivity) eluted with the void volume, Peak II (21%) co-chromatographed with (but was not identical to) glutathione adduct **15a**, and Peak III (43%) co-chromatographed with starting material **3** but lacked the UV absorption at 219 nm. The chemical identifications of these three radioactive peaks are described below. Table 3 shows the distribution of soluble radioactivity as a function of incubation time.

Peak I eluted with the solvent front at several pH values for the acetate buffer and we thus believed this to be [^{14}C]-acetate. This was demonstrated by distillation of volatiles from acidified Peak I material, addition of tritiated sodium acetate, formation of the *p*-bromophenacyl acetate, and recrystallization to constant ^{14}C/^{3}H ratio. Approximately 12% of the dpm in Peak I were recovered in this procedure.

Peak II appeared to coelute with the glutathione adduct **15a** and with cysteine adducts **16a,b** in the initial runs. A different solvent program was thus developed to allow complete resolution of tthe thioethers. Under these conditions of increased resolution, the peak II radioactivity was found not to coincide with any of the synthetic thioethers. Although we have not identified this material, we have nonetheless demonstrated the complete absence of the "expected" glutathione, cysteine, and N-acetylcysteine conjugates of this vinyl ketone among the major metabolites.

The identity of the first-produced detoxication product contained in Peak III was established by capillary gas chromatography to be the ethyl ketone **12**. The time course of production of **12** was established by incubation of **3** *in vitro* with tissue homogenates containing different cofactors and then monitoring the appearance of **12** and disappearance of **3** by quantitative GC analysis[6] (Fig. 3). Reduction of the unconjugated 13-ene was not detected during those incubations, although this product (**2**) can be resolved from **12** by chromatography of the soldier

Table 3. Time course of production of radioactive metabolities of vinyl ketone **3** as analyzed by hplc. The value $K' = (V - V_0/V_0)$ is the retention index for each peak. Values shown are relative % of recovered radioactivity

Incubation Time	Peak I k' = 2.0	Peak II k' = 9.1	Peak III k' = 14.5	% Recovery
4 hr	16.2	25.5	57.3	64
24 hr	35.6	20.5	43.9	82
48 hr	75	25	0	78

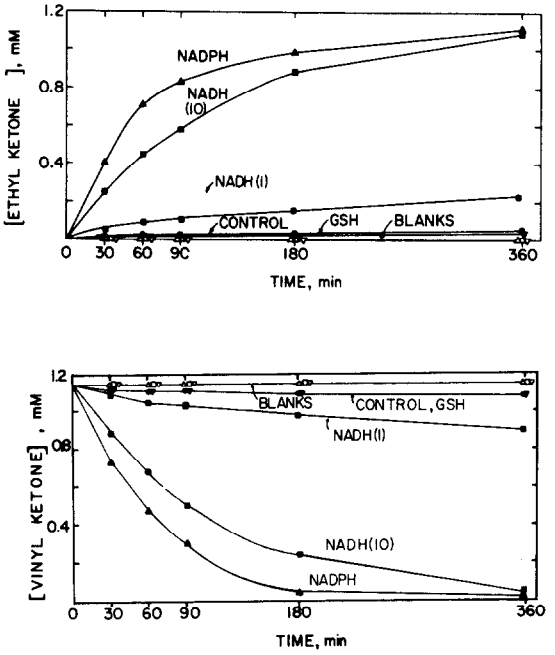


Fig. 3. Cofactor dependence of crude alkene reductase from homogenized *S. lamanianus* workers (details in experimental section) in 50 mM phosphate buffer (pH 7.2) containing 25 mM $MgCl_2$. Incubations were performed with 1.3 mM vinyl ketone **3** and added cofactors (solid figures) at the following concentrations: NADPH 10 mM (triangles); NADH (squares), 10 mM and 1 mM; reduced glutathione (inverted triangles), 4 mM; and with no cofactors (circles). Blank determinations on cofactor solutions lacking tissue homogenates are shown as open figures. *Top*: Appearance of saturated ketone **12**, measured by quantitative GLC of ethyl acetate extracts of incubation mixtures. Bottom: Disappearance of vinyl ketone **3**, also monitored by GLC.

secretion on a 50-m Carbowax 20M capillary column. It was apparent that the initial reductive detoxication of vinyl ketone **3** *in vitro* was independent of added glutathione but dependent on the presence of a reduced nucleotide cofactor. The half-life of **3** *in vitro* at 27° was less than one hour under conditions of ten-fold excess NADPH, and the more electron-poor conjugated alkene was reduced selectively. NADPH was more effective than NADH in promoting this transformation and when NADH was the limiting co-factor, *in vitro* reductions did not go to completion.

