Progress in the Chemistry of Organic Natural Products

A. Douglas Kinghorn · Heinz Falk
Simon Gibbons · Jun'ichi Kobayashi
Yoshinori Asakawa · Ji-Kai Liu *Editors*

109

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Volume 109

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ISSN 2191-7043 ISSN 2192-4309 (electronic)
Progress in the Chemistry of Organic Natural Products
ISBN 978-3-030-12857-9 ISBN 978-3-030-12858-6 (eBook)
https://doi.org/10.1007/978-3-030-12858-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, J.-K. Liu (eds.),
Progress in the Chemistry of Organic Natural Products, Vol. 109,
https://doi.org/10.1007/978-3-030-12858-6_1

Abbreviations

% *ee*	Enantiomeric excess
$[\alpha]_D$	Optical rotation at $\lambda = 589$ nm
′	Minutes
″	Seconds
18-crown-6	1,4,7,10,13,16-Hexaoxacyclooctadecane
2D-NMR	Two-dimensional nuclear magnetic resonance
8H-BINAP	2,2′-Bis(diphenylphosphino)-5,5′,6,6′,7,7′,8,8′-octahydro-1,1′-binaphthalene
9-BBN	9-Borabicyclo[3.3.1]nonane
abs	Absolute
Ac	Acetyl
acac	Acetylacetonate
AIBN	Azobisisobutyronitrile
aq	Aqueous
ATP	Adenosine-5′-triphosphate
ATPB	Acetonyltriphenylphosphonium bromide
BHT	2,6-Di-*t*-butyl-4-methylphenol
Bn	Benzyl
brsm	Based on recovered starting material
Bu	*n*-Butyl
Bz	Benzoyl
CAN	Cerium ammonium nitrate
cat.	Catalytic
CD	Circular dichroism
CHC	Cuticular hydrocarbon
COLOC	Correlation spectrometry of long-range coupling
COSY	Correlation spectrometry
Cp	Cyclopentadienyl
CSA	Camphorsulfonic acid
Cy	Cyclohexyl

Cyt	Cytochrome
d	Day(s)
DABCO	1,4-Diazabicyclo[2.2.2]octane
dba	Dibenzylidene acetone
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethyl azodicarboxylate
DET	Diethyl tartrate
DHF	4,5-Dihydrofuran
DHP	3,4-Dihydro-2*H*-pyran
DIAD	Diisopropyl azodicarboxylate
DIBAH	Diisobutylaluminum hydride
diglyme	Bis(2-methoxyethyl) ether
DMAP	4-(Dimethylamino)pyridine
DMAPO	4-(Dimethylamino)pyridine oxide
DME	1,2-Dimethoxyethane
DMF	Dimethylformamide
DMP	2,2-Dimethoxypropane
DMSO	Dimethyl sulfoxide
dppp	1,3-Bis(diphenylphosphino)propane
EDTA	Ethylenediamine tetraacetic acid
EPC	Enantiomerically pure compound
eq	Equivalents
ESIMS	Electronspray ionization mass spectrum
Et	Ethyl
ether	Diethyl ether
exc	Excess
FAB	Fast-atom bombardment
GLC	Gas-liquid chromatography
glyme	1,2-Dimethoxyethane (=dimethylglycol)
h	hour
HFP	Hexafluoropropan-2-ol
HMDS	Hexamethyldisilazane
HMPA	Hexamethylphosphoramide
HMQC	Heteronuclear multiple quantum coherence
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrum
HR-TOF-MS	High-resolution time-of-flight mass spectrum
HSQC	Heteronuclear single-quantum correlation
$h\nu$	Irradiation with light
IBX	2-Iodoxybenzoic acid
IMDA	Intramolecular Diels-Alder reaction
i-Pr	Isopropyl

IR	Infrared (spectroscopy)
IR-120	Acidic ion exchange beads
KHMDS	Potassium hexamethyldisilazide
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
LHMDS	Lithium hexamethyldisilazide
LIS	Lanthanide induced shift
MAD	Methylaluminum bis-(2,6-di-*t*-butyl-4-methylphenoxide)
MCPBA	*m*-Chloroperbenzoic acid
Me	Methyl
MEM	Methoxyethoxymethyl
MOM	Methoxymethyl
MoOPH	Oxodiperoxymolybdenum-(pyridine)-(hexamethylphosphoric triamide)
Mp	Melting point
MPTACl	(+)-α-Methoxy-α-(trifluormethyl)-phenylacetylchlorid (Mosher's reagent)
MS	Mass spectrum
Ms	Methanesulfonyl
MS	Molecular sieve
NaDPH	Nicotinamide-adenine dinucleotide phosphate
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
NMO	Morpholine *N*-oxide
NMR	Nuclear magnetic resonance (spectrometry)
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser and exchange spectroscopy
ORD	Optical rotation dispersion (spectroscopy)
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
Ph	Phenyl
$PhCH_3$	Toluene
PhH	Benzene
Piv	Pivaloyl
PMB	*p*-Methoxybenzyl
PMP	*p*-Methoxyphenyl
PP	Diphosphate
PPTS	Pyridinium *p*-toluenesulfonate
pyr	Pyridine
RCMT	Ring-closing metathesis
Redal®	Sodium bis(2-methoxyethoxy)aluminum hydride
rfl	Reflux
rt	Room temperature
sia	3-Methylbut-2-yl (=siamyl)
TADA	Transannular Diels-Alder reaction
TBAF	Tetrabutylammonium fluoride

TBS	*t*-Butyldimethylsilyl
t-Bu	*t*-Butyl
TEMPO	2,2,6,6-Tetramethylpiperidine *N*-oxide
t	*tertiary*
TES	Triethylsilyl
Tf (OTf)	Triflate
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
thexyl	2,3-Dimethyl-2-butyl
THF	Tetrahydrofuran, tetrahydrofuranyl
TIPS	Triisopropylsilyl
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TPAP	Tetrapropyl perruthenate
TPS	*t*-Butyldiphenylsilyl
Tr	Trityl = triphenylmethyl
Troc	Trichloroethoxycarbonyl
Ts	Tosyl = *p*-toluenesulfonyl
UV	Ultraviolet (spectroscopy)
y	Year
Z	Benzyloxycarbonyl
Δ	High temperature

1 Introduction

Termites (Isoptera), which are a suborder of the cockroaches, are the oldest eusocial insects [1–3]. It is assumed that they diverged from the Cryptocercoidae, wood-feeding cockroaches, in the Late Jurassic, predating the emergence of eusocial Hymenoptera (ants, wasps, bees) by at least 50 million years [1, 4, 5]. In the case of Hymenoptera, the order of insects with the most eusocial species, the evolution toward eusociality can be seen in several extant suborders with species ranking from solitary animals to animals with brood care within small groups up to the highest developed species with colonies of thousands of individuals and at least two castes. Contrary to extant Hymenoptera excepting the suborder of the ants, extant termites are exclusively eusocial, and even the most primitive species consist of more than one caste. The only indication of the evolution toward eusociality is found in comparison with the sister clade of the termites, the wood-feeding cockroaches, Cryptocercoidae. Symbiosis with flagellates containing cellulases allowed the common ancestor of both clades of these hemimetabolous insects feeding on wood. This had dramatic consequences. There was no shortage of food, but the symbionts had to be transferred to the offspring by feeding, which necessitated intense infant care by both parents. Since wood is poor in proteins, a prolonged development to adulthood followed, and offspring of different ages stayed with the parents (most of the symbionts are lost with each molting). It is thought that the splitting of the two

Plate 1 The last of its kind: *Mastotermes darwiniensis*, the only extant species of the most primitive termite family. Pseudergates and one soldier are depicted. Photograph: CSIRO, Creative Commons 3.0

clades started with the takeover of the feeding from the parents by the older offspring. The helping behavior in turn permitted more offspring thus reducing the ability of the parents to defend their offspring, which in turn necessitated the development of specialized defenders. Indeed, in the most primitive termites, a soldier caste occurs but no sterile worker caste. The abandonment of the defense by the parents allowed again higher production of offspring necessitating a stable helper caste—the sterile workers.

