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(3*Z*,6*Z*,8*E*)-3,6,8-DODECATRIEN-1-OL: SEX PHEROMONE IN A HIGHER FUNGUS-GROWING TERMITE, *Pseudacanthotermes spiniger* (ISOPTERA, MACROTERMITINAE)

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Abstract—The female sex pheromone of the fungus-growing termite *Pseudacanthotermes spiniger* (Termitidae, Macrotermitinae) was isolated from sternal glands of alates. The compound inducing attraction and excitation in males was identified as the (3*Z*,6*Z*,8*E*)-3,6,8-dodecatrien-1-ol by GC-MS, microhydrogenation, GC-FTIR, and NMR. This unsaturated alcohol is present in both sexes but in much higher quantities in females than in males (about 10 times). The hypothesis is suggested that this alcohol, which is detected at extremely low concentrations by the workers of *P. spiniger*, may be used either as a trail-following pheromone or a sex pheromone according to concentrations and to target castes. The presence of this alcohol in Macrotermitinae reinforces the idea of a phylogenetic proximity between this subfamily of higher termites and the lower termites Rhinotermitidae, where the unsaturated alcohol was previously found. The sternal glands of alates of *P. spiniger* also contain a geometric isomer of (3*Z*,6*Z*,8*E*)-3,6,8-dodecatrien-1-ol.

Key Words—Termites, Isoptera, Macrotermitinae, *Pseudacanthotermes spiniger*, insect sex pheromone, (3*Z*,6*Z*,8*E*)-3,6,8-dodecatrien-1-ol.

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INTRODUCTION

Termite colonies generally reproduce by seasonal dispersal flights of winged males and females (alates). The meeting and the pairing of these alates is mediated through various factors, among which chemical stimuli from abdominal glands are of prime importance. However, sex pheromones have been demonstrated only in a few cases: *Kalotermes* (Wall, 1971), *Zootermopsis* (Pasteels, 1972; Stuart, 1975), *Hodotermes* (Leuthold, 1977), *Reticulitermes* (Buchli, 1960; Stuart, 1969, 1975; Clément, 1982), *Trinervitermes* (Leuthold and Lüscher, 1974; Leuthold, 1977), and only one publication has dealt with the chemical nature of termite sex pheromone (Clément et al., 1989).

In this paper, we report on the isolation and the identification of the female sex pheromone of a fungus-growing termite, *Pseudacanthotermes spiniger*. This species is one of the rare species of termites in which pairing occurs in the air during the swarming flight (Grassé and Noirot, 1951; Grassé, 1984). The nests of *P. spiniger* are entirely subterranean, but epigeous turrets (up to 1.5 m high) are built by the workers just before the swarming season. The alates are gathered in these "swarming turrets" some weeks before the flight. For some years, *P. spiniger* has been becoming more abundant in the sugarcane plantations of equatorial Africa and is becoming economically important.

METHODS AND MATERIALS

Alates of *P. spiniger* were collected in the savannah near Franceville in Gabon, where the colonies of *P. spiniger* swarm at the end of the rainy season (May 1988, 1989, 1990). One hundred fifty thousand alates were collected either directly from swarming turrets or during the flight by covering these turrets with mosquito nets.

In *P. spiniger* sex attraction is intense and can be observed in the laboratory for several days and even several weeks. In Petri dishes, the females take up a "calling" posture with their highly raised abdomen exposing the sternal gland. There is no differentiated tergal gland in the alates of *P. spiniger*. The males are attracted by these calling females. As soon as they touch the females, a tandem run is released. The female is the leader, the male follows, palpating with antennae and mouthparts the female abdominal extremity. As already mentioned (Stuart, 1969), this suggests that in the sexual behavior both olfactory and tactile stimuli are involved. Therefore, two males were used for each sex attraction bioassay since a single male is deprived of tactile communication and shows rather unstable behavior.

Laboratory Bioassays. Sex attraction bioassays were carried out by introducing two males into 15-cm-ID Petri dishes. These dishes contained small bent

pieces of Whatman No. 1 filter paper (1 cm^2) on which 20 μl pentanic extracts were applied with a microsyringe. The solvent was evaporated immediately before the bioassay. A similar piece of paper treated with 20 μl pentane and evaporated to dryness was used as control. The two males were introduced at the same time and at the same distance from the pieces of paper. During 300 sec, the time spent by one or two males licking or palpating the different pieces of paper was measured. A bioassay was noted as positive for an extract when the time was longer than 150 sec. After each bioassay, the Petri dish was cleaned with ethanol and pentane; males and pieces of paper were replaced.