Twenty workers of the Floridian termite *Prorhinotermes simplex* were treated with 100 μg each of the labelled nitroalkene **1** by topical application. After 24 hr incubation, homogenization and workup of the methanol extract as described above, reverse-phase hplc analysis revealed three peaks of radioactivity. Peak I (72%) eluted with the void volume, Peak II (4%) co-eluted with adduct **13**, and Peak III (24%) co-chromatographed with starting nitroalkene **1** but lacked the chromophore at 267 nm. Analysis of Peak III by glc indicated the absence of **1** and the presence of the nitroalkane **9** after the 24 hr period. The low specific activity of the nitroalkene **1** frustrated attempts to obtain α-bromophenacyl acetate of constant ^{14}C/^{3}H ratio from Peak I. The identity of the Peak II conjugate also remains ambiguous.

Biosynthesis

Biosynthetic studies using the "less precious" domestic termite *P. simplex* were undertaken to establish the origin of the unusual nitroalkene **1**. Administration of the precursors by feeding it not practical in termites, since soldiers only ingest regurgitated food from workers. We therefore employed two techniques: *in vivo* biosynthesis, in which precursor solutions were injected into soldier or work abdomens,[4] and *in vitro*, in which labelled substrates were added to homogenates of whole termites in a phosphate buffer. We envisaged two schemes for the origin of the nitroalkene, the first based on an α-oxidation/transamination sequence beginning with palmitic acid, and a second sequence based on a modified sphingolipid pathway which requires a myristic acid equivalent and L-serine (Scheme 3).

Labelled palmitic acid (16-[^{14}C]) and sodium acetate (1-[^{14}C]) were tested using both *in vitro* and *in vivo* methods. After incubation, homogenates were extracted with hexane-ethyl acetate (1:1) or whole termites were crushed in the same solvent. The organic fraction was purified by pipette flash chromatography and analyzed by radio-glc and radio-hplc to determine the degree of incorporation of label into the nitroalkene **1** and nitroalkane **9**. Essentially no incorporation of 16-[^{14}C]-palmitate into nitroalkene **1** was detected, although substantial incorporation of acetate was observed. Incorporation of 1-[^{14}C]-myristic acid, 2-[^{14}C]-glycine, and U-[^{14}C]-L-serine are currently being studied.

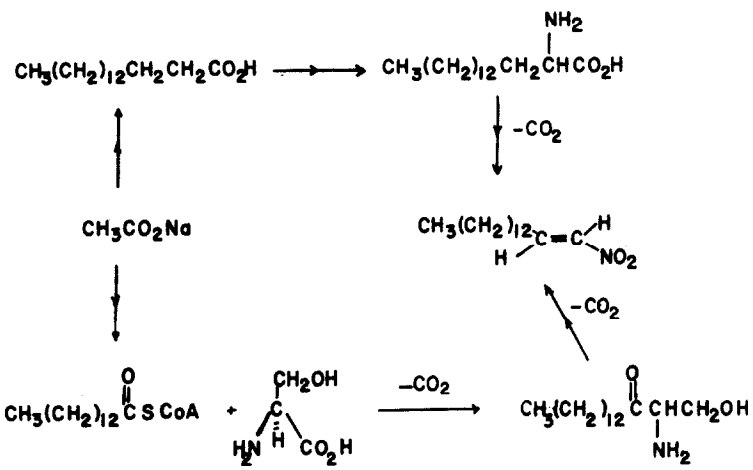
DISCUSSION

Soldiers of the advanced rhinotermitines have evolved an unusual ability to biosynthesize lipid-soluble contact poisons based on the modification of fatty acids into nitroalkenes, vinyl ketones, and β-ketoaldehydes. The toxicity of the defense secretions to biochemically unprotected species is 10–100 fold higher than the toxicity of the soldier secretion to conspecific workers.[6] Moreover, compounds **9** and **12** are an order of magnitude less toxic than the unsaturated compounds **1** and **3**.[6] It is, therefore quite clear that species-specific detoxication systems have evolved in these insectide-producing creatures. The detoxication of endogenously-produced insecticides had been rigorously demonstrated in only one other instance, that of a polydesmid millepede which both makes and disposes of HCN, phenol, and guaiacol.[14]

