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(*Z,E,E*)-DODECATRIEN-1-ol: A MINOR COMPONENT OF TRAIL PHEROMONE OF TERMITE, *Coptotermes formosanus* SHIRAKI

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Abstract—In the course of the elucidation of the primary structure of an isolated trail pheromone from *C. formosanus*, a minor component that had the same molecular weight as the major trail pheromone, (*Z,Z,E*)-3,6,8-dodecatrien-1-ol [(*Z,Z,E*)-DTE-OH], was detected in the mass chromatogram of *m/z* 180 of capillary GC-MS. The mass spectrum of the minor component showed a prominent pattern of dodecatrien-1-ol. Chemical analysis demonstrated that the complete structure was (*Z,E,E*)-DTE-OH. Furthermore, capillary GC-MS-HR-SIM analysis indicated that the component existed only in the workers of *Coptotermes formosanus* Shiraki and was not present in workers of *Reticulitermes speratus* (Kolbe). This minor component may be a species-specific factor of *C. formosanus*, although this was not suggested by a two-choice bioassay.

Key Words—Subterranean termite, *Coptotermes formosanus*, *Reticulitermes speratus*, Rhinotermitidae, Isoptera, trail pheromonal minor component, geometrical isomer, (*Z,E,E*)-3,6,8-dodecatrien-1-ol, capillary GC-MS-HR-SIM.

INTRODUCTION

Recent investigations demonstrated that the trail pheromones produced by two Japanese rhinotermitids, *Reticulitermes speratus* (Kolbe) and *Coptotermes formosanus* Shiraki (Yamaoka et al., 1987; Tokoro et al., 1989, 1991, 1992) had identical chemical structures. This compound was identical to

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(*Z*,*Z*,*E*)-3,6,8-dodecatrien-1-ol [(*Z*,*Z*,*E*)-DTE-OH], which was previously obtained from fungus-infected wood (Matsumura et al., 1968, 1969) and later identified as the trail pheromone of *R. virginicus* (Tai et al., 1969). However, Howard et al. (1976) suggested that termite species should produce specific trail pheromones, as demonstrated by the ability of four rhinotermitid species to recognize extracts containing their own trail pheromones. This result suggests that species specificity could be associated with chemical variation in trail pheromones. Previous workers suggested that chemical variation in trail pheromone may result in species specificity and that small quantities of chemical analogs may act in a multicomponent way conferring specificity (Kaib et al., 1982; Traniello, 1982; Rucie, 1987).

In the course of the isolation and identification of a trail pheromone from *C. formosanus*, a small amount of an unidentified material, which was not identical to (*Z*,*Z*,*E*)-DTE-OH but showed the trail-following activity, was obtained as described previously (Tokoro et al., 1992). Capillary gas chromatography–mass spectrometer (CGC-MS) analysis of the material suggested that it was an isomer of (*Z*,*Z*,*E*)-DTE-OH. Therefore, this material, a minor component of the trail pheromone, may impart specificity to *C. formosanus*.

Capillary GC-MS high-resolution selected-ion-monitoring (CGC-MS-HR-SIM) analysis is a recent and unique detection technique. This highly sensitive and highly selective analysis can essentially facilitate the quick identification of a compound whose molecular weight is known.

It was necessary to determine whether the minor component was actually biosynthesized by termites (in sternal glands) or whether the trail pheromone (*Z*,*Z*,*E*)-DTE-OH was isomerized into the minor component by the extraction procedure. Highly sensitive and selective analysis (CGC-MS-HR-SIM) can facilitate the detection of minor component before isomerization occurs. As the molecular weights of the minor component and trail pheromone are known (Tokoro et al., 1992), these compounds were detectable by means of CGC-MS-HR-SIM analysis.

The purpose of this experiment was to identify this compound and to determine whether it is a minor component of the trail pheromone of *C. formosanus* and *R. speratus* by using CGC-MS-HR-SIM. In addition, the species-specific function of the minor component was examined in a choice bioassay using the two termite species.

METHODS AND MATERIALS

Test Termites. Termites of *R. speratus* were obtained from small colonies at the campus of Kyoto University in Uji, Japan. Individual termites were removed from wood for extraction. The termites were reared at 26 ± 2°C and

60 ± 5% relative humidity for one month until use. *C. formosanus* were collected in Wakayama, Japan, and reared at the Wood Research Institute, Kyoto University, for about 10 years on Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) at 28 ± 2°C and ca. 80% relative humidity. The test termites were undifferentiated pseudergates, older than third instar as determined by the size of the termite body (hereafter referred to as "workers"). Termites from which extracts were made were fed moistened filter paper for about five days to minimize food or nest odor contamination before extraction. The workers for bioassay were placed in a Petri dish with a moistened filter paper for 1 hr prior to bioassay.