Extracts. Two kinds of extracts were prepared: (1) Extracts from whole individuals were prepared by washing dealate males or females in doubly distilled pentane for 1 hr at 4°C, 250 ml solvent was used for 4000 individuals. Samples were stored at −20°C. (2) Extracts from sternal glands were prepared by removing the glands under a stereomicroscope with microscissors and forceps from cold anesthesized males or females. The glands [dimensions of the gland (μm)—females: 1800/550/150; males: 600/250/80] were removed from adjacent tissues (hemolymph, fat body) with a paper towel and transferred to a vial of doubly distilled pentane (200 glands/2 ml solvent) and allowed to soak for 1 hr at 4°C; then the glands were removed and the extracts were used immediately for bioassays or stored at −20°C for chemical analyses.

For all bioassays, the extracts were tested at a concentration of one individual equivalent.

Chemical Analyses. Gas chromatographic analyses (GC) were carried out with a Packard 429 gas chromatograph fitted with a split–splitless injector and a flame-ionization detector. The capillary column used was either a DB-5 fused silica column (30 m × 0.32 mm ID, J&W Scientific) or a CP-WAX 58 CB column (25 m × 0.25 ID, Chrompack) with temperature programming from 50°C to 220°C, at 2°C/min. Injector and detector temperatures were, respectively, 230°C and 250°C. Helium was used as the carrier gas at a flow rate of 5 ml/min. The GC traces were recorded and integrated by a CR3A Shimadzu integrator.

For preparative GC, a Girdel 30 chromatograph fitted with a Pyrex column (3 m × 3.2 mm) packed with 5% SE 30 on 100–120 mesh Chromosorb AW-DMCS, and a flame-ionization detector was used with helium as the carrier gas (flow rate 15 ml/min). Oven temperatures were programmed from 100°C to 200°C, at 2°C/min. An effluent splitter was used that allowed 50% of the effluent to flow to the detector. Fractions were collected in U-shaped glass tubes (1 mm ID) cooled to 0°C.

Gas chromatographic–mass spectrometric (GC-MS) analyses were carried out with a Nermag R 10-10-C quadrupole mass spectrometer coupled to a Girdel 31 gas chromatograph fitted with a split–splitless injector and a DB-5 fused

silica capillary column (60 m × 0.32 ID). The GC conditions described above were used. The column was connected directly to the ion source of the spectrometer through a heated transfer line maintained at 260°C.

Electron impact (EI) mass spectra were obtained at 70 eV on an 0.8-sec cycle, the instrument scanning from 25 to 300 amu with the ion source maintained at 150°C. Positive chemical ionization (CI) mass spectra were generated at 90 eV using methane or ammonia (and deuterated ammonia) at a source pressure of 0.2 and 0.3 torr, respectively, with a source temperature of 90°C, and the instrument scanning from 60 to 300 amu in 0.7 sec. Negative CI was obtained with the hydroxyl anion OH^- by ionizing a 5:1 mixture of methane and nitrous oxide (CH_4–N_2O) at a source pressure of 0.3 torr.

Microhydrogenation was performed on line in the GC-MS system by means of a fused silica capillary reactor. The reactor, consisting of a 60-cm piece of deactivated fused silica capillary column (6 m × 0.32 mm ID), statically coated with a 0.5% CH_2Cl_2 solution of palladium acetyl-acetonate (Fluka) was connected to the outlet of the analytical column with a zero-dead volume capillary butt-connector (Supelco) (Le Quéré et al., 1989). Palladium metal was precipitated at 220°C under hydrogen flow. Hydrogen was then used as the carrier gas at a flow rate of 1.5 ml/min, and the GC conditions were those previously described.

Fourier transform infrared (FTIR) spectra were taken in the gas phase on a Bruker IFS 85 FTIR spectrometer coupled, via a Bruker gold-coated lightpipe (20 cm × 1.0 mm ID) maintained at 200°C, to a Carlo-Erba 5160 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m × 0.32 mm ID), an on-column injector, and a flame ionization detector. The carrier gas was helium at a flow rate of 3 ml/min and temperature programming from 30 to 60°C at 10°C/min and then to 200°C at 3°C/min.