The median size of the population of extant termite colonies is 40,000 individuals reaching from 100 individuals with *Cryptotermes piceatus* to 7 million with *Mastotermes darwiniensis* [6] (Plate 1). Nearly 3000 species belonging to ca. 280 genera are known to date, and up to 30 new species are described per annum. These small, soft-bodied, mostly blind insects are found throughout the world in tropical forests, tropical savannas, and semideserts extending to the subtropics [7]. Some of the so-called lower termites were able to colonize even temperate woodlands and temperate rain forests. The highest density in species is found in the northwestern Congolese rain forest. It is thought that termites evolved there.

The termites are divided into "lower termites," which are the evolutionary earlier termites characterized by their flagellate endosymbionts, and "higher termites" including the Termitidae which constitute the largest family with 70% of the termite species. Engel et al. suggested another division of the termites based on extant and extinct termite families [1].

These authors distinguish between the most basal termite family Mastotermitidae, the Euisoptera, termite families that possess no frontal gland, and families possessing a frontal gland, the Neoisoptera [1]. The evolutionary more recent termites (higher termites) have lost the symbiotic protists. In the case of the basal Termitidae, the Macrotermitinae, the flagellates were substituted by ectosymbionts, the basidiomycetous *Termitomyces*. It is thought that the cultivation of those fungi on combs, built from the feces of the termites, necessitated that these termites had to use next to their own feces soil to build their mounds. This led to the change of the gut symbionts and later on to the ability of the Termitidae to feed on soil. Thus,

extant termites feed on soil, wood (dry or damp, decaying), grass, litter, lichens, or the conidia of *Termitomyces*. Digestion is achieved by a large assembly of symbiotic bacteria, archaea, and, in case of the lower termites, flagellates in the hindgut. Predigestion occurs in the midgut by the termite's own cellulases, xylanases, and proteolytic enzymes [8, 9]. Often the feces are converted by microbes externally (bacterial or fungal comb growers) and taken up again by the termites (external rumen). The breakdown of cellulose under the anaerobic conditions in the hindgut as formulated by Brune et al. [10] generates the acetate, needed by the termites for nutrition as well as carbon dioxide and hydrogen:

$$(C_6H_{12}O_6) + 2H_2O = 2CH_3COO^- + 2H^+ + 2CO_2 + 4H_2$$

Hydrogen in turn is used by the symbiotic archaea for methanogenesis [11]. Since the food is generally poor in proteins, enrichment in nitrogen occurs by nitrogen-fixing symbionts [12, 13]. In the case of Macrotermitinae, most of the digestion is transferred to the *Termitomyces* fungi and in the case of *Sphaerotermes sphaerothorax* to bacteria [14, 15], which allows the most efficient exploitation of their food including lignocellulose.

Their feeding behavior has made around 10% of the species of termites into pests for humans by destroying wooden structures, timber, crops, and fruits [16]. A Chinese proverb even warns that a single termite hole can destroy a 1000 m soil dike. In addition to the damage to man-made structures and crops and the deterioration of rangeland [17], the release of methane into the atmosphere (2–5% of the world's atmospheric methane [1, 18, 19]), due to cellulolysis, has to be added. These negative effects are more than counterbalanced by the ecological benefit of the activity of termites. The foraging of these detritivores aerates and improves water infiltration of the soil and increases its nitrogen and phosphorus content [20–22]. Considering that termites constitute a large part of the animal biomass of the tropics and thus dominate (next to ants) the terrestrial ecosystem in the tropics, their ecological benefit is enormous.

All termite colonies contain one pair of reproductives, queen and king, most possibly the founding pair. In some species, especially of fungus-growing termites, colony foundation by multiple male and female reproductives (pleometrosis) is known [23]. The caste system varies and is more flexible in the lower termites than in the higher developed termites (Plate 2).

Lower termites have no sterile worker caste. The later instars of these hemimetabolous insects are arrested in their development. They are called pseudergates (false workers) and forage, build, feed, and groom parents and siblings and care for the eggs. At a later point in their lifetime, pseudergates may develop into soldiers or alates.

As an example of the caste system of the Termitidae, the developmental pathway (Plate 2) of *Nasutitermes exitiosus* (Plate 3) is described [24]: winged termites (alates) swarm (synchronous nuptial flight) from established mounds. After a partner is chosen and established by shedding the wings and tandem running, the pair seeks a suitable place to burrow their nest. Approximately 60 days after the royal cell is

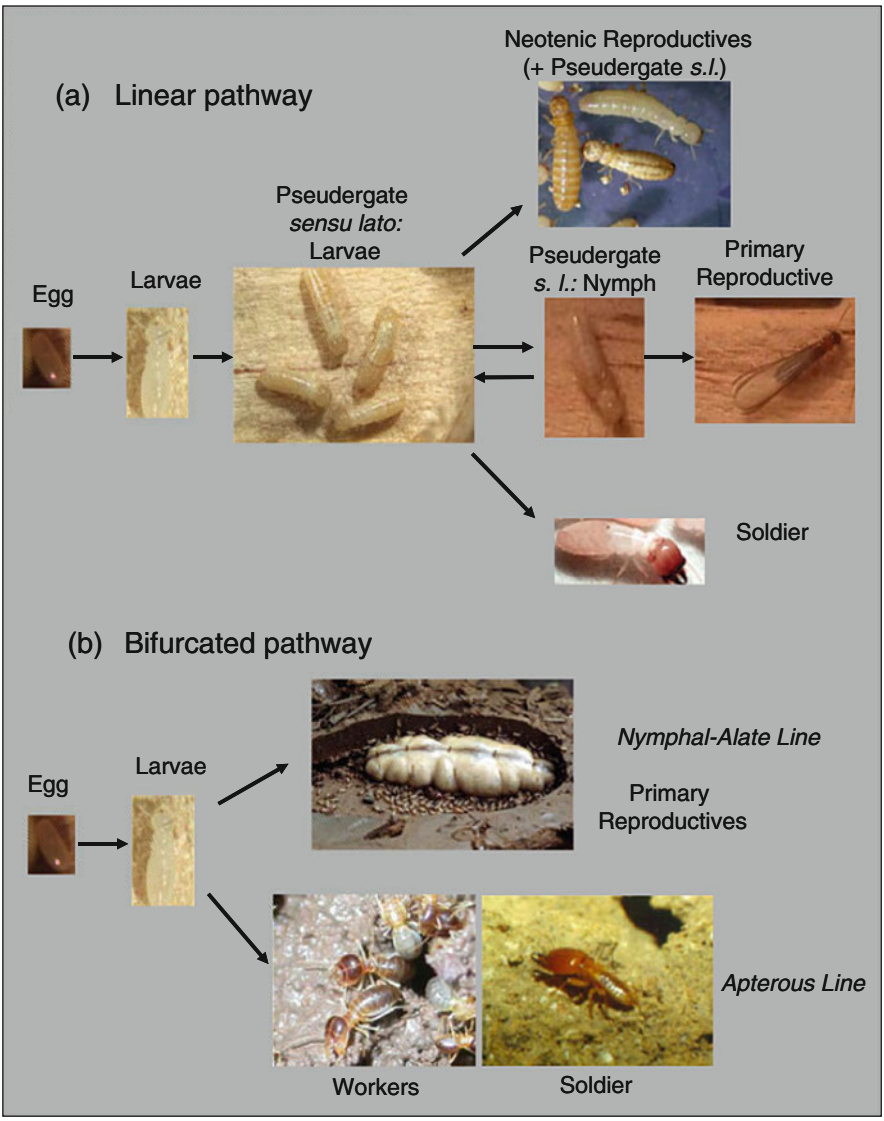


Plate 2 Social organisation and status of workers in termites. Lower and higher termites differ in their development. The hatched larvae of lower termites develop by (several) molting in totipotent pseudergates (false workers), which may develop into reproductives (either winged queens or kings or neotenics) or into soldiers. In higher termites the hatched larvae are transformed by molting either in nymphs with wingpads and further on to reproductives, or become sterile workers, which may stay as workers or develop into soldiers. Plate courtesy of Roisin Y, Korb J (2011). pp 133–164, chapter 6, in Biology of termites: A modern synthesis. Springer Dordrecht, Heidelberg, London New York. Eds Bignell DE, Roisin Y, Lo N, p 137 (Fig. 6.1)

Plate 3 At the other end of the evolutionary development: *Nasutitermes exitiosus*. Their trail-following pheromone was the first termite pheromone isolated. True workers, nymphs with wingbuds, and in their midst one "small" soldier, recognizable by its snout. Photograph: Entomology, CSIRO, Creative Commons 3.0

sealed, the first hatchlings appear, which are fed by queen and king until they have molted several times. Thereafter, the offspring begins to forage and feed and tend the royal pair and the successive siblings (up to 3000 per day). Meanwhile, the queen's abdomen will extend up to 500–1000 times.