Other worker termites (200 individuals) used for sternal gland excision were immobilized in a refrigerator at −20°C for 10 hr. Two hundred abdominal fifth sternites at which the sternal gland was positioned were carefully dissected from the bodies of worker termites under a binocular stereoscopic microscope. The dissected parts were extracted with *n*-hexane as sternal gland extract.

Chemicals. The authentic DTE-OH isomers were supplied by Dr. H. Yamamoto (Nagoya University); all other reagents were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Isolation and Identification of Minor Component. Workers (300,000) were extracted with *n*-hexane for three days. The minor component was isolated and identified as described previously for the major component of the trail pheromone (Tokoro et al., 1989, 1992). Subsequently, the isolated minor components were identified by means of capillary GC-MS and capillary GC-FTIR analysis combined with microscale chemical reactions (acetylation, partial reduction, ozonolysis) as described elsewhere (Yamaoka et al., 1987; Tokoro et al., 1992).

Authenticity of Minor Component by CGC-MS-HR-SIM Analysis. Three analytical samples were prepared as follows. For sample 1, 500 whole body workers were extracted in *n*-hexane for 20 hr, after which the excess solvent was evaporated with nitrogen gas. This extract was redissolved in 30 μl *n*-hexane and purified by silica-gel pipet flash column chromatography with EtOAc/Hx eluants. For sample 2, 50 whole body workers were extracted with diethyl ether for 5 min, and excess solvent was evaporated with nitrogen gas. The extract was redissolved in 5 μl *n*-hexane. For sample 3, one whole body worker was extracted with *n*-hexane for 40 min, and excess solvent was evaporated with nitrogen gas. The extract was redissolved in μl *n*-hexane. Three samples each were prepared for both termite species. One microliter of each sample was used for each analysis. All samples were subjected to CGC-MS-HR-SIM immediately following preparation.

These samples were first analyzed by capillary GC and subsequently analyzed by CGC-MS-HR-SIM. The capillary GC-MS apparatus was a Hewlett Packard (HP) model 5890 gas chromatograph combined with a model M-80B mass spectrometer (Hitachi, Ibaraki, Japan), equipped with a model 0101 on-line

data system. A fused-silica WCOT capillary column (25 m × 0.25 mm ID, liquid phase PEG-HT; GL Science Ltd. Tokyo, Japan) was used with this system. The analytical conditions were as follows: The ion source temperature was 180°C, and ionization energy was 70 eV. Samples were dissolved in 1 μl of *n*-hexane injected with a Grob-type splitless injector. The oven temperature was increased from 60 to 210°C at a rate of 30°C/min for a 5-min period, and the injection temperature was 210°C. The resolution was approximately 6000 units, and the SIM monitor was set at *m/z* 180.1513, the precise molecular weight of DTE-OH.

Bioassay Method. In order to determine the threshold response levels for trail-following and species specificity of trail pheromone, choice tests (Y-tests) were conducted.

A modification of the Howard et al. (1976) choice bioassay was used to examine the preferential difference between the two rhinotermitid termites in trail-following activity of some substances (Figure 1). One microliter each of a dissolved sample was streaked from the junction to the distal end of the branched line (1.5 cm long) and stem (3 cm long). Another 1 μl was streaked from the junction to the end of basal point of the Y-shaped pencil guideline (ca. 1.5 cm long and the angle formed by the branches is 45°). One microliter of another dissolved sample was streaked along another branch (1.5 cm long) and the same stem of the Y-shaped pencil guideline drawn on the clay-coated paper with a 5-μl micropipet. The same was done for the other branched and basal lines with the other dissolved sample. After evaporation of the solvent (at least 10 sec), a plastic cylinder (1 × 1.5 cm ID) was placed on the paper at a side of the test arena. One opening of the cylinder directed the termite toward the test arena. A worker termite was then introduced into the cylinder, and a red-colored Petri

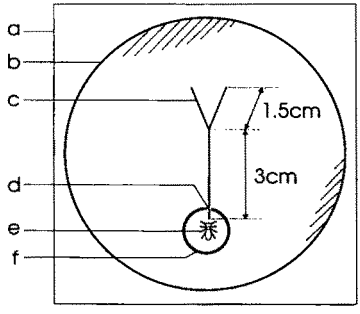

FIG. 1. Scheme of modified choice bioassay: (a) fine-quality clay-coated paper, (b) Petri dish lid (.5 × 9 cm), (c) pencil guideline, (d) opening, (e) test termite, (f) plastic cylinder.

dish lid (1 × 5.7 cm ID) was placed above it in order to minimize the influence of air movements and light.