For NMR analysis, the trapped component was rinsed from the tube into a Teflon-lined capped vial with distilled pentane, evaporated to dryness, and redissolved in deuterochloroform ($CDCl_3$, 99.96% D, CEN-CEA, Gif sur Yvette). The purity of collected fractions was monitored by capillary GC.

[^{1}H]NMR spectra were recorded on a Bruker WM400 instrument in 5-mm-ID NMR tubes. The signal due to the residual protons of the deuterated solvent was used as internal reference (δ = 7.24).

RESULTS

Origin Sex Pheromone. In *P. spiniger*, the males are attracted by the females; the females are never attracted by the males. In bioassays, the females are neither attracted by the male extracts nor by the female extracts (0 positive out of 30 bioassays performed).

The results obtained with the males (Table 1, A) show that the bioassays are not biased when using two males, since these two individuals never joined together for a long time on control papers in the Petri dish.

The males were strongly attracted by female extracts (Table 1, B). In most of the cases, the males started running rapidly toward the female extracts and met each other on the female extract in few seconds. Then, they could stay on the extract during the entire bioassay, while licking and palpating the piece of paper and each other alternately. In a few cases (3/20), males were attracted by the male extracts. This is not completely surprising since male–male homosexual tandems have been observed sometimes.

If given a choice between extracts from female sternal glands and extracts from whole females without sternal gland, the males always choose the sternal gland extract (Table 1, C). It could be concluded that the females of *P. spiniger* attract males by means of a sex pheromone that is secreted by their sternal gland.

Isolation of Sex Pheromone. The GC patterns of male and female washes were similar. However, a compound with a retention time of 57 min was about 10 times more abundant in females than in males. The same peak was observed in the sternal gland extracts of both sexes, and it was also about 10 times higher in female glands than in male glands (Figure 1, compound A). Compound B, with a retention time of 62 min, is also more abundant in females than in males.

The extracts from whole individual washes were fractionated by gas chro-

TABLE 1. SEX ATTRACTION BIOASSAYS: ORIGIN OF FEMALE SEX PHEROMONE OF *Pseudacanthotermes spiniger*[a]

Bioassay	Extract	Positive responses (%)	T (sec) ($\bar{X} \pm$ SD)
A	Control 1	0	0.8 ± 0.5
	Control 2	0	1 ± 1.1
B	Female wash	85	240.8 ± 45
	Male wash	15	40.5 ± 38
	Control 2	0	0
C	Female sternal gland	95	240.9 ± 24
	Female without sternal gland	0	0.7 ± 0.4
	Control 2	0	0.5 ± 0.4

[a] Choice tests for male dealates between filter paper impregnated with various extracts. All extracts were tested to one individual or one gland equivalent. A bioassay was considered as positive when males licked and palpated extracts for at least 150 sec. (Duration of the bioassay = 300 sec.) T: mean time ($\pm$SD) spent by males on extracts. Control 1: piece of filter paper. Control 2: filter paper previously impregnated with pentane. Number of replicates: 20.