The detoxication of the termite secretions by an alkene reductase was unprecedented and surprising. We had originally undertaken this project with the expectation that both the mechanism of toxicity and the autodetoxication scheme would involve the addition of biological nucleophiles to the electrophilic α,β-unsaturated systems by conjugate addition.[1,9b,10] The α,β-unsaturated ketone and nitro compounds are well known to react preferentially with sulfhydryl groups *in vivo*,[15] and these reactions can lead to Michael-type adducts with cysteine or glutathione[16] and with enzymes possessing -SH groups at the active site. While the latter process may result in loss of enzyme function,[17] the former process is a key detoxication pathway which facilitates excretion of xenobiotics by rendering reactive lipophilic compounds less reactive and more water-soluble.[16,18]

Although the uncatalyzed reaction of sulfhydryl-containing molecules with conjugated ketones and nitro compounds occurs slowly under physiological conditions,[16] enzymes which catalyze the conjugation of xenobiotics with glutathione have evolved in many organisms, both vertebrates[19] and invertebrates.[20] The glutathione S-transferases generally show reactivity with a broad range of electrophilic alkylating agents. Glutathione S-transferase activity has been found in flies,[21] caterpillars,[22] cockroaches,[23] earthworms,[24] and beetle larvae[20a] and is implicated in resistance to chloronitrobenzenes, diazinon, and a variety of organophosphorus insecticides.[18,21] Thus far, only two insect GSH transferases have been purified: a housefly enzyme using gel permeation and ion-exchange chromatography[25] and a larval moth enzyme using affinity chromatography.[26] Termites in this study were found to have GSH, GSSG, and GSH transferase levels in the range of those reported for resistant flies.[27,28]

The interspecific toxicity of the two defense secretions was determined by exposing workers of *P. simplex* (nitroalkene-producing soldiers), *S. lamanianus* (vinyl ketone-producing soldiers), and the eastern subterranean termite *Reticulitermes flavipes* to filter pads containing a hundred-fold dose range of unlabelled **1** or **3** for a 48 hr



Scheme 3. Possible biogenetic origins for nitroalkene **1**.

period (Fig. 4). For vinyl ketone **3**, *S. lamanianus* workers exhibited the highest tolerance, followed by *P. simplex* and *R. flavipes*. For nitroalkene **1**, *P. simplex* survived the highest doses, followed by *S. lamanianus* and *R. flavipes*. The alkene reductase is effectively absent in *R. flavipes*, and shows substrate specificity in both higher rhinotermitids. We also demonstrated that the reduced "detoxication products" **9** and **12** were less toxic by an order of magnitude than the corresponding α,β-unsaturated compounds.

The rapid substrate-specific reduction of endogenous reactive unsaturated compounds may be the result of evolutionary pressures on termites to develop detoxication pathways which are nitrogen-conserving.[6,29] Detoxication of a defense substance by reduction of the chemically reactive functionality followed by the recycling of its carbons *via* oxidative catabolism may be a novel adaptation for the avoidance of nitrogen loss inherent in the excretion of glutathione or cysteine conjugates of insecticides.

Biogenetic schemes for the derivation of the vinyl ketones, 2-alkanones, and β-ketoaldehydes *via* modified fatty acid β-oxidation pathways were proposed earlier.[10] No further evidence has yet been obtained, although this is an area of ongoing interest in our group.