In being hemimetabolous insects, no metamorphosis occurs, and the hatchlings look like diminutive, colorless adults. Unlike the lower termites, the differentiation of the castes of the higher termites starts with the first molt. In the case of *Nasutitermes exitiosus* (Plate 3), this leads to so-called nymphs, which are arrested in their development to winged adults, but show wing buds and may develop into supplementary reproductives called neotenics. The main bulk of the second instar is split into smaller and larger instars. After (a) further molt(s), they split into four castes: small sterile workers, small sterile presoldiers, large sterile workers, and large sterile presoldiers. One more molt transforms the presoldiers into soldiers. The development of workers and soldiers is slow; they mature within one year, and their life span is approximately 5 years. The founding royal pair may live up to 25 years. After their death, supplementary reproductives will substitute for them. The most populous castes are those of the sterile workers. The soldiers differ in a more pronounced manner from the workers and nymphs. Their heads are enlarged, pigmented, and highly sclerotized. Whereas the workers are engaged in foraging; tending to the royal pair, the younger siblings, and the eggs; feeding soldiers; building and restoring the nest (mound) and tubes or tunnels; and partly defending, the smaller soldier caste is involved in pioneering, guarding, and defending the mound and foraging or mound- and tube-building workers. The task of the larger soldiers, which usually do not leave the nest, seems to constitute the protection of the brood and the royal pair. Termite colonies are very structured, and the caste proportion is restored rapidly after disturbance.

These small, mostly blind insects are very vulnerable in being not or only barely sclerotized. Thus, they are easy prey even to small predators. For example, dispersing queens, which are better protected than all other castes, because they are more sclerotized and possess defensive substances at this stage [25], survive to less than 1% to found a new colony [1]. Due to their limited sclerotization, termites are very sensitive to temperature variations, light, and desiccation. Thus, to survive they need protection and fortification. The most important way of protection is through their nests. Abe distinguished between one-piece nesters, which live within wood and thus never have to leave their nests to forage; a small group of intermediate nesters, for example, the most basal termites *Mastotermes darwiniensis* (Plate 1); and separate-piece nesters, where nest and food source are separate. Most primitive termites are wood feeders, and thus they live within wood and are protected by the wood. With the development of a true worker caste and other food than wood, the nestings became more complex, spanning from rather diffuse subgeal nests with long subterraneous galleries for foraging to nests that reach high above the ground, called mounds, or carton nests in trees, connected by tunnels (tubes) with the foraging sources. Only a few species of the separate-piece nesters have developed the ability to forage without the protection of mud tunnels in the open air [26]. The most elaborate mounds are found with the fungus-cultivating Macrotermitinae. These structures, built mostly from the feces of termites, contain up to several millions of termites and their fungus combs. The mounds are separated into different chambers with thermoregulation around 30°C, constant humidity, and gas exchange and are the most complex known architectural features in the animal kingdom. Despite their admirable fortifications [27], termites have to defend themselves against many predators, especially against ants. This is the task of the soldier caste. As mentioned above, the heads of soldiers are highly sclerotized, and thus they are able to physically defend the colony. In some species, soldiers plug entry holes or small galleries with their body. In most other species, the physical defense is accomplished by the enlarged sclerotized mandibles. Gradually to this physical defense, chemical defense was added until in the highest developed termite species, defense by soldiers relies entirely on chemical weapons. Mostly from one exocrine gland, toxins, irritants, antihealants, and sticky substances are released. The diversity of compounds isolated from these glands of different families, genera, and species is enormous. Several reviews have dealt with the physical and chemical defense of termites in the past [28–32]. The chemistry of these secondary metabolites is part of the present contribution.

Living in colonies of up to a few million individuals, infections are a high risk especially due to the easily penetrable cuticle of termites and to inbreeding via neotenics. Defense against microbes is achieved by the termites' innate immune system, by antimicrobial peptides [33], and partly by antibiotically active terpenoids of the defensive secretion [34]. Against multicellular parasites, encapsulation is used. Diseased nestmates are either cannibalized or sealed off [35, 36], and cadavers are buried under the soil [37, 38].

Eusociality is defined by three traits: cooperation in caring for the young, reproductive division of labor with more or less sterile individuals working on behalf of individuals engaged in reproduction, and overlap of at least two generations of life

stages capable of contributing to colony labor [39]. Advanced eusociality is defined as a superorganism, in which interindividual conflict for reproductive privilege is diminished and the worker caste is selected to maximize colony efficiency in intercolony competition [40, 41]. In such a superorganism as a termite colony, communication is all-important. Most termites are, with the exception of the alates, blind and live subterranean; thus communication seems to be restricted to vibration, touching with antennas, body contact, smell (olfactory), as well as taste (contact chemoreception). Therefore, communication is mostly conducted by chemicals. Wilson distinguished within the intraspecific chemical communication between pheromones that trigger an immediate behavioral response—the releaser pheromones—and pheromones that initiate long-lasting physiological changes, the primer pheromones. One surprising fact of the chemical communication system of termites is the parsimony of chemicals used. Pheromonal parsimony, or the use of one compound (pheromone) for different interactions, may have been a driving force in expanding the horizons of eusociality as Blum suggests [42]. The cryptic lifestyle of termites, their smallness, the difficulty of rearing these animals in the laboratory as well as the semiochemical parsimony have made the search, isolation, and structure determination of the compounds secreted in picograms from the relatively few (exocrine) glands and the designation of the biological activity very difficult. Despite these disadvantages, contemporary isolation techniques, especially solid-phase microextraction (SPME) [43–45], modern spectrometric methods, and synthesis have permitted the identification of volatile pheromones of more than 100 species. The chemistry of the pheromones of termites is a further part of this contribution.

The small size of the termites, their cryptic lifestyle, their feeding behavior, and the difficulty in maintaining termites in the laboratory have impeded the study of the biochemical pathways of their secondary metabolites particularly. Relevant information unraveled completes the present contribution.

2 Pheromones

Eusocial insect organizations are based on communication and information transfer with semiochemicals being at the center [46]. It is tempting to look for pheromones of termites by analogy to those of the much more investigated pheromones of the hymenopterans, but caution is necessary due to the large evolutionary distance between the homometabolic hymenopterans and the heterometabolic Blattodea. Whereas the ants, which resemble superficially the termites, being also, with the exception of the reproductive caste, flightless, eusocial insects, have a multitude of exocrine glands (>60), morphological data reveal the presence of $\geq$20 different exocrine structures in termites [47] (and literature cited therein, [48, 49]). The scarcity of glands necessitates the use of a relatively small number of semiochemicals for more than one interaction. This so-called pheromonal parsimony [42] as well as the fact that these mostly subterranean living creatures are, with the exception of the alates, blind, thus missing an important sense most hymenopterans

use extensively, raises the question as to how is it possible that termites are able to maintain ordered communities with up to several million members. Their communication seems to be reduced to vibration [50–55], contact (antennation), smell (olfactory), and taste (contact chemoreception). The investigation of the pheromones of the termites is hampered due to the difficulties in rearing the animals in the laboratory and thus to observe and investigate the behavior of such subterranean, light-sensitive organisms. To these impediments are added the small size of the animals themselves and thus of their glands, which secrete the semiochemicals in picogram quantities. Also, the chemical components of these small and few glands have to perform a multitude of semiochemical tasks. Depending on concentration and in combination with further components of the same or other glands, different activities may be initiated. Modern isolation techniques like solid-phase microextraction (SPME) [43–45], electroantennograms (EAG) [56–59], spectrometric methods, and synthesis have permitted the identification of volatile pheromones of ca. 100 species. In contrast, the contact pheromones, with the exception of the cuticular hydrocarbons, are mostly unknown [60]. The structure and activity of primer pheromones of termites and thus of caste differentiation and developmental retardation are with few exceptions left to speculation.