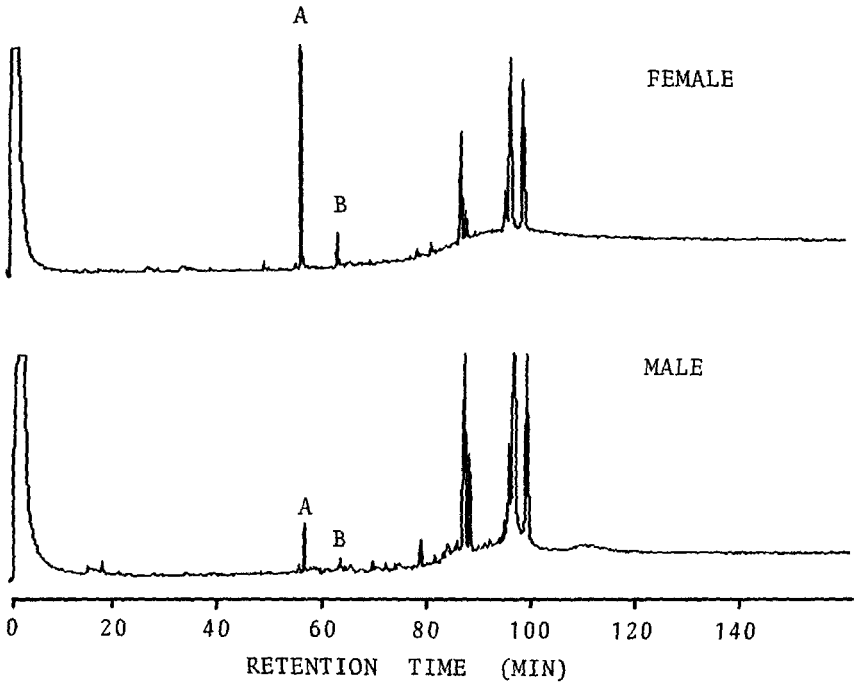


FIG. 1. Gas chromatograms of sternal gland extracts from female (upper) and male (lower) alates *Pseudacanthotermes spiniger*. Compounds A and B are much more abundant in females than in males. A is the female sex pheromone of *P. spiniger*.

matography, and the fractions were tested in bioassays at the concentration of one individual equivalent (Figure 2 and Table 2). Only fractions 2 and 4 were attractive for males and able to induce sexual behavior in these individuals (quick moving, licking, antennal palpating). Fraction 2, which only contained compounds A and B, was as active as the whole female wash. When only fractions 1 and 3 were tested, there was no positive response. Fractions 4 and 5 enabled us to separate compounds A and B: only fraction 4 with compound A was active. Compound A is the female sex pheromone of *P. spiniger*.

Identification of Sex Pheromone. The mass spectrum of compound A is presented in Figure 3. A spectral search in various MS data bases gave no plausible candidate. However, a fragment ion at *m/z* 31 suggested a primary aliphatic alcohol (Budzikiewicz et al., 1964), and the fragmentation pattern was found typical of a polyunsaturated compound. The molecular weight, suggested by an important ion at *m/z* 180, was confirmed by chemical ionization GC-MS, with methane and ammonia as reagent gases. The methane-CI mass spectrum displayed pseudomolecular ions at *m/z* 179 $(M - 1)^+$, 180 $(M)^+$ and 181 (M

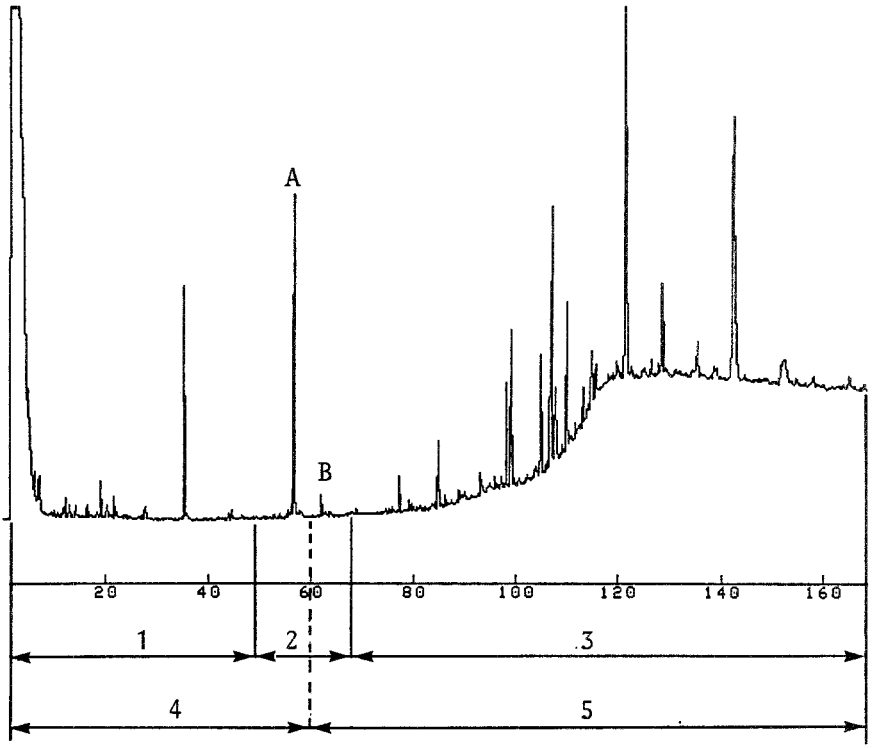


FIG. 2. Chromatographic fractions of washes of female alates *Pseudacanthotermes spiniger*. Fraction 2 contains compounds A and B, which are especially abundant in females. Fraction 4 contains A , fraction 5 contains B. Only fractions 2 and 4 induced attraction and excitation in male dealates.