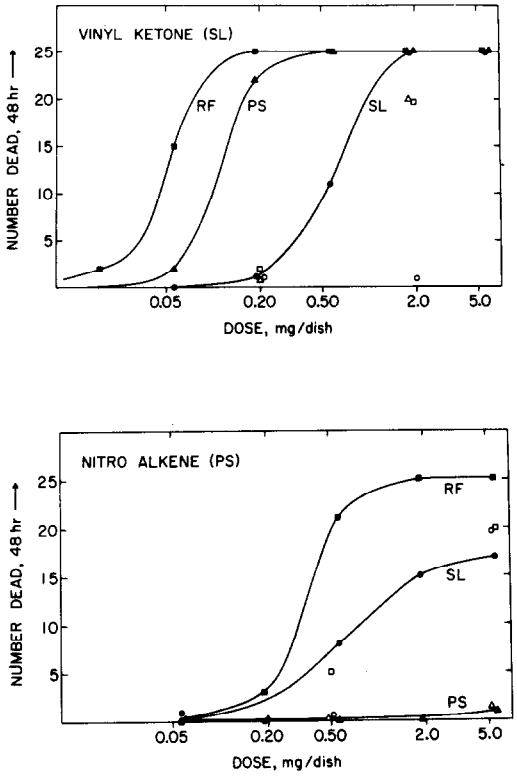


Fig. 4. Interspecific toxity of vinyl ketone **3** and nitro alkene **1** to workers of three rhinotermitids. Twenty-five workers of each species were exposed to a hundred-fold dosage range of pure synthetic compounds absorbed onto moistened cellulose pads. Solid figures show the number of dead termites per dish after 2 days at 28°C, plotted as a function of the dosage (logarithmic scale). No mortality was observed in any control dishes. RF = *Reticulitermes flavipes*; PS = *Prorhinotermes simplex*; SL = *Schedorhinotermes lamanianus*. Open figures show the number of dead termites per dish (20 maximum) for two doses of the "detoxified" ethyl ketone **12** (top) and the nitroalkane **9** (bottom).

The biogenesis of the nitroalkene constitutes another intriguing problem, since nitro compounds are rare in nature. It is even more puzzling to find a nitrogen-containing defense secretion in an organism known to have a nitrogen-deficient diet and to possess elaborate behavioral and biochemical adaptations to conserve nitrogen.[29] Our preliminary studies[27] have shown that acetate is efficiently incorporated by soldiers *in vivo* and *in vitro* into the nitroalkene **1**. However, palmitate was not incorporated intact, and the very low incorporation obtained was rationalized in terms of initial β-oxidation to acetate. This result casts considerable doubt on our first proposal shown in Scheme 3, which would have required α-amination of palmitic acid, oxidation at nitrogen, decarboxylation, and finally dehydration to give **1**.

Our second hypothesis invokes a modification of the sphingolipid pathway. Condensation of the C_{14} fatty acid myristic acid (or its equivalent) with L-serine would give, after decarboxylation, an α-amino β-keto alcohol. Oxidation at nitrogen, reduction at C-3, oxidation decarboxylation at C-1, and dehydration would lead to the nitroalkene. If glycine were involved in the initial condensation instead of serine, the biogenesis could be shortened by several transformations. Further exploration of these possibilities is in progress to establish both the biosynthetic pathway for the nitroalkene *in vivo* and to understand how this termite can "afford" to waste valuable organically-bound nitrogen in colony defense.

EXPERIMENTAL

General procedures. Organic solvents were purified before use: (a) tetrahydrofuran (THF) was distilled under N_2 from Na metal and benzophenone immediately before use; (b) pyridine was distilled from BaO and stored over 4Å molecular sieves; (c) DMSO, bp 50–55° at *ca.* 1.0 mm of Hg, was distilled from a soln of dimsyl sodium in DMSO using the red color of triphenylmethide anion as an indicator and stored over 4 Å molecular sieves; (d) nitromethane was washed with a soln containing 25 g of $NaHSO_3$ and 25 g of $NaHCO_3$ per liter, then with water, 5% H_2SO_4, water, and sat $NaHCO_3$, then dried over $CaCl_2$, and distilled at 60° at 160 mm of Hg; (e) hexanes were washed with conc H_2SO_4, then 30% fuming H_2SO_4, water, sat $NaHCO_3$, dried over $MgSO_4$, and distilled from 4 Å molecular sieves at 68–72° at 1 atm; (f) 1,4-dioxane was distilled under N_2 from LAH immediately before use.

In the extraction procedure "brine" refers to sat NaCl aq, "bicarbonate" to sat $NaHCO_3$ aq, and 12% acid refers to 3N HCl. Solns were dried over $MgSO_4$ and filtered through a layer of Florisil (100–200 mesh). Solvents were removed under reduced pressure with a Büchi Rotovapor-RE.

IR spectra were taken on a Perkin–Elmer 727 spectrophotometer either as a thin film between NaCl salt plates or as a 10% soln in CCl_4. Gas chromatography was performed on a Varian 3700 instrument equipped with (a) 2m × 2mm I.D. glass column packed with 3% OV-17 on Gas Chrom Q, (b) 50-m WBOT glass capillary coated with Carbowax 20M. FID data were processed using a Vista CDS-401 data reduction system. Low-resolution electron-impact mass spectra were obtained using a Hewlett Packard Model 5980A mass spectrometer interfaced to an HP5710A GC equipped with a 1% SP-2100-packed glass column. High resolution mass spectra were obtained on an MS-30 instrument interfaced to an HP7210A GC and a DS-50 data system. NMR spectra were obtained on Varian Associates CFT-20 instruments operating at 20 MHz for ^{13}C and 80 MHz for ^{1}H. High resolution ^{1}H-NMR spectra were obtained on a Bruker 360 spectrometer. Shifts are reported for deuteriochloroform solutions as ppm downfield from $(CH_3)_4Si$, using the $CHCl_3$ resonance as the internal standard for ^{1}H and the $CDCl_3$ resonance for ^{13}C. Microcell ^{13}C-NMRs required Wilmad 8-mm,