When a worker termite succeeded in moving along the sample streaked Y-shaped line within 1 min, it was considered that a "basic activity" was induced. When the termite completed a trail-following and reached one distal end of the branched part of a Y-shaped line or deviated from the trail at least 2 cm, the termite was removed from the test stage immediately. Ten replicates of Y-shaped lines were prepared, and 10 termites (one at a time) were tested on each line. The numbers of test worker termites that selected either of the branched parts were recorded. A given sample was considered to have elicited a threshold trail-following response if more than 50% of 100 test termites showed a basic activity. Twofold dilution series of each sample were employed to determine the trail-following activity of the test materials. Bioassays were carried out at approximately 26 ± 2°C and 60 ± 5% relative humidity under the fluorescent lighting to estimate the trail-following activity. No termite was used for more than one bioassay. All the data were analyzed by a chi-square statistical analysis using the Yates correction for continuity (Yates, 1934).

RESULTS AND DISCUSSION

Isolation and Identification of Minor Component. We estimated that the mean amount of the minor substance extracted from each individual worker termite was approximately 5 pg on the basis of GC analysis.

The capillary GC-MS data of the minor component (Figure 2A) and of the authentic DTE-OH isomers (Figure 2B) indicated that the two samples were similar. The peaks (scan numbers 333 and 346) had similar retention times, and the mass spectra showed a prominent molecular ion at *m/z* 180 ($M^+ \cdot$). A series of diagnostic ions at *m/z* 91, 105, 119, 133 (C_nH_{2n-7}) were also found, as reported previously (Tokoro et al., 1992), suggesting that the minor component was an isomer of DTE-OH.

The partially hydrogenated products of the minor component were analyzed by capillary GC-MS. Seven peaks were obtained from each compound resulting from the partial hydrogenation procedure. These were identified as *n*-dodecanol, (*Z*)-3-dodecen-1-ol, (*E*)-6-dodecen-1-ol + (*E*)-8-dodecen-1-ol, (*Z*,*E*)-3,8-dodecadien-1-ol, (*Z*,*E*)-3,6-dodecadien-1-ol, (*E*,*E*)-6,8-dodecadien-1-ol, and (*Z*,*E*,*E*)-3,6,8-dodecatrien-1-ol [(*Z*,*E*,*E*)-DTE-OH]. Results of ozonolysis and capillary GC-FTIR analysis upheld these results. It was concluded that the minor component was identical to (*Z*,*E*,*E*)-DTE-OH.

Capillary GC and Capillary GC-MS-HR-SIM Analyses of Whole-Body Extracts. Results of capillary GC analyses of the whole-body extract (sample 1) and that of authentic DTE-OH isomers are shown in Figures 3A and 4B,

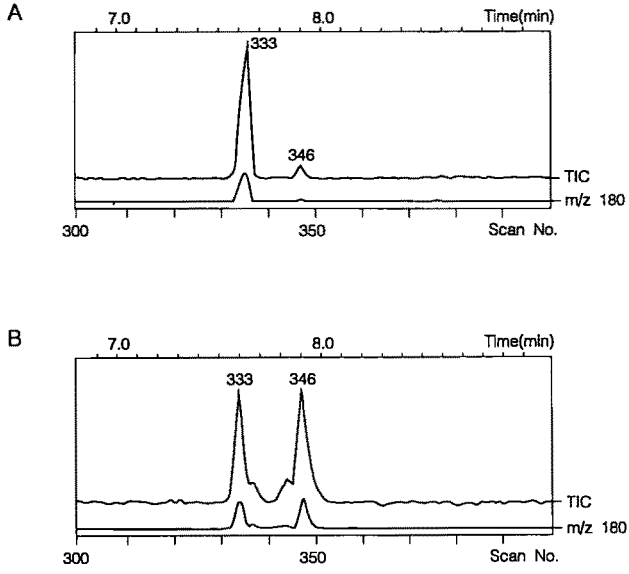

FIG. 2. Total ion chromatogram and mass chromatogram (*m/z* 180) of the trail pheromone and the minor component isolated from *C. formosanus* and authentic (*Z,E,E*)-DTE-OH. (A) Trail pheromone (scan no. 333) and the minor component (scan No. 346); (B) authentic DTE-OH isomers: scan No. 333, (*Z,Z,E*)-DTE-OH; scan No. 346, (*Z,E,E*)-DTE-OH.

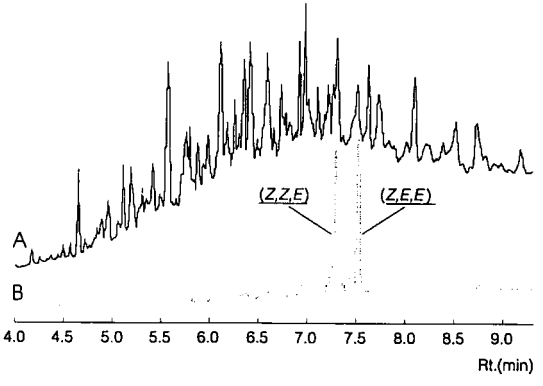

FIG. 3. Capillary GC data of the whole body extracts from *C. formosanus* (A) and authentic DTE-OH isomers (B).

respectively. The chromatogram in Figure 3A shows many peaks consisting of the components of the extracts. However, because of the large number of peaks, it is impossible to distinguish between the target components and the impurities.

