Novel free ceramides as components of the soldier defense gland of the Formosan subterranean termite (*Coptotermes formosanus*)

Masaya Ohta,[1,*] Fumito Matsuura,[*] Gregg Henderson,[†] and Roger A. Laine[§,]**

Department of Biotechnology,* Faculty of Life Science and Biotechnology, Fukuyama University, Gakuen-cho, Fukuyama-shi, Hiroshima 729-0292, Japan; Department of Entomology,† Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803; and Departments of Biological Sciences,§ and Chemistry,** Louisiana State University and A&M College, Baton Rouge, LA 70803

Abstract **Of the lipid extracts of the defense secretion from the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, on high-performance thin-layer chromatography analysis, no glycolipids or phospholipids were detected, but free fatty acids and three novel ceramides were found (termed TL-1, TL-2, and TL-3). Free fatty acids were confirmed to be lignoceric acid (C24:0) and hexacosanoic acid (C26:0), as described previously [Chen, J., G. Henderson, and R. A. Laine. 1999. Lignoceric acid and hexacosanoic acid: major components of soldier frontal gland secretions of the Formosan subterranean termite (*Coptotermes formosanus*). *J. Chem. Ecol.* 25: 817–824]. TL-1, TL-2, and TL-3 were characterized as ceramides differing in hydrophobicity based on results of matrix-assisted laser desorption-ionization time-of-flight mass spectrometry analysis, mild alkaline treatment, GC-MS analysis of fatty acid methylesters, and GC-MS analysis of sphingoid long-chain bases (LCBs) as trimethylsilyl derivatives. Fatty acids in TL-1 and TL-2 were C18:0, C20:0, and C22:0, and those in TL-3 were 2-hydroxy C18:0, C20:0, and C22:0. The most predominant LCB in TL-2 was a novel trihydroxy C_{14}-sphingosine, 1,3,9-trihydroxy-2-amino-6-tetradecene. TL-3 contained C_{18}-sphinganine and two kinds of novel sphingadienines, 1,3-dihydroxy-2-amino-7,10-hexadecadiene and 1,3-dihydroxy-2-amino-11,14-eicosadiene. Although examination of the biological activities of these novel ceramides was beyond the scope of these studies, because of the minuscule quantities available from termite secretions, it will be interesting in the future to synthesize these molecules for biological testing.**—Ohta, M., F. Matsuura, G. Henderson, and R. A. Laine. **Novel free ceramides as components of the soldier defense gland of the Formosan subterranean termite (*Coptotermes formosanus*). *J. Lipid Res.* 2007.** 48: **656–664.**

Supplementary key words sphingoid base • osmium tetroxide oxidation • matrix-assisted laser desorption-ionization time-of-flight mass spectrometry

Many termite species, including the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, have a soldier caste that is morphologically and physiologically specialized for defense (1). The physiological defense strategies are used by mechanically and chemically armed soldier termites. Weapons of the soldiers include mandibles modified for crushing and, most interesting, a defense secretion sprayed from its frontal gland, ejected from a foramen at the front of the head (2). The secretion has several properties, including immobilization, acting like a glue, and as an irritant against predators such as ants. The secretion apparently contains an unidentified alarm pheromone that repels workers and attracts soldiers (3). Furthermore, chemical signals in the secretion may stimulate workers exposed to the fluid to morph into soldiers during their next two molts (4). Chemical analyses of the defense gland secretions from the families Rhinotermitidae and Termitidae have revealed an impressive diversity of chemical components: monoterpenes, diterpenes, sesquiterpenes, quinones, macrocyclic lactones, alkanes, alkenes, nitroalkenes, vinyl ketones, and aldehydes (5, 6).

In Coptotermitinae, chemical analyses of the frontal gland components have been performed in only a few species. A heterogeneous suspension of *n*-alkanes and mucopolysaccharides was reported in *Coptotermes lacteus* (7). Hexadecanal and heptadecanal were found in *Coptotermes testaceous* (8). Recently, we reported that the free fatty acids, lignoceric acid and hexacosanoic acid, were major components of soldier frontal gland secretions in *C.*

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Abbreviations: FAME, fatty acid methyl ester; HPTLC, high-performance thin-layer chromatography; LCB, long-chain base; MALDI-TOF MS, matrix-assisted laser desorption-ionization time-of-flight mass spectrometry; TMS, trimethylsilylsilane.