Of the 20 distinct exocrine glands known to date, the contents and function(s) of their secretion are known only in part for six glands:

(a) The labial or salivary gland [61], which exists in all species and castes. Labial gland secretions serve various functions during nest construction, colony defense (especially in the basal termites that possess no frontal glands (Euisoptera and *Mastotermes darwiniensis* (Plate 1)), colony hygiene, and aggregation at gnawing sides.
(b) The sternal gland is found in all castes and in most termite families. It secretes the trail-following pheromone, which in many families is also a sexual pheromone.
(c) The posterior sternal gland delivers the sex pheromone in Macrotermitinae [62].
(d) The tergal gland is found in the last tergites of alates and secretes sexual pheromones. In 2012, Costa-Leonardo detected tergal glands also in the soldiers of the subfamily Syntermitinae [63, 64].
(e) The frontal gland, the best investigated exocrine gland in termites, is found in the head of all Neoisoptera (≡ Termitidae, Serritermitidae, and Rhinotermitidae) as an unpaired gland. It varies considerably between families and genera, and it is well developed only in soldiers. In most taxa this gland is restricted to the head; in Rhinotermitidae it extends into the abdomen. Its contents, irritants, glues, or contact poisons are secreted from a frontal pore, the fontanelle. The secretion serves the defense but may have other functions as well. For example, it may regulate caste determination, has fungistatic and bacteriostatic activities, and induces alarm and aggregation.

    Of the other glands very little is known; the contents of the secretion as well as the activities these secretions initiate are not known as yet. For example, the

recently discovered clypeal gland is only found in the reproductive caste and may contain queen or king pheromones [49].

(f) The cuticle, which some researchers see as the largest exocrine gland, contains a mixture of chemicals. The hydrocarbons are thought to effect nestmate recognition and protection against dehydration. The differences in the hydrocarbons (straight-chain and methyl-substituted hydrocarbons) within the colony may influence caste differentiation. The cuticle also contains antibiotics and proteinaceous compounds; the function of these compounds is still unknown.

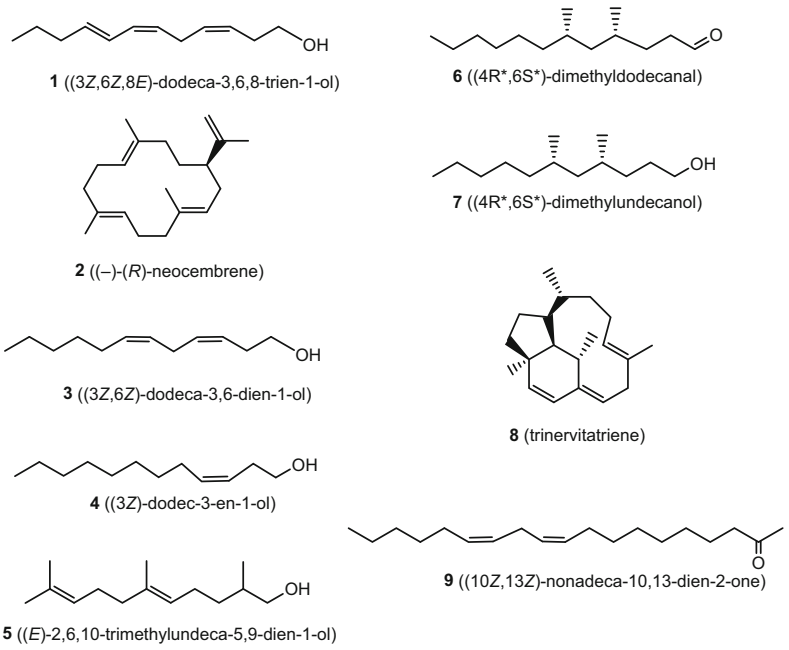
2.1 Releaser Pheromones

Termites have few, highly conserved releaser pheromones used to determine a certain behavior, as, e.g., for trail-laying, sex-pairing, home-marking (?), food-marking, phage stimulation, aggregation, alarm, worker-arresting, egg-recognition, etc. These are found in most termite families.

2.1.1 Trail-Following Pheromones

The trail-laying behavior of termites [62–76] has been described several times since 1911 [77–79] (and literature cited therein). However, the source and nature of the secretion remained unknown until Lüscher and Müller investigated the trail-laying behavior of *Zootermopsis nevadensis* [77]. By partially covering ventral body parts of the insects, they found the sternal gland as the source of the biologically active secretion. Further they determined that the active compounds are soluble in ether. Setting the animals on a wire gauze above an artificial trail of sternal gland secretion initiated trail-following, thus demonstrating the volatile nature of the secretion.

For the mostly blind termites, chemical communication when foraging is essential. This is especially true for separate-piece nesters. However, trail-laying pheromones are also found in one-piece nesters, probably to guide nestmates to breaches in the nest or to mark their nest. Some termite species use additional cues for guidance. Hodotermitinae have compound eyes and thus are the only termite workers able to use optical signs [80]. Rickli and Leuthold demonstrated that *Trinervitermes geminatus* uses magnetic guidance [81]. Evans et al. list several species that support the chemical cues by vibrational signals [51–53]. Pheromone trails may well be templates of the construction of tunnels and galleries [27]. Kaib et al. described the search for food in the subterranean termite *Reticulitermes flavipes* (formerly *R. santonensis*) [82, 83]. A few pioneer workers explore the territory in every direction laying a dotted trail; when food is discovered, they return to the nest laying a continuous trail. This trail is followed within seconds by other workers. Soldiers are less sensitive and thus follow when a larger group of workers explores the trail laid. Consequently, galleries or tunnels are built for the main group of foragers. The chemistry and biology of trail-following communication have been described for around 70 species from all families of termites [69]. So far only nine



Scheme 1 Trail-following pheromones of termites

active compounds are known [72, 84]. Most likely these pheromones are derived from the sex pheromones of the alates [85].

The first structure determined of a trail-following pheromone was (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol (**1**) (Scheme 1), which is found in most families of termites [70, 72, 73, 75, 84, 86–88]. For this unsaturated primary alcohol, the threshold for trail-following in *Rhinotermes flavipes* is 0.01 pg/cm trail, whereas stereoisomers and derivatives are only slightly active, more than seven orders of magnitude less than **1** [87, 88]. Later on, **1** was detected as the general trail-following pheromone of the Reticulitermitinae and found in other genera of Rhinotermitidae also. Saran et al. found for *Reticulitermes hesperus* that the pheromone stimulus lasted around 48 h and that an unbelievable 0.05 fg/cm of **1** stimulated the termites [66]! In the secretion of the sternal gland of *Coptotermes formosanus* (Rhinotermitidae), **1** is the main compound accompanied by small amounts of the isomeric (3*Z*,6*E*,8*E*)-dodeca-3,6,8-trien-1-ol, but its function is unknown as yet [89, 90]. Compound **1** is thought to be the general pheromone for orientation, whereas recruitment is initiated by species-specific pheromones.