Results of capillary GC-MS-HR-SIM analyses of the minor component and (*Z*,*E*,*E*)-DTE-OH demonstrated that the DTE-OH isomers (i.e., trail pheromone isomers) could be detected by HR-SIM at *m*/*z* 180.1513 and that both could be isolated from impurities. Two samples (2 and 3) from both termite species exhibited several impurity peaks due to diethyl ether solvent impurities (R_f 6.2: sample 2) or the background impurity from the column liquid phase (sample 3). In the case of *C. formosanus* (Figure 4A), all test samples exhibited the prominent (*Z*,*Z*,*E*)-DTE-OH peaks. Samples 1 and 2 also exhibited the clear peaks

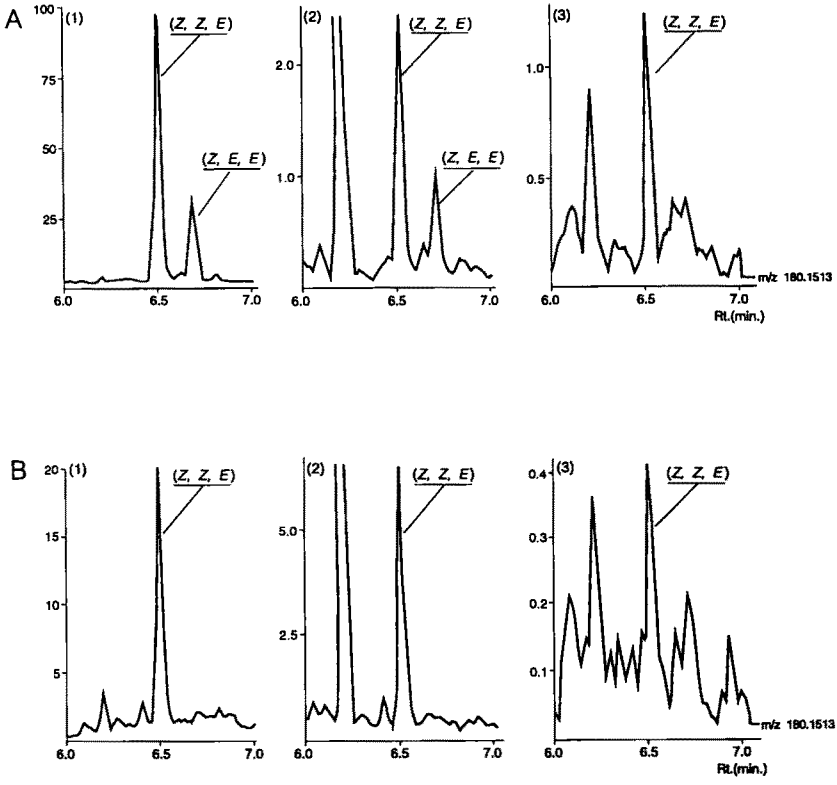

FIG. 4. Capillary GC-MS-HR-SIM data of the whole-body extracts from *C. formosanus* (A) and *R. speratus* (B). HR-SIM chromatograms at *m*/*z* 181.1513 (WE = worker equivalent). Sample 1: 16.7 WE/μl; sample 2: 10 WE/μl; sample 3: 1 WE/μl.

of (*Z*,*E*,*E*)-DTE-OH, which were each equivalent to 35% (w/w) of the quantity of (*Z*,*Z*,*E*)-DTE-OH.

All test samples from *R. speratus* (Figure 4B), also exhibited the prominent (*Z*,*Z*,*E*)-DTE-OH peaks. Thus, in these two rhinotermitid termites, the minor component, (*Z*,*E*,*E*)-DTE-OH, may be produced only by *C. formosanus*. As the extraction procedure was the same for the two species, these results suggest that the minor component was not a product of isomerization of the trail pheromone (*Z*,*Z*,*E*)-DTE-OH.

In the CGC-MS-HR-SIM analyses, the amounts of detected trail pheromone and minor component from a worker for each termite species was determined with an external standard technique. (*Z*,*Z*,*E*)-DTE-OH was extracted in ca. 15 ng, 142 pg, and 46 pg quantities in samples 1, 2, and 3, respectively, for *C. formosanus*. The minor component, (*Z*,*E*,*E*)-DTE-OH, amounted to 525 pg and 50 pg in samples 1 and 2, respectively. For *R. speratus*, the extract of (*Z*,*Z*,*E*)-DTE-OH consisted of 313 pg, 301 pg, and 32 pg in samples 1, 2, and 3, respectively. As a result, the minor component, (*Z*,*E*,*E*)-DTE-OH, was apparently extracted from the workers of *C. formosanus* in concentrations equivalent to 35% (w/w) of (*Z*,*Z*,*E*)-DTE-OH. Thus, the CGC-MS-HR-SIM analysis is very effective for the detection of pheromone isomers, and it allows detection with high sensitivity and high selectivity from crude lipid extracts.