[1] To whom correspondence should be addressed.
e-mail: ohta@bt.fubt.fukuyama-u.ac.jp

formosanus (9), and a novel lysozyme was discovered (10) in addition to the hydrocarbons and mucopolysaccharides that had been reported by Prestwich (11). It is well known that the use of volatile fatty acids as an ant repellent is a widespread phenomenon among insects (12). Fatty acid esters are known to be fly and yellowjacket repellants (13, 14). However, it is unknown whether long-chain free fatty acids such as lignoceric acid and hexacosanoic acid have the same irritant quality as volatile fatty acids. Waller and LaFage (15) reported that soldiers and workers of this species were killed at the same rate in laboratory arenas by ants and that ants removed workers, but not soldiers, from the foraging arena. Therefore, the gland secretions of this species can be assumed to have other possible functions and may contain other bioactive chemical substances.

This article reports the occurrence of free ceramides containing a variety of unique long-chain bases (LCBs) in the soldier frontal gland secretions from *C. formosanus*. Although specific defense functions of these compounds are not yet defined, free ceramides are well known as potent signaling molecules in apoptotic pathways (16, 17).

MATERIALS AND METHODS

Termites

Five different colonies of Formosan subterranean termites were collected from infested houses in New Orleans (four colonies) and Lake Charles (one colony), Louisiana, and placed in plastic trash cans maintained at room temperature.

Preparation of lipids from defense gland secretions

A white liquid drop obtained from the frontal gland of 100 soldiers from each colony was obtained by stimulating the head, collected with clean fine forceps, and dissolved in 10 ml of chloroform-methanol-water (1:2:0.8, v/v/v) (18). Ten milliliters of chloroform and water was added to this solution, and the two phases were separated by centrifugation. The chloroform layer (crude lipid fraction) was evaporated under reduced pressure and subjected to chemical analyses.

Fractionation of ceramides from the lipid fraction

The crude lipid fraction was dissolved in diethyl ether and applied to a column (0.5 × 5 cm) packed with Iatrobeads (6RS-8060; Iatron Laboratories, Tokyo, Japan). The column was eluted with 20 ml of ether and 20 ml of chloroform-methanol (9:1, v/v). The simple lipid fraction eluted with chloroform-methanol was further fractionated by TLC on silica gel G (Analtech, Inc.) using chloroform-methanol-acetic acid (98:2:1, v/v/v). After lipid spots were detected under ultraviolet light after spraying with primuline reagent (Nacalai Tesque, Kyoto, Japan), each spot and the silica gel was removed from the plate with a razor blade and extracted with chloroform-methanol (1:1, v/v).

High-performance thin-layer chromatography

High-performance thin-layer chromatography (HPTLC) (silica gel 60; Merck) gels were developed with either *1*) chloroform-methanol-water (65:25:4, v/v/v) or *2*) chloroform-methanol-acetic acid (98:2:1, v/v/v). Components were detected by charring with 50% H_2SO_4 and alternatively with the ninhydrin reagent to detect free amines.

Analysis of fatty acids

Bound fatty acids from each lipid were released by methanolysis in 2.5% methanolic HCl (0.3 ml) at 80°C for 12 h. Fatty acid methyl esters (FAMEs) were extracted from the reaction mixture three times with *n*-hexane, and analyzed by GC (Hewlett Packard 5890A gas chromatograph) on a capillary column (DB-5; 0.25 mm × 30 m; J&W Scientific) or by GC-MS. The temperature program was 60°C for 1 min, 8°C/min to 260°C, and hold for 14 min. Free fatty acids were determined according to Hashimoto, Aoyama, and Shioiri (19). The dried sample was dissolved in 0.9 ml of methanol-benzene (2:7, v/v); 0.1 ml of a 10% solution of trimethylsilyldiazomethane in hexane (GL Sciences, Tokyo, Japan) was added and reacted for 30 min at room temperature, dried under nitrogen, and dissolved in hexane for gas chromatography.

Analysis of LCBs

For component analysis, the purified ceramides were methanolysed in aqueous methanolic HCl at 70°C for 18 h as devised by Gaver and Sweeley (20). FAMEs were extracted with hexane, and LCBs were extracted with chloroform by the method of Ando and Yu (21). A portion of the LCB fraction was trimethylsilylated with 50 μl of pyridine-hexamethyldisilazane-trimethylchlorosilane (5:6.5:4, v/v/v) at 60°C for 30 min and analyzed by GC-MS. Another portion of the LCB fraction was dissolved in 1.2 ml of 20 mM $NaIO_4$ in 90% methanol and kept in the dark at room temperature for 3 h, 1 ml of water was added, and the mixture was extracted with CH_2Cl_2. The aldehydes were analyzed by GC-MS as their free forms or as trimethylsilylsilane (TMS) derivatives. LCB double bond positions were determined by GC-MS of TMS derivatives of polyhydroxylated products of periodate oxidation followed by osmium tetroxide oxidation. The aldehydes were reduced to alcohols with $NaBH_4$, and the reaction was terminated with acetic acid and dried under nitrogen. The resultant alcohols were subjected to OsO_4 oxidation according to Niehaus and Ryhage (22). The locations of vicinal -OTMS residues were determined by GC-MS using TMS derivatives.