120 μl spherical cavities and data acquistion was performed in the double precision mode. Microprobe ^{13}C-NMR was performed in 1.7 mm capillaries in C_6D_6. Liquid scintillation counting was performed principally on a Packard TriCarb with quench correction by both automatic external standardization and channels ratio methods.

Termites

Prorhinotermes simplex (Hagen) colonies were obtained (December, 1978) from rotten pine in Miami, Florida by M. E. Melcer and P. Luykx. *Schedorhinotermes lamanianus* (Sjöstedt) was collected (March, 1980) from a carton nest in a fallen tree (Jadini Forest, Diani, Kenya). Both have been maintained on aged sweetgum or pine in trash cans at 27° and 85% relative humidity since their arrival at Stony Brook. *Reticulitermes flavipes* (Kollar) colonies were obtained on campus and held under similar laboratory conditions. Individuals were removed from stock colonies using soft forceps within 24 hr of use in experiments, and were held on moistened filtered paper during this time.

High-performance liquid chromatography. Twenty termites were homogenized in 2 mL MeOH in a Microflex vial, and insoluble material was removed by centrifugation (100 g) followed by filtration through a Millipore HPLC sample clarification kit. Radioactivity was determined in aliquots of soluble and tissue-digested (Scintigest, 60°, 2d) insoluble fractions. The MeOH was removed *in vacuo* and the residue was dissolved in 2 mL water buffered to pH 5.6 with 0.1% AcOH and NH_4OH. A portion of this material was injected onto a Waters liquid chromatograph equipped with a water-equilibrated reverse-phase column (Whatman PXS 10/25 ODS) and eluted at 1.5 mL/min following a linear gradient to 100% MeOH. Eluent was monitored continuously by fixed wavelength (254 nm) and variable wavelength (219 nm) detectors. Aliquots of samples collected at 2 min intervals were counted using a Packard TriCarb, with quench corrections by automatic external standardization. In later experiments, a Packard RAM 7500 radioactivity monitor interfaced to the Vista CDS-401 data system provided on-line monitoring of HPLC effluent radioactivity.

Synthesis of labelled and unlabelled defense compounds

1-*Tosyloxytridecane* (**7**). A soln of 1.362 g (6.79 mmole) of 1-tridecanol in 50 mL Ca_2Cl_2 and 2.0 mL Et_3N was cooled to 4°, treated with 2.023 g (10.61 mmole) of *p*-toluenesulfonyl chloride (recrystallized from hexane) and stirred for 5 days at 4°. Lactic acid was then added and the soln was stirred for one day at 4°. The soln was washed with bicarbonate and 12% acid, and dried. Concentration of the soln yielded 2.103 g (87%) of the tosylate. The m.p. after recrystallization from hexanes was 43.5–44.0°. IR: $\lambda_{max}^{CCl_4}$ = 1380 and 1180 cm^{-1} (SO_2 stretch). ^{1}H-NMR spectrum: δ7.75 (d, J = 8Hz, 2H), 7.25 (d, J = 8Hz, 2H), 3.6 (t, J = 6Hz, 2H), 2.4 (s, 3H), 1.0–1.8 (br s, 22H), 0.9 (br t, 3H).

1-*Tetradecanenitrile.* Dry NaCN (48 mg; 0.99 mmole was dissolved in 2 mL dry DMSO and then 388 mg (1.09 mmole) of 1-tosyloxytridecane was transferred in 0.5 mL of hexanes. The reaction was stirred for 24 hr at room temp. The soln was diluted with water and extracted with EtOAc. The EtOAc layer was dried and concentrated. The crude reaction product was applied to a 1.5 × 15 cm silica gel (100–200 mesh) column and developed with 30% EtOAc in hexanes to yield 269 mg (quantitative) of the nitrile. Glc (3% OV-17) indicated a purity of 98%. IR: λ_{max}^{film} = 2250 cm^{-1} (–C≡N). ^{1}H-NMR: 2.3 (t, J = 6 Hz), 1.1–1.6 (br s, 22H), 0.9 (br t, 3H).