Results of Y Test Using Sternal Gland Extract and Authentic DTE-OH. In order to determine species specificity, Howard et al. (1976) first employed a choice bioassay technique. Preliminary trials that employed the choice bioassay technique of Howard et al. supported that termites showed a statistically equal response to the two chemicals of the same activity level generating 50/50 distribution. However, it was noticed during the bioassay that a group of termites undertook tandem running, and some termites seemed to disturb the natural behavior of the others in the test arena. Therefore, a single worker termite at a time was tested instead of a group of 15 termites to rule out group effects. In addition, the shorter branched lines were considered to result in the evenness of the streaked quantity of test chemicals along the trail line.

Results obtained in the Y tests are shown in Tables 1–10. As expected, two samples that had the same trail-following activity level generally induced a 50/50 distribution. The results suggested the applicability of the bioassay technique to examine the species specificity using two termite species. Each threshold quantity, which could induce the basic activity in more than 50% of test workers, of the samples was estimated as follows in the case of *R. speratus*: (*Z*,*Z*,*E*)-DTE-OH 100 fg, (*Z*,*E*,*E*)-DTE-OH 50 pg/1.5-cm trail, *R. speratus* sternal gland extract (R.s.-SGE) 10^{-4} WE/1.5-cm-trail, *C. formosanus* sternal gland extract (C.f.-SGE 5×10^{-5} WE/1.5-cm trail. In the case of *C. formosanus* workers: (*Z*,*Z*,*E*)-DTE-OH 100 fg, (*Z*,*E*,*E*)-DTE-OH 25 pg/1.5-cm trail, R.s.-SGE 10^{-4} WE/1.5 cm-trail, C.f.-SGE 5×10^{-5} WE/1.5-cm trail. Since

TABLE 1. DISTRIBUTION OF TERMITES IN Y TEST WHEN SEVERAL OF THE SAME (*Z, Z, E*)-DTE-OH CONCENTRATIONS OR (*Z, E, E,*)-DTE-OH CONCENTRATIONS ARE APPLIED TO *R. speratus* WORKERS

Test samples (weight/1.5 cm)	Number of termites choosing			Chi-square[a]
	Left		Right	
(*Z,Z,E*)-DTE-OH				
50 ng	8	(72)[b]	20	4.32
5 ng	46	(13)	41	0.18
500 pg	54	(0)	46	0.49
50 pg	48	(0)	52	0.09
5 pg	41	(6)	53	1.29
500 fg	54	(6)	40	1.80
250 fg	38	(12)	50	1.38
100 fg	46	(10)	44	0.00
50 fg	18	(66)	16	0.03
25 fg	15	(82)	3	6.72
5 fg	0	(100)	0	
(*Z,E,E*)-DTE-OH				
50 ng	41	(2)	57	2.30
5 ng	48	(1)	51	0.04
500 pg	39	(4)	57	3.01
50 pg	41	(9)	50	0.70
25 pg	21	(65)	14	1.03
10 pg	8	(88)	6	0.07
5 pg	14	(78)	6	2.45
500 fg	1	(94)	5	1.50

[a] Chi-square analysis made on the hypothesis that if the two-sample trail is identical, a 1 : 1 distribution will result, and the value of chi-square will be less than 3.84 at the 95% level of significance.
[b] Number of termites deviating.

the active threshold level of (*Z,E,E*)-DTE-OH was at least 100 times higher than that of (*Z,Z,E*)-DTE-OH (Tables 1 and 2), a behavioral effect of the component to the termite might be insignificant by itself. It is necessary to show whether the (*Z,E,E*)-DTE-OH and (*Z,Z,E*)-DTE-OH synergistically induce workers of *C. formosanus* to species-specific trail-following behavior.

Two kinds of solutions of (*Z,Z,E*)-DTE-OH were used for the Y tests. Solution A was a hexane solution of (*Z,Z,E*)-DTE-OH as an artificial trail pheromone of *R. speratus*, and solution B was a mixture of (*Z,Z,E*)-DTE-OH and (*Z,E,E*)-DTE-OH [35% (w/w) of DTE-OH] as an artificial trail pheromone of *C. formosanus*.