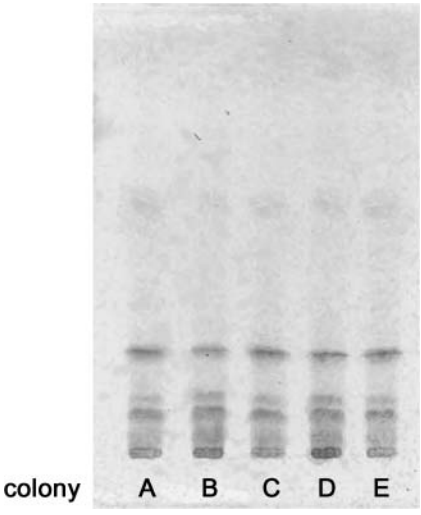

Fig. 1. High-performance thin-layer chromatography (HPTLC) of total lipids from frontal gland secretions of Formosan subterranean termite soldiers in five different colonies. The plate was developed with chloroform-methanol-acetic acid (98:2:1, v/v/v). Spots were detected by charring with 50% H_2SO_4.

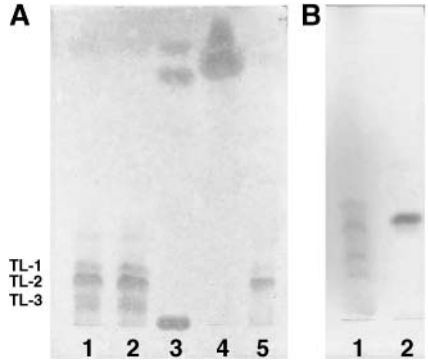

Fig. 2. HPTLC of the simple lipid fractions obtained by Iatrobeads column chromatography. A: Lane 1, the lipid fraction eluted with chloroform-methanol (9:1, v/v); lane 2, mild alkaline hydrolysates (0.5 N NaOH, 37°C, 6 h) of the chloroform-methanol fraction; lane 3, methanolysates (2.5% methanolic HCl, 80°C, 12 h) of the chloroform-methanol fraction; lanes 4, 5, fatty acid methyl ester and ceramides, respectively, from bovine brain. B: Lane 1, methanolysates of the chloroform-methanol fraction; lane 2, sphingosine from bovine brain. The plates were developed with chloroform-methanol-acetic acid (98:2:1, v/v/v) for A and with chloroform-methanol-water (65:25:4, v/v/v) for B. The spots were detected with 50% H_2SO_4 for A and with ninhydrin reagent for B.

Gas liquid chromatography-mass spectrometry

All samples were analyzed on a Shimadzu GC-17A with a QP5050A mass spectrometer, using a DB-1 column (0.25 mm × 30 m; J&W Scientific) at 60°C for 1 min, increasing to 260°C at 8°C/min, and held at 260°C at 14 min. The electron ionizing energy was 70 eV.

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Voyager DE STR; PerSeptive Biosystems) of native ceramides and peracetylated derivatives was performed as described previously (23). The matrix solution contained 10 mg/ml 2,5-dihydroxybenzoic acid in 10% ethanol. One microliter of matrix solution and 1 μl of the samples in chloroform were mixed on the sample plate directly and dried to avoid crystallization.

Peracetylation

The ceramides were peracetylated in 100 μl of acetic anhydrate-pyridine (1:1, v/v) at 80°C for 2 h, before MALDI-TOF MS.

RESULTS

Properties of lipids from frontal gland secretions

HPTLC using solvent I (data not shown) showed several spots from the total lipid extract in the region where simple lipids migrated but indicated no glycolipids or phospholipids. HPTLC of total lipids using solvent II is shown in **Fig. 1**; all lipid components of the five colonies gave several spots that had the same migration behavior. For component analysis, the total lipids were degraded in methanolic HCl at 80°C for 12 h, upon which all original TLC spots disappeared and a new spot was detected in a FAME mobility position (data not shown). These fatty acid compositions were normal and had hydroxylated fatty acids from C18 to C24 as the major species. The positions