$+ 1)^+$ and a prominent ion at m/z 163 $(M - 17)^+$, characteristic of an unsaturated alcohol of molecular weight 180 (Sarris et al., 1985). This was confirmed by the ammonia-CI mass spectrum, which showed intense pseudomolecular ions at m/z 181 $(M + 1)^+$ and 198 $(M + NH_4)^+$, and a weak ion at m/z 163 $(M - 17)^+$. These molecular ions and their isotopic contributions suggested a molecular formula $C_{12}H_{20}O$ and three sites of unsaturation.

The functionality was confirmed with a CI-MS experiment with deuterated ammonia (ND_3) as reagent gas. The pseudomolecular ions at m/z 183, $(M - H + D + D)^+$ and 203, $(M - H + D + ND_4)^+$ revealed one exchangeable proton (Lin and Smith, 1979).

Online microhydrogenation (Le Quéré et al., 1989) gave a mass spectrum typical of a straight-chain primary alkanol, with no molecular ion. However, an ion at m/z 168, tentatively attributed to $(M - H_2O)^+$, suggested a molecular

TABLE 2. SEX ATTRACTION BIOASSAYS: ATTRACTIVENESS OF CHROMATOGRAPHIC FRACTIONS DERIVED FROM FEMALE WASHES OF *Pseudacanthotermes spiniger*[a]

Bioassay	Extract	Positive responses (%)	T (sec) ($\bar{X} \pm$ SD)
A	Fraction 1	0	0.13 ± 0.15
	Fraction 2	100	280.2 ± 8.2
	Fraction 3	0	0.03 ± 0.06
	Control	0	0
B	Fraction 1	0	0.3 ± 0.3
	Fraction 3	0	1.1 ± 0.9
	Control	0	0.03 ± 0.06
C	Fraction 4	100	278.5 ± 10.9
	Fraction 5	0	1.4 ± 1.05
	Control	0	0.2 ± 0.17
D	Whole extract	46.6	131.4 ± 51.6
	Fraction 2	50	154.1 ± 50.9
	Control	0	0.9 ± 0.7

[a]Choice tests for male dealates between different chromatographic fractions of female pentanic washes. All fractions were tested to one individual equivalent. See table 1 for abbreviations. Number of replicates: 30, except for bioassay B where N = 10.

weight of 186, and thus, the structure 1-dodecanol. This was confirmed by spectral search in MS data bases and by OH^- CIMS [m/z 185 $(M - 1)^-$], revealing three carbon–carbon double bonds in the original compound, accounting for the three sites of unsaturation, and consequently, the structure dodecatrien-1-ol.

The GC-FTIR spectrum of A (Figure 4) confirmed the hydroxyl group (ν OH: 3657 cm^{-1}; ν C–O: 1049 cm^{-1}). The C–O stretching frequency was characteristic of a primary alcohol (Nyquist, 1984). The OH stretching frequency was rather low for a primary alcohol, suggesting an intramolecular hydrogen bonding, probably with one of the carbon–carbon double bonds, in a $(5 + \pi)$ system (Nyquist, 1984), revealing a 3-alken-1-ol subunit. Other important features of the vapor-phase infrared spectrum were the C=C stretching frequency at 1659 cm^{-1} and the CH out-of-plane deformation frequency (δ CH) at 719, 949, and 980 cm^{-1}. The former (719 cm^{-1}) was attributed to a *Z*-disubstituted ethylenic bond and the pair 949 and 980 cm^{-1} to *Z,E*-conjugated double bonds.

Finally, the structure of compound A, isolated by preparative GC, was established by [^{1}H]NMR, including two-dimensional chemical shifts correlation (COSY) and selective decoupling experiments. The 400-MHz [^{1}H]NMR spec-