Results of the Y test with solutions A and B are shown in Tables 5 and 6. In all cases, the observed preference of termites corresponded well to the

TABLE 2. DISTRIBUTION OF TERMITES IN Y TEST WHEN SEVERAL OF THE SAME (*Z, Z, E*)-DTE-OH CONCENTRATIONS OR (*Z, E, E*)-DTE-OH CONCENTRATIONS ARE APPLIED TO *C. formosanus* WORKERS[a]

Test samples (weight/1.5 cm)	Number of termites choosing			Chi-square
	Left		Right	
(Z,Z,E)-DTE-OH				
50 ng	11	(83)	6	0.94
5 ng	39	(30)	31	0.70
500 pg	55	(2)	43	0.23
50 pg	42	(3)	55	1.48
5 pg	46	(2)	52	0.26
500 fg	46	(7)	39	0.42
250 fg	47	(0)	33	2.11
100 fg	48	(22)	30	3.71
50 fg	24	(58)	18	0.60
25 fg	0	(96)	4	2.25
5 fg	0	(100)	0	
(Z,E,E)-DTE-OH				
50 ng	54	(2)	57	2.30
5 ng	44	(1)	51	0.04
500 pg	58	(3)	39	3.34
50 pg	56	(6)	38	3.07
25 pg	40	(28)	32	0.68
10 pg	5	(92)	3	0.13
5 pg	2	(96)	2	0.25
500 fg	1	(99)	0	0.00

[a] See footnotes to Table 1.

50/50 distribution at the 95% level of significance. These results indicated that *R. speratus* workers could not distinguish between solutions A and B. Contrary to expectation, *C. formosanus* could not discern the two solutions either. Thus, the minor component (*Z,E,E*)-DTE-OH could not elicit a specific trail-following behavior in both *C. formosanus* and *R. speratus*.

Y tests were concurrently conducted with the sternal gland extracts (as natural pheromone) of the two species to examine whether a termite species could distinguish its own extracts from other extracts. As demonstrated in Tables 3 and 4, threshold trail-following levels of sternal gland extract from *C. formosanus* were two times higher than those from *R. speratus*, regardless of termite species. Therefore, test concentrations were selected based on the trail-following activities. In other wards, the concentrations that succeeded in inducing the same level of trail-following behavior to both termite species were streaked along the Y shaped line in choice bioassay.

TABLE 3. DISTRIBUTIONS OF TERMITES IN Y TEST WHEN SEVERAL OF THE SAME STERNAL GLAND EXTRACTS ARE APPLIED TO *R. speratus* WORKERS

Test samples (WE[a]/1.5 cm)	Number of termites choosing			Chi-square[b]
	Left		Right	
R.s-SGE[c]				
0.1	44	(6)[d]	50	0.27
0.01	46	(2)	52	0.26
0.005	50	(0)	50	0.01
0.001	42	(0)	58	1.40
0.0005	53	(8)	39	1.84
0.00025	48	(11)	41	0.40
0.0001	44	(13)	43	0.00
0.00005	18	(54)	28	1.76
0.000025	12	(72)	18	0.83
0.0000125	0	(96)	4	2.25
0.000005	0	(100)	0	
C.f-SGE[e]				
0.1	50	(1)	49	0.00
0.01	52	(2)	46	0.26
0.005	59	(0)	41	2.89
0.001	44	(6)	50	0.27
0.0005	52	(0)	48	0.09
0.00025	43	(3)	54	1.03
0.0001	40	(6)	54	1.80
0.00005	28	(49)	23	0.31
0.000025	12	(76)	12	0.04
0.0000125	2	(98)	0	0.50
0.000005	0	(100)	0	

[a] Worker equivalent.
[b] Chi-square analysis made on the hypothesis that if the two-sample trail is identical, a 1 : 1 distribution will result, and the value of chi-square will be less than 3.84 at the 95% level of significance.
[c] Sternal gland extract of *R. speratus*.
[d] Number of termites deviating is in parentheses.
[e] Sternal gland extract of C. formosanus.

Results of the Y test with interspecific sternal gland extract are shown in Tables 7 and 8. When the samples with the same trail-following activity were tested, any termite showed nonselective behavior to the extracts from both species. Termites could not recognize their own sternal gland extract trail under such test conditions but were rather more sensitive to quantitative differences. These results are different from the data of the previous report by Howard et al. (1976), who worked on *R. virginicus*, *R. flavipes*, *R. tibialis*, and *C. formosanus* and concluded that trail pheromones were species specific. This dif-

TABLE 4. DISTRIBUTIONS OF TERMITES IN Y TEST WHEN SEVERAL OF THE SAME STERNAL GLAND EXTRACTS ARE APPLIED TO *C. formosanus* WORKERS[a]