Tetradecanal (**8**). A soln of 234 mg (1.10 mmole) 1-tetradecanenitrile in 5 mL benzene under argon was cooled to 0°. To the cooled soln was added 1.2 mL of 1 M diisobutylaluminum hydride in hexanes and then the reaction was allowed to warm to room temp. and stir for 3 hr. MeOH was added dropwise until all foaming had stopped. 1,4-Dioxane (10 mL) was added to the soln and then 1 mL of 3N HCl was added and stirred for 1 hr. The mixture was poured into brine and extracted with EtOAc, and the organic layer was dried and concentrated to yield 239 mg (quantitative) of tetradecanal which was 95% pure by GLC and coeluted with an authentic sample.

1-*Nitro-2-pentadecanol* (**17**). A soln of 239 mg (1.13 mmole) of tetradecanal in 50 mL nitromethane was cooled to 0°. To soln was added 0.5 mL of 3N KOH in MeOH and the soln was allowed to warm to room temp. After 2 hr the soln was acidified to pH 2 with conc H_2SO_4 and extracted with EtOAc. The EtOAc layer was washed with brine, dried, and concentrated to yield 250 mg (81%) of 1-nitro-2-pentadecanol. λ_{max}^{film} 3550 (O–H), 1560, 1380 cm^{-1} (NO_2).

(E)-1-*Nitro-1-pentadecene* (**1**). To a soln of 250 mg (0.92 mmoles) of crude 1-nitro-2-pentadecanol in 30 mL pyridine was added 1 mL Ac_2O and the reaction was stirred for 1 hr. The soln was diluted with 12% acid and extracted with EtOAc. The extract was washed with bicarbonate and brine, dried, and concentrated. The resulting oil was chromatographed on silica gel (100–200 mesh) column (1.5 × 17 cm) with 5% EtOAc in hexanes to yield 135 mg (58%) of the 1-nitro-1-pentadecene. IR: $\lambda_{max}^{CCl_4}$ 1660 (C=C), 1530, 1380 cm^{-1} (NO_2). ^{1}H-NMR: δ7.1 (ABX_2, J_{AB} = 14 Hz, 2H), 1.9–2.2 (m, 2H), 1.1–1.75 (br s, 22H), 0.9 (br t, 3H). The ^{13}C shifts for the nitroalkene and related compounds are summarized in Table 1.

2-^{14}C-(E)-1-*Nitro-1-pentadecene* (**1**). The synthesis from tosylate **7** was repeated starting with 46.7 mg Na^{14}C N (500 μCi) to yield 106 mg (48%) 1-nitro-2-pentadecene with a specific activity of 51 μCi/mmole). Radioachemical purity was established using a Packard proportional counter interfaced to an oxidizing oven and a HP5720A gas chromatograph.

1-*Nitropentadecane* (**9**). A soln of 150 mg (60 mmole) of (*E*)-1-nitro-1-pentadecene in 30 mL EtOH was added over a 20 min period to a suspension of 86 mg (2.3 mole) $NaBH_4$ in 20 mL EtOH. After 2 hr, the reaction was acidified with 10% H_2SO_4, diluted with brine and extracted with EtOAc. The organic layer was dried and concentrated. The crude product was evaporatively distilled at 110° at 125 mtorr to yield 140 mg (88%) of **9**. IR: λ_{max}^{film} = 1560, 1385 cm^{-1} (NO_2). ^{1}H-NMR: δ4.25 (t, J = 6Hz, 2H), 1.9 (m, 2H), 1.1–1.3 (br s, 24H), 0.9 (br t, 3H).

10-*Undecen-1-ol.* 10-undecenoic acid (**10**), 1.81 g (9.8 mmol), was added to 10 ml benzene, the soln was added dropwise to 20 ml 30% $NaAlH_2(OCH_2CH_2OCH_3)_2$ and refluxed for 1 hr. 7 mL of 3 M HCl was added dropwise with cooling. The mixture was diluted with 20 mL H_2O and extracted several times with ether. These extracts were washed with sat NaCl, water, dried over $MgSO_4$ and filtered through Florisil to yield 1.48 g (90%) of product: ^{1}H NMR δ5.75 (ddt, J = 9.5, 17, 6 Hz, 1H), 4.75–5.15 (m, 2H), 3.2–3.7 (m, 3H), 1.8–2.2 (broad m, 3H), 1.25 (broad s, 14H). IR: 3500(OH), 3080(C=CH_2), 1640, 1000, 910 cm^{-1} (C=CH_2).