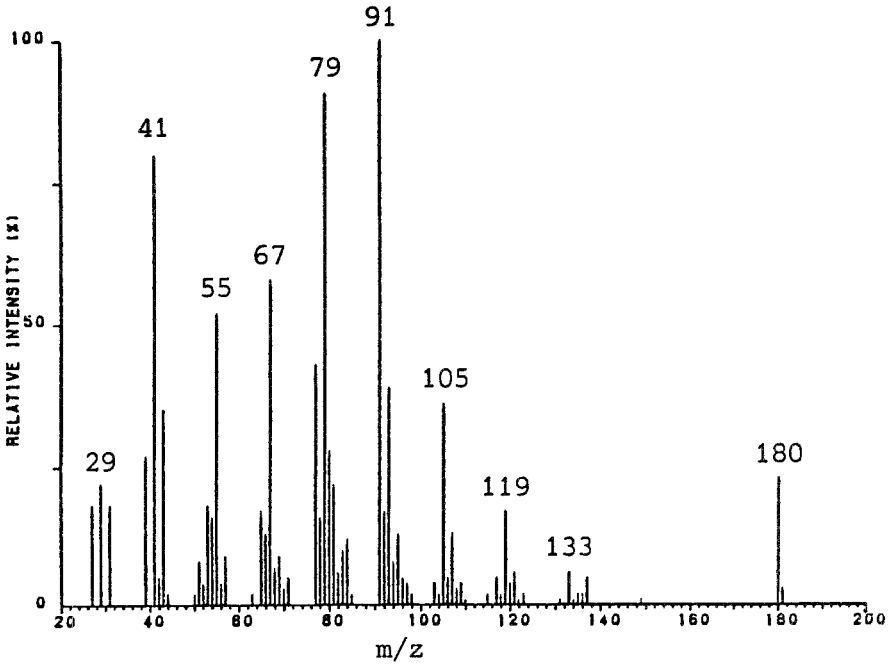


FIG. 3. Mass spectrum (70 eV) of compound A.

trum (Table 3) revealed well-resolved multiplets due to six olefinic hydrogens at δ 6.32, 5.98, 5.70, 5.58, 5.42, and 5.25 ppm.

The proton at δ 6.32 (dd) was coupled (J = 15 Hz) to a vinyl hydrogen (δ 5.70), confirming one *E*-double bond, and coupled (J = 10.9 Hz) to another olefinic proton (δ 5.98), confirming the conjugated double bonds. The *Z* geometry of this second double bond was confirmed by the coupling constant (J = 10.7 Hz) measured in the olefinic resonance at δ 5.98 (dd) which, itself, revealed a coupling with the vinyl hydrogen at δ 5.25.

The olefinic proton at δ 5.70 (dt) was also coupled (J = 6.9 Hz) to a methylene resonance at 2.09 (dt) which was connected (J = 7.0 Hz) to another methylene signal at δ 1.42 (tq), itself coupled to a methyl triplet (J = 7.4 Hz) at δ 0.92. Therefore, the partial structure $CH_3-CH_2-CH_2-CH=CH-CH=CH-$ could be deduced.

The olefinic multiplet at δ 5.25 (dt) was also coupled to a methylene group at δ 2.95 (dd, J = 7.4 Hz), itself coupled (J = 7.4 Hz) to another vinyl hydrogen at δ 5.58. The latter was coupled to the olefinic resonance at δ 5.42 with a coupling constant (J = 10.7 Hz) characteristic of a *Z*-ethylenic bond. This olefinic signal (dt) revealed a coupling (J = 7.3 Hz) with a methylene resonance

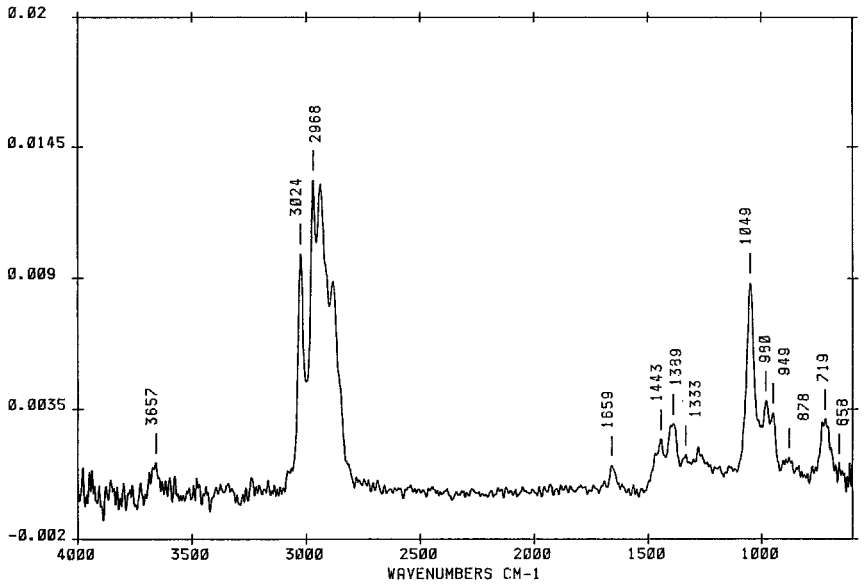
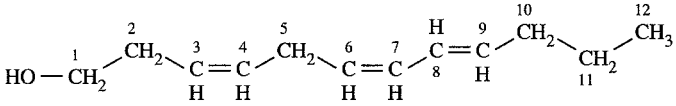