Test samples (WE/1.5 cm)	Number of termites choosing			Chi-square
	Left		Right	
R.s-SGE				
0.1	43	(8)	49	0.27
0.01	47	(5)	48	0.00
0.005	44	(9)	47	0.04
0.001	45	(8)	47	0.01
0.0005	50	(2)	48	0.01
0.00025	48	(11)	41	0.40
0.0001	39	(18)	43	0.11
0.00005	21	(52)	27	0.52
0.000025	12	(78)	10	0.05
0.0000125	9	(87)	4	1.23
0.000005	4	(95)	1	
C.f.-SGE				
0.1	51	(1)	48	0.04
0.01	48	(4)	48	0.01
0.005	48	(6)	46	0.01
0.001	45	(8)	47	0.01
0.0005	46	(10)	44	0.01
0.00025	44	(9)	47	0.04
0.0001	40	(8)	52	1.32
0.00005	33	(34)	33	0.02
0.000025	24	(57)	19	0.37
0.0000125	14	(68)	18	0.28
0.000005	11	(75)	14	0.16

[a] See footnotes to Table 3.

TABLE 5. Y TEST OF SOLUTION A [(*Z,Z,E*)-DTE-OH] TRAIL VERSUS SOLUTION B [(*Z,Z,E*)-DTE-OH + (*Z,E,E*)-DTE-OH] TRAIL FOR *R. speratus* WORKERS

Quantity of (*Z,Z,E*)-DTE-OH (weight/1.5 cm)	Number of termites choosing			Chi-square[b]
	Solution A		Solution B[a]	
5 ng	32	(20)	48	2.81
500 pg	56	(4)	40	2.34
50 pg	47	(1)	52	0.16
5 pg	45	(1)	54	0.65
500 fg	48	(10)	42	0.28
100 fg	27	(32)	41	2.49

[a] Chi-square analysis made on the hypothesis that if the two-sample trail is identical, a 1 : 1 distribution will result, and the value of chi-square will be less than 3.84 at the 95% level of significance.
[b] (*Z,E,E*)-DTE-OH was added to solution A at the rate of 35% of (*Z,Z,E*)-DTE-OH quantity.
[c] Number of termites deviating.

TABLE 6. Y TEST OF SOLUTION A [(*Z*,*Z*,*E*)-DTE-OH) TRAIL VERSUS SOLUTION B [DTE-OH + (*Z*,*E*,*E*)-DTE-OH] TRAIL FOR *C. formosanus* WORKERS[a]

Quantity of (*Z*,*Z*,*E*)-DTE-OH (weight/1.5 cm)	Number of termites choosing			Chi-square
	Solution A		Solution B	
5 ng	30	(30)	40	1.16
500 pg	49	(7)	44	0.17
50 pg	44	(7)	49	0.17
5 pg	40	(6)	54	1.80
500 fg	36	(8)	55	3.56
100 fg	24	(36)	38	2.73

[a]See footnotes to Table 3.

TABLE 7. Y TEST OF STERNAL GLAND EXTRACT FROM *R. speratus* VERSUS THAT FROM *C. formosanus* USING *R. speratus* WORKERS

Test samples (WE[a] 1.5 cm)		Number of termites choosing			
(a) R.s.-SGE[c]	(b) C.f.-SGE[d]	a		b	Chi-square[b]
0.02	0.01	40	(0)[e]	60	3.61
0.002	0.001	38	(5)	57	3.41
0.0002	0.0001	43	(11)	46	0.04
0.0001	0.00005	42	(3)	55	1.48
0.03	0.01	57	(1)	42	1.98
0.003	0.001	76	(0)	24	26.01
0.0003	0.0001	57	(3)	39	3.01
0.01	0.01	12	(5)	83	51.58
0.001	0.001	30	(5)	65	12.17
0.0001	0.0001	16	(8)	76	37.84
0.01	0.02	13	(1)	86	52.36
0.001	0.002	12	(1)	87	55.31
0.0001	0.0002	10	(1)	89	61.45

[a]Worker equivalent.
[b]Chi-square analysis made on the hypothesis that if the two-sample trail is identical, a 1 : 1 distribution will result, and the value of chi-square will be less than 3.84 at the 95% level of significance.
[c]Sternal gland extract of *R. speratus*.
[d]Sternal gland extract of *C. formosanus*.
[e]Number of termites deviating is in parentheses.