The monocyclic diterpene neocembrene (cembrene A, (*E*)-6-cembrene, (1*E*,5*E*,9*E*,12*R*)-1,5,9-trimethyl-12-(1-methylethenyl)cyclotetradeca-1,5,9-triene) (**2**) was the first termite pheromone isolated. In this contribution the name neocembrene is used and the numbering follows the one introduced by Moore: (1*R*,3*E*,7*E*,11*E*)-3,7,11-trimethyl-1-(1-methylethenyl)cyclotetradeca-3,7,11-triene. This numbering was used consistently not only for neocembrene itself but also for the polycyclic diterpenes derived from neocembrene up to the most recent

syntheses of the tetracyclic diterpenes of termites. Moore chose an abundant Australian *Nasutitermes* species for his investigation and demonstrated that the isolated pure compound was a trail-following pheromone [91, 92]. Much later, **2** was also identified as trail pheromone of the lower termite subfamily, Prorhinotermitinae [93]. The electroantennogram of the gas chromatogram (GC/EAD) of the sternal gland secretion showed next to neocembrene (**2**) a small peak at the retention time of (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol (**1**), which could explain the difference in attractivity of pure neocembrene and the sternal gland extracts for *Prorhinotermes* sp. [94].

More than 30 years passed until the next trail-following pheromones were characterized. (*Z*,*Z*,*E*)-Dodeca-3,6,8-trien-1-ol (**1**), (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (**3**), and (3*Z*)-dodec-3-en-1-ol (**4**) are the respective main compounds of the sternal glands of two Macrotermitinae [95, 96]. (3*Z*)-Dodec-3-en-1-ol (**4**) was found in several more Macrotermitinae shortly afterward [97]. Later on, **4** was found as the main trail-following pheromone of the family of the Euisopteran Kalotermitidae [98].

In the last two decades, investigation of lower termites led to the characterization of the norsesquiterpene alcohol (*E*)-2,6,10-trimethylundeca-5,9-dien-1-ol (**5**) as a trail-following pheromone (*Mastotermes darwiniensis* (Plate 1)) [99], *syn*-4,6-dimethyldodecanal (**6**) as the main trail-following pheromone of *Zootermopsis* species (Archotermopsidae) [100, 101], and *syn*-4,6-dimethylundecan-1-ol (**7**) as the main trail-following pheromone of *Hodotermopsis sjöstedti* (Archotermopsidae) [102].

Although many termite species follow trails scented with **1**, the mixture of compounds secreted from the sternal gland is species specific. The explanation for this phenomenon is that **1** is the general orientation signal that is supplemented by species-specific components. Possibly cuticular hydrocarbons (CHCs), which are not only species specific but also colony specific, enhance the attractivity of the trail pheromone for nestmates. One further cyclic diterpene, the tricyclic (11*E*)-trinervita-1(14),2,11-triene (**8**), was detected in glands of a *Nasutitermes* species, and it is assumed to have trail-following activity [103, 104]. Recently, a new unusual trail-following pheromone, (10*Z*,13*Z*)-nonadeca-10,13-dienone (**9**), was isolated from the sternal gland of *Glossotermes oculatus* (Serritermitidae) [69]. This compound has a surprisingly high boiling point although it should be active only for a short time. (10*Z*,13*Z*)-Nonadeca-10,13-dienone (**9**) has not been detected in any other termite family. Only this single compound was detected and confirmed by synthesis [69]. The threshold for detection of trail-following pheromones depends on caste and age. Depending on the species, older workers or soldiers are the pioneers and have the lowest threshold for the trail-following pheromone. After food has been detected, the pheromone trail is reinforced, which leads to mass recruitment. Wen et al. were able to demonstrate that the ratio of compounds **3** and **4** varied depending on the behavior of the forager [71]. The open-field foraging Macrotermitinae *Odontotermes formosanus* was chosen, and the ratio of the trail pheromones was determined [71]. These examinations revealed that **4** induced exclusively orientation, whereas **3** is responsible for orientation as well as recruitment. The fact that the ratio of pheromone components is variable depending on searching for food or recruiting was detected in eusocial insects for the first time.

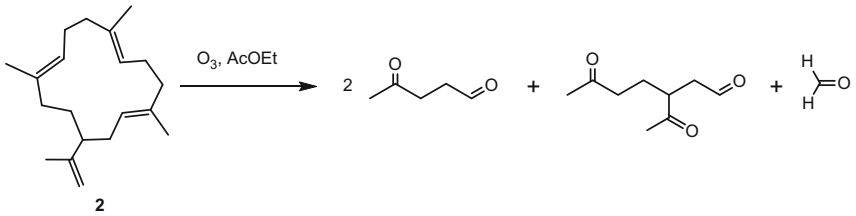
Isolation and Structure Determination of Trail-Following Pheromones

A few years after Butenandt et al. isolated and characterized the first insect pheromone bombycol ((10*E*,12*Z*)-hexadeca-10,12-dien-1-ol) [105–107], Moore published the isolation of the first (trail-following) pheromone of a termite [91]. Moore's task was even more difficult than Butenandt's. The rearing of the domesticated silkworm *Bombyx mori* was relatively easy, and the larger size of the insect yielded larger amounts of secretion from its glands. In contrast, Moore had to deal with small, cryptic living, wild animals. Thus, capture and rearing of the animals as well as the isolation, structure determination, and bioassays conducted required much more effort.

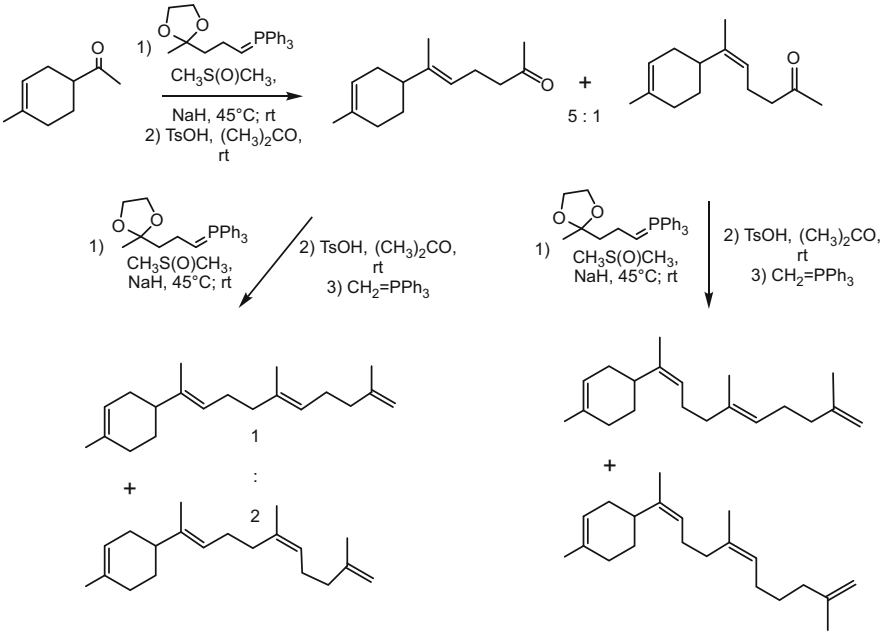
Moore chose the abundant, relatively easy to rear Australian termite *Nasutitermes exitiosus* (Termitidae, Plate 3) for his investigations. He started his research by extracting whole animals in alcohol and after evaporation of the solvent heated the residue to higher temperatures. This procedure led to the isolation of ellagic acid and isomers, which may well be part of the digested food of the termite workers [108]. In his next attempt, the termites were homogenized in ice water with 0.1% quinol as antioxidant. Steam distillation resulted in volatile, oily monoterpenes (α-pinene, β-pinene, and β-phellandrene) [109]. To detect the so far evasive trail-following pheromone(s), he then extracted homogenized termites in light petroleum [91]. The extract was treated with aqueous base, and the unsaponifiable fraction was chromatographed on alumina. The active fraction was vacuum distilled with *n*-eicosane as carrier and chromatographed subsequently on silica gel. The two compounds obtained were separated by preparative GC. The molecular weight as well as the fragmentation pattern of the mass spectra of the pure compound pointed to a diterpene. The structure was narrowed down by means of IR and UV spectra and microhydrogenation followed by mass spectrometry to a monocyclic diterpene with four isolated double bonds, but the amount of the pure compound was insufficient for a complete structure elucidation. Moore continued the structure elucidation by developing a microozonization method [110] and by looking for more abundant sources of the compound in plants guided by bioassays.