10-*Undecen-1-yl* p-*toluenesulfonate.* 10-undecen-1-ol (1.20 g, 7.1 mmol) was added to 25 mL CH_2Cl_2 and 2 mL Et_3N. After cooling to 0°, 3.57 g (18.7 mmol) TsCl was added and the mixture was stirred (0°, 5d). Lactic acid (1.05 g, 11.7 mmol) was added and stirred for 1 day. The reaction was diluted with 50 mL ether, extracted with 3M NaOH, 3M HCl, and sat NaCl, dried over $MgSO_4$ to yield 2.20 g (95%) of the product. ^{1}H NMR: δ7.75 (d, J = 8 Hz, 2H), 7.25 (d, J = 8 Hz, 2H), 5.75 (ddt, J = 9.5, 17, 6 Hz, 1H), 4.75–5.15 (m, 2H), 4.1 (t, J = 6 Hz, 2H), 2.45 (s, 3H), 1.80–2.20 (m, 2H), 1.25 (broad s, 14 H). IR: 3080 (C=CH_2). 1640 (C=CH_2), 1370, 1180 cm^{-1} (SO_2).

11-*Dodecenenitrile* (**6**). NaCN (0.36 g, 7.3 mmol) was added to 18 mL dry DMSO, 2.13 g (6.6 mmol) of tosylate was added and stirred for 24 hr. The mixture was diluted with 50 mL of water and extracted with ether. The ether layer was washed with sat NaCl and dried over $MgSO_4$ yielding 1.12 g (95%) of the product. ^{1}H NMR: δ5.75 (ddt, J = 9.5, 17, 6 Hz, 1H), 4.75–5.15 (m, 2H), 2.30 (t, J = 6 Hz, 2H), 1.80–2.30 (m, 2H), 1.1–1.8 (broad s, 14H). IR: 3080 (C = CH_2), 2255 (C≡N), 1640, 1000, 910 cm^{-1} (C=CH_2).

11-*Dodecenal* (**11**). Nitrile (980 mg, 5.5 mmole) was dissolved in 40 mL benzene, and 6 mL (6 mmole) of DIBAL-H in hexane was added dropwise. After 1 hr the mixture was cooled to 0° and MeOH was added until no further reaction was observed. The mixture was diluted with 20 mL ether and 5 mL HCl; the ether layer was separated and dried with $MgSO_4$ to yield 710 mg (70%) of product. ^{1}H NMR: δ9.75 (t, J = 2 Hz, 1H), 5.75 (ddt, J = 10, 17, 6 Hz, 1H), 4.75–5.15 (m, 2H), 2.31 (t, J = 7 Hz, 2H), 1.80–2.20 (m, 2H), 1.00–1.80 (m, 14H) ppm. IR: 2720 (CHO), 1730 (C=O), 3080 (C=CH_2), 1640, 990, 910 cm^{-1} (C=CH_2).

1,13-*Tetradecadiene-3-ol* (**18**). Mg metal (160 mg, 6.4 mmole) was placed in 1 mL dry THF, and Grignard formation was started with I_2 and 1,2-dibromoethane. Ten mL THF was added and excess vinyl bromide (in THF) was added while refluxing the THF until all Mg metal was consumed. Aldehyde **11** (560 mg, 3.1 mmole) was added as soln in 5 mL THF at 0°. After 1 hr, the reaction was quenched with ether (H_2O saturated), diluted with hexanes, washed with H_2O, $NaHCO_3$, sat NaCl, and dried over $MgSO_4$ to yield 560 mg (87%) of crude product. ^{1}H NMR: δ5.50–6.00 (m, 2H), 4.75–5.25 (m, 4H), 4.0 (m, 1H), 2.75 (s, 1H), 1.80–2.20 (m, 2H), 1.00–1.80 (m, 16H). IR: 3400 (O–H), 3080 (C=CH_2), 1640, 1000, 920 cm^{-1} (C=CH_2).