TABLE 8. Y TEST OF STERNAL GLAND EXTRACT FROM *R. speratus* VERSUS THAT FROM *C. formosanus* USING *C. formosanus* WORKERS[a]

Test samples (WE/1.5 cm)		Number of termites choosing			
(a) R.s.-SGE	(b) C.f.-SGE	a		b	Chi-square
0.02	0.01	39	(6)	55	2.69
0.002	0.001	40	(2)	58	2.95
0.0002	0.0001	43	(10)	47	0.16
0.0001	0.00005	27	(30)	43	3.21
0.03	0.01	68	(6)	26	17.88
0.003	0.001	62	(2)	36	6.38
0.0003	0.0001	62	(11)	27	12.99
0.01	0.01	22	(10)	68	22.50
0.001	0.001	26	(4)	70	19.26
0.0001	0.0001	6	(6)	88	69.80
0.01	0.02	7	(3)	90	69.32
0.001	0.002	15	(4)	81	44.01
0.0001	0.0002	10	(6)	84	56.69

[a] See footnotes to Table 7.

ference may be due to the different test termites. However, it is of great interest to point out that termites always chose the line with a sample of two to three times higher trail-following activity (Tables 9 and 10). Such sensitive preference might be directly related to orientation activity, as demonstrated with *R. hesperus* by Grace et al. (1988). We also found similar results as the artificial trail studies conducted by Howard et al. (1976) with these two termite species.

Although (*Z,E,E*)-DTE-OH did not induce species-specific trail-following behavior in either species in this bioassay, the minor component may possibly impart a species-specific mechanism to the *C. formosanus* trail pheromone. Possibly, this bioassay condition was unsuitable for distinguishing between pheromone trails of the two species. On the other hand, it is possible that this minor component is only a by-product of the biosynthesis of (*Z,Z,E*)-DTE-OH. It is necessary to determine specific and colonial or seasonal variety of the quantitative ratio of (*Z,Z,E*)-DTE-OH and (*Z,E,E*)-DTE-OH to all rhinotermitid termites.

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TABLE 9. DISTRIBUTION OF TERMITES IN Y TEST WHEN SEVERAL OF THE SAME STERNAL GLAND EXTRACTS ARE APPLIED TO *R. speratus* WORKERS

Test samples (WE[a]/1.5 cm)	Number of termites choosing			Chi-square[b]
R.s.-SGE[c]	×2[d]		×1[d]	
0.1	71	(1)[e]	28	17.82
0.01	80	(2)	18	37.97
0.005	92	(0)	8	68.89
0.001	97	(0)	3	86.49
0.0005	90	(6)	4	76.86
0.0001	69	(12)	19	27.28
0.00005	40	(55)	5	25.69
0.000025	14	(83)	3	5.88
R.s.-SGE	×3		×1	
0.1	94	(3)	3	83.51
0.01	94	(0)	6	75.69
0.001	100	(0)	0	98.01
0.0001	96	(4)	0	94.01
C.f. SGE[f]	×2		×1	
0.1	83	(1)	16	44.00
0.01	80	(2)	18	37.97
0.005	92	(0)	8	68.89
0.001	85	(2)	13	51.44
0.0005	95	(0)	5	79.21
0.0001	82	(2)	16	8.00
0.00005	87	(5)	8	64.04
0.000025	32	(62)	6	16.45
C.f.-SGE	×3		×1	
0.1	95	(2)	3	84.50
0.01	94	(1)	5	78.22
0.001	98	(0)	2	90.25
0.0001	89	(2)	9	63.68

[a] Worker equivalent.
[b] Chi-square analysis made on the hypothesis that if the two-sample trail is identical, a 1 : 1 distribution will result, and the value of chi-square will be less than 3.84 at the 95% level of significance.
[c] Sternal gland extract of *R. speratus*.
[d] Number means a multiple of test concentration.
[e] Quantity of applied sample per trial.
[f] Sternal gland extract of *C. formosanus*.

TABLE 10. DISTRIBUTION OF TERMITES IN Y TEST WHEN SEVERAL OF THE SAME STERNAL GLAND EXTRACTS ARE APPLIED TO *C. formosanus* WORKERS[a]

Test samples (WE/1.5 cm)	Number of termites choosing			Chi-square
R.s.-SGE	×2		×1	
0.1	68	(6)	26	17.88
0.01	65	(4)	31	11.34
0.005	82	(5)	13	48.67
0.001	58	(4)	38	3.76
0.0005	74	(9)	17	34.46
0.0001	64	(18)	18	24.70
0.00005	56	(18)	26	10.26
0.000025	22	(74)	4	11.12
R.s.-SGE	×3		×1	
0.1	83	(7)	10	55.74
0.01	79	(3)	18	37.11
0.001	80	(9)	11	50.81
0.0001	84	(10)	6	65.88
C.f-SGE	×2		×1	
0.1	78	(6)	16	39.59
0.01	71	(2)	27	18.87
0.005	60	(5)	35	6.06
0.001	70	(3)	27	18.19
0.0005	66	(15)	19	24.89
0.0001	58	(20)	22	8.00
0.00005	57	(18)	25	11.72
0.000025	62	(22)	16	25.96
C.f-SGE	×3		×1	
0.1	94	(2)	4	80.83
0.01	74	(8)	18	32.88
0.001	84	(4)	12	52.51
0.0001	76	(18)	6	58.06

[a]See footnotes to Table 9.

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