The ozonization products (Scheme 2) and the ^{1}H NMR spectra of **2** pointed to one disubstituted and three trisubstituted double bonds. These data led the authors to the erroneous assumption that the compound is a substituted cyclohexene. To prove this assumption, the possible substituted cyclohexenes were synthesized by methods developed for carotene syntheses [92] (Scheme 3).

None of the synthesized compounds showed significant trail-following in termites. Therefore, guided by biosynthesis reasoning, the authors concluded that geranylgeranyl pyrophosphate, the open-chain precursor of cyclic diterpenes, closes to the 14-membered ring of the cembrenes [111–116]. However, the compound was



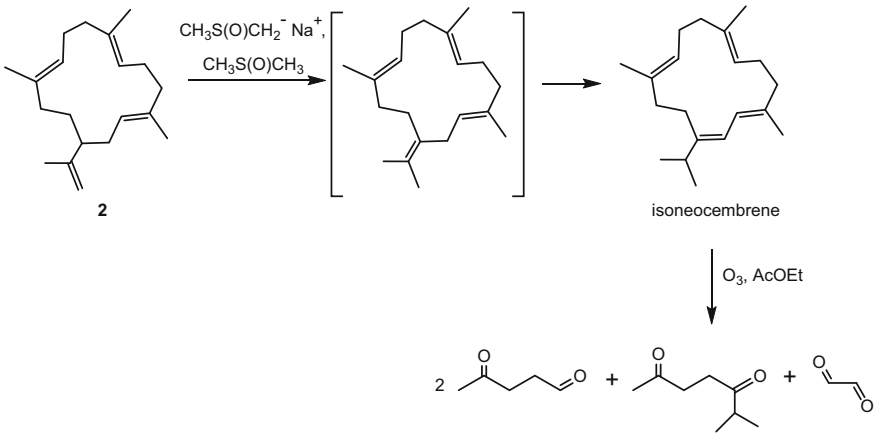
Scheme 2 Ozonization of neocembrene (**2**)



Scheme 3 Synthesis of substituted cyclohexenes

not identical with cembrene [117]. An improved isolation from *Nasutitermes exitiosus* (Plate 3) permitted further experiments.

Isomerization of the purified trail-following pheromone with strong base and subsequent microozonization pointed to the structure of neocembrene (**2**) (Scheme 4). The structure determination was corroborated by comparison with a plant product isolated and characterized at that time [118]. Although the positions of the double bonds were ascertained, their configuration had to be determined by



Scheme 4 Isomerization of neocembrene (**2**) and subsequent degradation

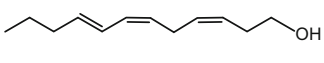
synthesis [119–122]. Six years and the cooperation of Australian and Japanese researchers [92, 123] were necessary to characterize the monocyclic diterpene as neocembrene (**2**). Resolution of synthetic racemic neocembrene indicated that both enantiomers are biologically active [124]. Kato, Moore et al. by synthesizing two additional stereoisomers of neocembrene demonstrated that only neocembrene (**2**), with all double bonds (*E*)-configured, showed trail-following activity in *N. exitiosus* [124]. Traniello et al. found that **2** effects only orientation in termites (*N. corniger* = *N. costalis*) but no recruitment and it is not as active as the secretion of the sternal gland [125].

Indeed, in more recent years, several examples of two-component trail-following pheromones with neocembrene (**2**) as one of the components have been described [93, 94]. Since Moore's first isolation of neocembrene (**2**), this monocyclic diterpene has been isolated as trail-following pheromone from *Nasutitermes* species [104, 117, 125, 126] but, surprisingly, also from a subfamily of lower termites, the Prorhinotermitinae [93, 94]. Neocembrene was perceived as sex pheromone of some Nasutitermitinae [103], and it was found in the frontal glands of soldiers of some species of the Cubitermitinae [127, 128]. Prestwich was able to gather enough neocembrene from the defensive secretion of *Cubitermes glebae* to determine its absolute configuration as (−)-(*R*)-neocembrene (**2**) [128]. The same absolute configuration of neocembrene (**2**) and of its polycyclic derivatives was found in the secretion of all investigated termite species independent of family, caste, or gland. As mentioned above, neocembrene was isolated from plants [118, 129] but also from animals (soft corals [130], Pharaoh's ant [131], and the Chinese alligator [132]).

While Moore, Kato et al. were involved in the structure determination of neocembrene (**2**), Coppel et al. investigated the trail pheromone(s) of a termite species of the family Rhinotermitidae [86, 87]. The observation that wood decayed

by the fungus *Lenzites trabea* (*Gloephyllum trabeum*) attracts termites [88] initiated Coppel's search for the trail-following pheromone of *Reticulitermes virginicus*, a species especially attracted. For the isolation 385 g of termites and 15 kg of decaying wood were homogenized, extracted with *n*-pentane, and after evaporation codistilled with mineral oil. Chromatography on Florisil and further separation by preparative GC led to pure compounds. The respective compounds obtained from both sources had the same R_f value. Comparison of the spectra of the 100 µg pure compound derived from the decaying wood with the imperfect spectra of the 1 µg of pure compound derived from the termites showed no deviations. The bioassays corresponded qualitatively and quantitatively. Thus, the identity of the two compounds was accepted. The absorption at 234 µm in the UV spectrum pointed to a conjugated diene. The ^{1}H NMR spectrum revealed signals corresponding to one methyl group; three methylene groups, two of them allylic methylene groups and one diallylic methylene group; and a primary alcohol group (Table 1). The mass spectrum with the molecular peak at 180 mu and the IR spectrum confirmed the primary alcohol group. Thus, the researchers concluded that the compound is a *n*-dodecatrien-1-ol. Microhydrogenation produced the known *n*-dodecan-1-ol. The most likely structure fitting those data was a dodecatrienol with $\Delta^{3,4}$- and $\Delta^{8,9}$-double bonds. The position of the third double bond as well as the configuration of the double bonds was determined by synthesis of several isomers of dodecatrienol. Bioassays showed that the most active compound (>0.1 pg for 10 cm trail) was (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol (**1**), which has identical spectra to the natural

Table 1 NMR spectrometric data of the abundant trail-following pheromone (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol (**1**) Ref. [133]

Structure, Nr.	Position	^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz)	^{13}C NMR (δ/ppm)
 (3*Z*,6*Z*,8*E*)-Dodecatrien-1-ol (**1**)	1	3.65 q $J_{1,2} = 6$	62.2
	2	2.37 dt $J_{2,3} = 7$, $J_{2,1} = 6$	30.8
	3	5.41 dtt $J_{3,4} = 11$, $J_{3,2} = 7$, $J_{3,5} = 1$	125.7
	4	5.56 dtt $J_{4,3} = 11$, $J_{4,5} = 7$, $J_{4,2} = 1$	131
	5	2.95 t $J_{5,4} = J_{5,6} = 7$	26.1
	6	5.25 br dt $J_{6,7} = 11$, $J_{6,5} = 7$	127.1
	7	5.98 br t $J_{7,6} = J_{7,8} = 11$	129.0
	8	6.32 ddq $J_{8,9} = 15$, $J_{8,7} = 11$, $J_{8,10} = J_{8,6} = 1$	125.4
	9	5.69 dt $J_{9,8} = 15$, $J_{9,10} = 7$	135.3
	10	2.09 q $J_{10,9} = J_{10,11} = 7$	34.9
	11	1.42 sext $J_{11,10} = J_{11,12} = 7$	22.5
	12	0.91 t $J_{12,11} = 7$	13.8
	OH	1.68 t $J_{OH,1} = 6$	

product. Thus, (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol (**1**) was determined as the first known structure of a termite (trail-following) pheromone.