FIG. 4. Gas-phase infrared spectrum of compound A.

TABLE 3. [^{1}H]NMR SPECTRUM OF COMPOUND A IDENTIFIED AS (*Z,Z,E*)-3,6,8-DODECATRIEN-1-OL[a]



Compound A: (*Z,Z,E*)-3,6,8-Dodecatrien-1-ol

6.32 (1H, dd, J = 15 and 10.9 Hz, H_8)
5.98 (1H, dd, J = 10.9 and 10.7 Hz, H_7)
5.70 (1H, dt, J = 15 and 6.9 Hz, H_9)
5.58 (1H, dt, J = 10.7 and 7.4 Hz, H_4)
5.42 (1H, dt, J = 10.7 and 7.3 Hz, H_3)
5.25 (1H, dt, J = 10.7 and 7.4 Hz, H_6)
3.67 (2H, t, J = 6.4 Hz, H_1)
2.95 (2H, dd, J = 7.4 and 7.4 Hz, H_5)
2.37 (2H, dt, J = 7.3 and 6.4 Hz, H_2)
2.09 (2H, dt, J = 7 and 6.9 Hz, H_{10})
1.42 (2H, tq, J = 7 and 7.4 Hz, H_{11})
1.20 (1H, br s, —OH)
0.92 (3H, t, J = 7.4 Hz, H_{12})

[a] 400.13 MHz, δ ppm/TMS, $CDCl_3$. Chemical shift (integration, multiplicity, coupling constants, assignment).

at δ 2.37 (dt), which was connected (J = 6.4 Hz) to another methylene group at δ 3.67 (t). The chemical shift of the latter was characteristic of an oxygen-bearing methylene. Finally, a broad singlet at δ 1.20 was tentatively attributed to the hydroxyl proton resonance.

Thus, the structure of compound A was demonstrated to be (*Z*,*Z*,*E*)-3,6,8-dodecatrien-1-ol as depicted in Table 3.

Compound A was identical to the product identified as the trail-following pheromone of various subterranean termites (Matsumura et al., 1968; Tai et al., 1969; Tokoro et al., 1989, 1990). The spectroscopic data are in good agreement with those published for the trail-following pheromone. Particularly, the mass spectrum and the [^{1}H]NMR spectrum were identical (Yamaoka et al., 1987; Eya et al., 1990).

There is about 15–20 ng (3*Z*,6*Z*,8*E*)-3,6,8-dodecatrien per female and 1–3 ng per male. Until now, it was not possible to test the activity of the synthetic alcohol on male alates of *P. spiniger*.

The other product of fraction 2 (compound B), with a longer GC retention time, gave exactly the same EI and CI mass spectra; it was therefore identified as a geometric isomer of the major product. The complete assignment of its structure is in progress.

Attempts to Detect Dodecatrienol in Food. P. spiniger is a fungus-growing termite that eats dead plants. The debris of wood or leaves are first packed together in the nest in small sawdust heaps and then incorporated into fungus gardens before ingestion by the workers. Several samples of sawdust (10 g/25 ml pentane) and fungus combs (100 g/250 ml pentane) were extracted during 2 hr with distilled pentane and analyzed by gas chromatography. No trace of the dodecatrienol was found in either sawdust or fungus combs.