1,13-*Tetradecadiene-3-one* (**3**). The crude alcohol (530 mg, 0.29 mmole) was stirred in hexanes with 2.0 g of active MnO_2 for 24 hr. The mixture was filtered through Celite/Florisil to yield 400 mg (76%) of product. Mass spectrum (70 ev) *m/z* (relative intensity): 70 (97), 55 (100). ^{1}H NMR: δ6.05–6.25 (m, 2H), 5.40–6.00 (m, 2H), 4.75–5.10 (m, 2H), 2.45 (t, J = 10 Hz, 2H), 1.80–2.10 (m, 2H), 14H). IR: 3080 (C=CH_2), 1640 (C=CH_2 unconjugated), 1620 (C=CH_2 conjugated), 1685 cm^{-1} (C=O). The mass spectrum and glc retention time were compared with those obtained for the natural product isolated from the termite.[9b]

3-[^{14}C]-1,13-*Tetradecadiene-3-one* (**3**). The synthesis from 10-undecenyl tosylate were repeated starting with 83 mg (1.7 mmoles, 1.0 mCi) of [^{14}C]-NaCN to yield 188 mg of 3-[^{14}C]-1,13-tetradecadien-3-one (**3**) (53% chemical, 56% radioactive, 0.32 mCi/mmole). Radiochemical purity was established using a Packard proportional counter interfaced to an oxidizing oven and a HP5720A gas chromatograph.

13-*Tetradecen-3-one* (**12**). A soln of 180 mg (1.0 mmole) of **11** was added to 3 mL (3 mmole) of EtMgBr soln. After 1 hr the reaction was transferred to sat NH_4Cl aq, extracted with EtOAc, dried and concentrated. Flash chromatography (1.5 cm × 20 cm) with 10% EtOAc yielded 120 mg (58%) of 13-tetradecen-3-ol. The alcohol was oxidized with a suspension of 420 mg (1.92 mmole) of pyridinium chlorochromate in 20 mL CH_2Cl_2. After 1.5 hr, the reaction was filtered through Florisil and concentrated to yield 120 mg (quantitative) of saturated ketone **12**. ^{1}H-NMR: δ5.50–6.10 (ddt, J = 10,17,6 Hz, 1H) 4.75–5.20 (m, 2H), 2.25–2.75 (m, 4H), 1.80–2.20 (m, 14H), 1.00 (t, 3H). IR: λ_{max}^{film} = 3100 (C–H), 1720 (C=O), 1640, 1000, 910 cm^{-1} (C=CH_2). ^{13}C NMR resonances are listed in Table 2. The high resolution mass spectrum gave the molecular ion at *m/z* 210.1968 ($C_{14}H_{26}O$ requires 210.1984).

Synthesis of thioether conjugates

General procedure for thioethers by trimethylsilylthioether method (*Method B*). The thiol compound (reduced glutathione, cysteine, N-acetylcysteine, or the trifluoroacetyl dimethyl ester derivative of glutathione) (0.3 mmole) was suspended in 50 mL dry 1,2-dichloroethane and treated with 1 eq. of Et_3N followed by 1 eq. of chlorotrimethylsilane per active hydrogen. The suspension clears after stirring 1–6 hr at room temp, and then a soln of 1 eq. vinyl ketone **3** or nitroalkene **1** in 1,2-dichloroethane is added. The reaction is stirred 12 hr at 20° and quenched by addition of 1 mL of H_2O and removal of all solvents *in vacuo*. Product purification is effected by dissolving the solids in H_2O, adjusting pH to 2.0, and collecting the product by centrifugation (1,000 g, 4°C, 20 min). Carbon-13 resonances for hydrochloride salts of the adducts in $CDCl_3$ solution are reported in Tables I and II.

Protected glutathione conjugated of **1** (**13b**) was obtained in 62% yield by the method above, except that purification was by flash chromatography using 50% ethyl acetate-dichloromethane.

Cysteine conjugate of **1** (**14a**) was obtained using Method A. Nitroalkene **1** was stirred with 1 equivalent of cysteine for 2 hr in dioxane with solid $NaHCO_3$ as the base. Aqueous workup and precipitation at pH 4 gave 70% of the adduct **14a**.

Glutathione conjugate of **3** (**15a**) was obtained in 87% yield by the general procedure above except the product was precipitated at a pH value of 4.0.

Protected glutathione conjugate of **3** (**15b**) was obtained in 45% yield by the general procedure above except for the purification. The conjugate was flash chromatographed (17 × 1.5 cm, 230–400 mesh silica gel) with 50% ethyl acetate-dichloromethane.

Cysteine conjugate of **3** (**16a**) was obtained in 84% yield by the general procedure above, except the product was precipitated at a pH value of 4.0.

N-acetylcysteine conjugate of **3** (**16b**) was obtained in 51% yield by the procedure above. A high resolution mass spectrum of its methylester was obtained which showed *m/z* 385.2257 ($C_{20}H_{35}NO_4S$ requires 385.2286).

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