Despite the rapid improvement of separation techniques and spectrometric methods in the following 30 years and the knowledge of the lipophilic nature of these pheromones, only **1** was isolated from several termite species in more than one family [70, 74, 76, 89, 90, 134–136]. As an example of the search for trail-following pheromones at that time, Tokoro's investigation of *Coptotermes formosanus* may be described [89, 90]. Around 200,000 pseudergates (561 g), reared on red pine in a darkened laboratory at 28°C and 80% humidity for 5 years, were separated in 10 portions and fed with filter paper for 5 days to substitute for intestinal material and prevent the isolation of dietary materials. Then the termites were soaked in *n*-hexane for 3 days. After filtration the portions were combined, and the solvent evaporated yielding 51 g material. Fractionating by chromatography on silica gel with *n*-hexane/ethyl acetate mixtures led to 350 mg active material, which was further purified on silver nitrate-impregnated silica gel. Two further separation steps consisted of HPLC and GC on a nonpolar and then on a polar column. Each fraction was tested by the following simple bioassay: the dissolved fraction was streaked along a cycle ($d = 4.7$ cm) on filter paper in a Petri dish covered with a red-colored lid. When at least a third part of the test animals stayed on the cycle for more than 2 min, "basic activity" was assumed. With these fractions the activity was further investigated by dilution steps. The pure active compound was analyzed by capillary GC combined with MS and FTIR [134]. A small molecular peak and several characteristic fragmentation ions, the UV spectrum, and several microreactions revealed the structure as identical with **1**. This was confirmed by comparison of the spectra with the spectra of synthetic **1**, those of synthetic stereoisomers, and those of synthetic (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (**3**).

In 1990, Pawliszyn introduced "solid-phase microextraction" (SPME) as a new isolation technique [43], which allowed the extraction of small body parts without the use of solvents [44, 45, 137]. Primarily this method, along with the introduction of excision of body parts (e.g., glands) with microscissors under a stereomicroscope, the detection of bioactivity by electroantennogram (EAG), as well as the use of computer-assisted statistical analyses has facilitated and accelerated the discovery of new trail-following pheromones and led to the confirmation that in several termite species, more than one substance is involved in trail-following.

In 2001, an additional trail-following substance was characterized as (*Z*)-dodec-3-en-1-ol (**4**) [95]. Bordereau et al. chose the fungus-growing termite *Macrotermes annandalei* not only to employ these new methods but also to compare them with the established techniques. Whole bodies of workers or excised glands were immersed in solvents. No significant differences in the biological activity were detected when changing the solvent (*n*-pentane, *n*-hexane, or dichloromethane) or between extracts of the whole animals and of the sternal glands. The extraction by SPME was carried out under a stereomicroscope by rubbing the surface of the exposed sternal gland with a polydimethylsiloxane/divinylbenzene fiber, which then was inserted in the injection port of a gas chromatograph. The *n*-hexane extracts of whole animals as well as of excised glands were purified by chromatography on silica gel, and the

fractions were tested by bioassays. On the filter paper a Y was drawn. At the stem and one branch of the Y, a small part of a fraction was drawn with a syringe, and at the other branch, pure solvent was drawn. The time and distance the test animal traveled were measured. The active fractions were further purified by preparative GC on capillary columns. Characterization was carried out by GC/MS. Electron-impact as well as chemical-ionization mass spectra were investigated. The peaks of the capillary gas chromatogram were compared with standardized retention indices [138–141]. The mass spectrometric data revealed a dodecen-1-ol. The position of the double bond was elucidated by the addition of dimethyl disulfide to the unsaturated primary alcohol and analysis of the fragmentation patterns of the mass spectra of the obtained adduct. The stereochemistry of the double bond was established by synthesis [95]. Comparison of the gas chromatogram of an extract of the sternal gland surface and an intertergal membrane surface by SPME showed that four compounds were specific to the gland surface including (3*Z*)-dodec-3-en-1-ol (**4**).

In a similar way, the sternal gland extract of another Macrotermitinae, *Ancistrotermes pakistanicus*, and SPME of the sternal gland surface permitted the characterization of (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (**3**) [96], which was confirmed by synthesis.

The use of electroantennography (EAG) [56] coupled with gas chromatography (EAD) [57] allowed the detection of a two-component trail-following pheromone for the first time [94]. The trail-following pheromone of *Prorhinotermes simplex* had been investigated earlier [93], and neocembrene (**2**) was the single substance that was bioactive. Even by the more sensitive detection technique of two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC/TOF-MS), no other bioactive gland-specific compound could be detected. Using EAD revealed a second bioactive substance in the extract of the sternal gland of pseudergates of *P. simplex*. The retention indices and MS fragmentation pattern pointed to (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol (**1**) as a second active component. Experiments with neocembrene (**2**) and synthetic **1** showed that *Prorhinotermes simplex* preferred a mixture of **2** to **1** (10^{-1} ng/cm:10^{-8} ng/cm) to pure **2** and even to a trail made of sternal gland extract.

In the same manner, the trail-following pheromone of several Nasutitermitinae was investigated and found to consist of neocembrene (**2**) as main compound and dodecatrienol **1** as minor component [104]. In several of the Nasutitermitinae investigated, trinervitatriene **8**, found earlier as a sex pheromone [103], supplemented the gland extracts. The short lifetime of laboratory-reared Nasutitermitinae prevented the delineation of the function of this diterpene [104].

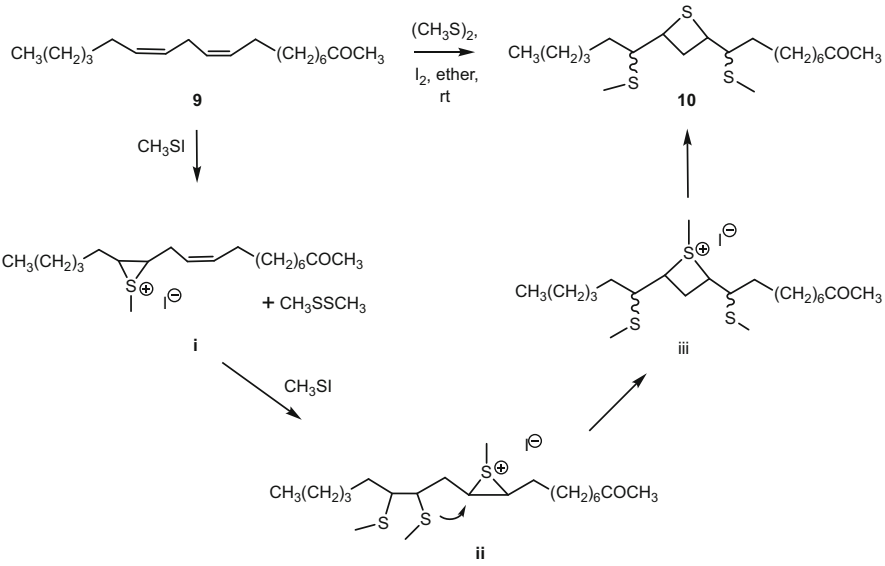
Basal termites possess sternal glands and are able to follow sternal gland extracts although the function of the trail pheromones of these one-piece nesters is disputed. More recently the structures of trail-following pheromones of basal termites have been elucidated [99, 101, 102].