DISCUSSION

(3*Z*,6*Z*,8*E*)-3,6,8-dodecatrien-1-ol (dodecatrienol) was first isolated and identified by Matsumura et al. (1968, 1969) from total extracts of workers of *Reticulitermes virginicus* and from food of this termite, which was wood infected by the fungus *Gloephyllum trabeum*. As this alcohol was detected by the workers at extremely low concentrations, it was considered to be the trail pheromone. However, it was never identified in the sternal gland itself, which produces the trail pheromone in termite workers. Some authors considered that dodecatrienol could be either an exogenous diet-related pheromone or just an attractant (Stuart, 1970; Ritter and Coenen-Saraber, 1969; Tschinkel and Close, 1973). Nevertheless, dodecatrienol has been found recently in workers of *Reticulitermes speratus* (Tokoro et al., 1990) and *Coptotermes formosanus* (Tokoro et al., 1989) fed with filter paper and it actually seems to be a trail-following pheromone of termites.

Our results in *P. spiniger* show for the first time that dodecatrienol occurs in the sternal gland of termites. As there is no dodecatrienol in the food, this substance must be synthesized by the termites themselves. In *P. spiniger* dodecatrienol is found in the swarming imagoes and is about 10 times more abundant in the female sternal glands than in male glands. Dodecatrienol has an unquestionable power of attraction and excitation over the male alates. It therefore can be considered as the sex pheromone of *P. spiniger*. It is interesting to note that tetradecyl propionate, the ester identified as a sex pheromone in *Reticulitermes flavipes*, is also present in both sexes but in much larger quantities in female alates than in males (Clément et al., 1989).

In *P. spiniger* the role of dodecatrienol might be not restricted to the only pairing of the alates. Some observations (Bordereau et al., 1991) show that the dealate imagoes are able to follow artificial trails prepared with pentanic solutions of dodecatrienol at very low concentrations. This would reunite partners after an accidental separation during the nuptial promenade. Moreover, dodecatrienol is also detected at extremely low levels by the workers of *P. spiniger* (Bordereau et al., 1990). In *P. spiniger*, dodecatrienol could be so used either as a sex or as a trail-following pheromone. This double function would depend on a variation of the capacity of detection according to target caste. At high concentrations, it acts as a female sex pheromone, eliciting attraction and excitation of the males, whereas at low concentrations it would act as a trail-following pheromone for workers and swarming imagoes.

The observations of Quennedey and Leuthold (1978) in *Trinervitermes bettonianus* are in good agreement with this hypothesis of a functional duality, as the sternal gland extracts of the workers, when applied in adequate concentrations, have the same capacity for male attraction as the female sternal gland extracts. In this species, the cembrene A identified as a trail-following pheromone is present in workers and in very much larger quantities in alates; however, its biological activity in sex attraction is not known (McDowell and Oloo, 1984).

It may seem surprising that a compound can be used as both a trail-following pheromone, which is detected at a very short distance, and a sex pheromone, which is considered to act at a long distance. Dodecatrienol is not an highly volatile component and cannot be detected beyond some tens of centimeters. However, in termites, sexual attraction never occurs at great distance, the only exception being *Hodotermes mossambicus* (Leuthold, 1977). In most species and in *P. spiniger* in particular the conditions of the dispersal flight favor the meeting of sexes. Flights are usually synchronous, and the alates do not disperse very much. In *P. spiniger*, where pairing occurs in the air, the alates have a zigzag and whirling flight, which greatly favors meetings between individuals.

Therefore, it is suggested that *P. spiniger* represents a very parsimonious

pheromonal system in which a single compound can assume at least two functions. This agrees with the economy principle often observed in social insects (Blum and Brand, 1972; Pasteels, 1976), and this interpretation would explain the presence of dodecatrienol in male alates. In these individuals, it would function only as a trail pheromone. However, the presence of dodecatrienol in both sexes could be also a vestigial character. In some primitive species such as *Kalotermes flavicollis* (Wall, 1971) or *Zootermopsis* (Castle, 1934; Pasteels, 1972), males and females may attract reciprocally.

Finally, the presence of dodecatrienol both in Rhinotermitidae, which are lower termites, and in Macrotermitinae, which are higher termites, must be underlined. This reinforces the phylogenetic proximity of Rhinotermitidae and Macrotermitidae, which was previously suggested on the basis of morphological and anatomical characters (Emerson, 1955; Deligne, 1985). Moreover, a great number of species of lower and higher termites are very sensitive to dodecatrienol (Ritter and Coenen-Saraber, 1969; Matsumura et al., 1972; Howard et al., 1976; Kaib et al., 1982). This suggests that dodecatrienol could be a basic pheromonal component common to many termite species while additional components would provide the specificity. This could explain the anatomical diversity and the complexity of the termite sternal gland (Quennedey, 1977).

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