The evolutionary oldest extant termite species *Mastotermes darwiniensis* (Mastotermitidae, Plate 1) is not a one-piece nester and possesses contrary to all other termites not one but three sternal glands. Whole pseudergates and their excised sternal glands were immersed in solvents differing in polarity. Additionally, a

polydimethylsiloxane/divinylbenzene fiber was applied for the SPME by rubbing the exposed sternal glands. Extracts of sternal glands were fractioned by preparative GC and the fractions tested in trail-following bioassays. The same isolation techniques were employed for the isolation of the trail-following pheromone(s) of the basal one-piece nesters *Porotermes adamsoni* and *Stolotermes victoriensis*. Comparing the GC/MS of the SPME of all three species revealed that they used a common trail-following pheromone. The accumulated SPME extracts of two of the species were fractioned by preparative GC. Surprisingly, the mass spectra of the bioactive compound suggested a terpenoid structure. The authors concluded, based on the retention indices, the molecular peak, and the fragmentation pattern, that the compound is the norsesquiterpene, 2,6,10-trimethylundeca-5,9-dien-1-ol (**5**). Syntheses of both possible stereoisomers established not only the suggested structure but also its stereochemistry as (*E*)-2,6,10-trimethyl-5,9-undecadien-1-ol (**5**). The same techniques (SPME, GC/MS with electron ionization as well as chemical ionization, retention indices, FTIR, "T-maze" bioassay, and syntheses) were employed for the search of the trail and sex pheromones of the basal termites exemplified by two *Zootermopsis* species [101] and one Hodotermopsinae [102]. Infrared spectra, the molecular peak, and the fragmentation pattern in the mass spectra sufficed to determine that pseudergates of *Hodotermopsis sjöstedti* use (4*R**,6*S**)-dimethylundecan-1-ol (**7**) as trail-following pheromone. The stereochemistry was determined by synthesis [102]. In the same way, the trail pheromone of the pseudergates of *Zootermopsis nevadensis* and *Z. angusticollis* was characterized as (4*R**,6*S**)-4,6-dimethyldodecanal (**6**) [101].

After isolation and characterization of the trail-following pheromones of around 60 species and finding only few bioactive hydrocarbons and primary alcohols of moderate boiling point over a period of nearly 60 years, it was surprising when the sternal glands of a species of the little-investigated family Serritermitidae yielded an unsaturated long-chain methyl ketone. The excised glands of *Glossotermes oculatus* were extracted in *n*-hexane, and the complex mixture of compounds was fractionated by chromatography on silica gel [69]. The bioactive fractions were further separated by preparative GC. Two-dimensional GC coupled with TOF-MS was used to characterize the substance as nonadecadien-2-one (**9**).

The positions of the double bonds of **9** were confirmed (Scheme 5) by the fragmentation pattern in the mass spectra of the dimethyl disulfide adducts **10**. This method was developed by Vincenti et al. for linear nonconjugated dienes [142, 143]. Iodine converted dimethyl disulfide into the methylthioiodide, which added to the double bond forming the thiiranium salt **i**. The generated iodide attacked a further dimethyl disulfide. The formed methylthiolate attacked the charged thioepoxide of **i**, and methylthioiodide formed the next charged thioepoxide **ii**. Intramolecular attack of the sulfide opened the thioepoxide by simultaneous formation of charged thietane. According to Vincenti et al., no five-membered ring was observed. Iodide transformed **iii** into **10**. Electron ionization occurs preferentially at the electron-rich sulfur atoms, thus yielding characteristic fragments. The stereochemistry of the double bonds was determined by the facile syntheses of **9**.



Scheme 5 Addition of dimethyl disulfide and iodine to skipped double bonds

The much-improved analytical techniques allowed Wen, Ji, and Sillam-Dusses to demonstrate with *Odontotermes formosanus* (Macrotermitinae) the dependence of the trail pheromone composition on caste as well as on behavioral context within a caste [71]. Solid-phase microextraction-GC, SPME-GC/MS, GC/EAD, retention indices, synthetic reference compounds, Y-shaped trail-following bioassays, the recorded video files, and statistical analysis showed that workers use two components, **3** and **4**, as trail pheromones, whereas soldiers use only one component, namely, **4**. The proportion of the two components in the secretion of the sternal gland of workers depends on the activity of the animal. Heavy work, such as brood care and wood gnawing, needs a higher proportion of **3** than trail-laying or construction of fungus gardens. Older termite workers having the largest sternal glands serve as the pioneers. A hungry worker is able to follow a trail made by 1 fg/cm! The authors found no synergistic effects between the two pheromone components. The bioassays indicated that **4** is responsible for orientation and **3** for orientation and recruitment. Trail-following activity increases with the number of workers having laid a trail, but longer exposure to **3** makes the antenna of workers insensitive and leads to chaotic behavior at the food side.

2.1.2 Sex-Pairing Pheromones

Although most of the behavior of termites [62–64] is initiated by chemical stimuli, present knowledge of sexual pheromones is still scanty. Due to the cryptic lifestyle

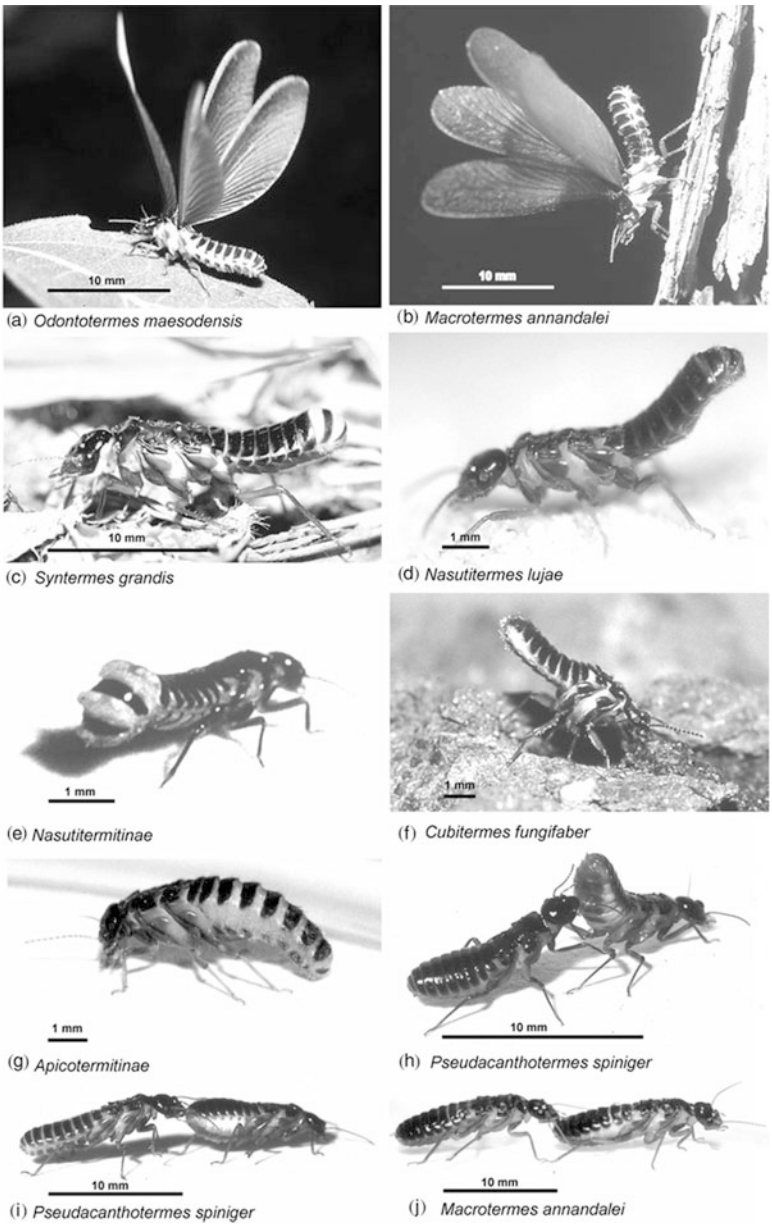


Plate 4 Pheromones and chemical ecology of dispersal and foraging in termites. (a)–(f) Calling females exposing their tergal and/or sternal glands to lure males with their sexual pheromones. (g) shows a female between callings. (h)–(j) After meeting, male and female plug their wings and decide by tandem running, with the male antennating and licking, if they fit together and look for a suitable place to found a new colony. Plate courtesy of Bordereau C, Pasteels JM (2011) Biology of termites: a modern synthesis, eds Bignell DE, Roisin Y, Lo N. Springer Dordrecht, Heidelberg, London New York, pp 279–320, chapter 11, p 282 (Fig. 11.1)