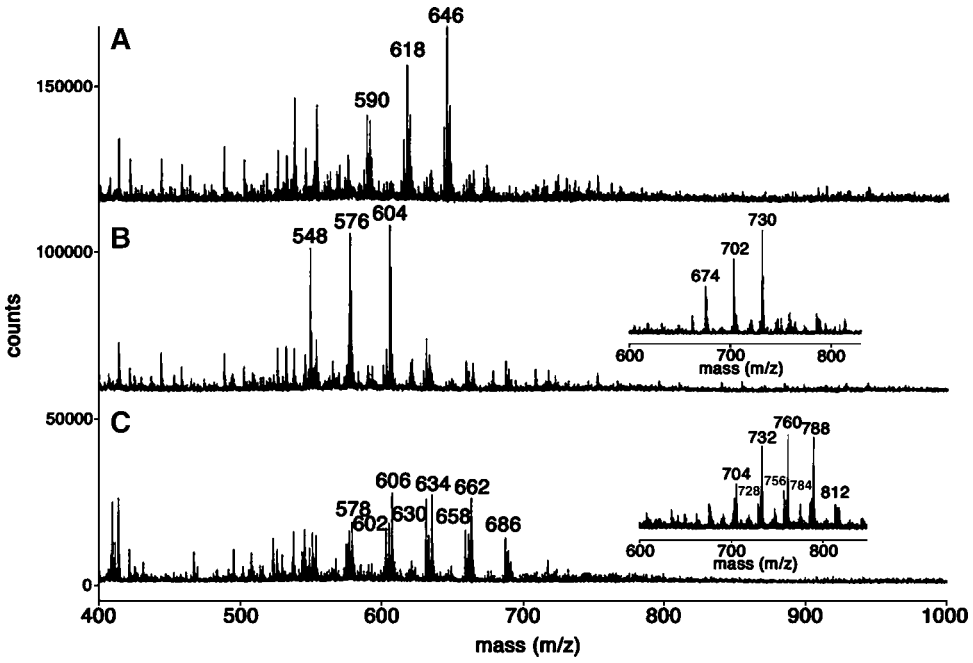

Fig. 3. Analysis of the ceramides isolated from the frontal gland secretion of termite soldiers by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. TL-1 (A), TL-2 (B), and TL-3 (C) were analyzed in the positive-ion reflectron mode using 2,5-dihydroxybenzoic acid as matrix. Corresponding pseudomolecular ions ($[M+Na]^+$) are marked in the insets. The internal small figures show the mass spectra of peracetylated TL-2 and TL-3, respectively.

of hydroxy groups of the hydroxylated fatty acids were determined by GC-MS. Diagnostic molecular ions ($[M]^+$) and characteristic fragment ions ($[M-59]^+$) (24) (data not shown) indicated the presence of the 2-hydroxy FAMEs of C18h:0 to C24h:0. Chen, Henderson, and Laine (9) found lignoceric acid and hexacosanoic acid as two major components of the frontal gland secretions. Free fatty acids were determined by methyl esterification using trimethylsilyldiazomethane without hydrolysis, confirming lignoceric acid and hexacosanoic acid by GC analysis but no free 2-hydroxy fatty acids (data not shown).

Purification of alkali-stable lipids in frontal gland secretions

Lipid fractions from frontal gland secretions were fractionated by Iatrobeads column chromatography using stepwise elution with *1*) ether and *2*) chloroform-methanol (9:1, v/v). HPTLC of the fraction eluted with chloroform-methanol as shown in **Fig. 2** gave three spots (denoted TL-1, TL-2, and TL-3) migrating with standard bovine free ceramides. These were stable in mild alkaline hydrolysis (0.5 N NaOH, 37°C, 6 h) (Fig. 2A, lane 2), and after methanolysis, they gave FAME- and ninhydrin-positive spots that corresponded to sphingosine bases (Fig. 2A, lane 3, 2B, lane 1), suggesting that the alkaline-stable compounds (TL-1, TL-2, and TL-3) were free ceramides. These components were further isolated by preparative TLC using chloroform-methanol-acetic acid (98:2:1, v/v/v).

Analysis of ceramides TL-1, TL-2, and TL-3

The relative distribution of fatty acids in TL-1 and TL-2 were C18:0 (20.2% and 12.7%), C20:0 (32.2% and 24.9%), and C22:0 (38.1% and 37.8%), but TL-3 contained the 2-hydroxy fatty acids C18h:0 (23.5%), C20h:0 (32.5%), and C22h:0 (28.9%) as the major species.

MALDI-TOF MS of TL-1 gave predominant pseudomolecular ions $[M+Na]^+$ at *m/z* 590, 618, and 646 (**Fig. 3A**). LCBs obtained from the hydrolysate were trimethylsilylated and analyzed by GC-MS. The TMS derivative yielded a single peak (TL-1-I) that eluted at the same retention time as that of authentic C_{18}-dihydrosphingosine (**Fig. 4A**). In the electron-impact mass spectrum of TL-1-I (**Fig. 5A**), intense characteristic ions for the TMS ether of sphingosine were observed at *m/z* 73 and 132. The ions at *m/z* 445 $[M]^+$, 430 $[M-15]^+$, and 342 $[M-103]^+$ indicated C_{18}-dihydrosphingosine.

On MALDI-TOF MS, TL-2 showed major pseudomolecular ions $[M+Na]^+$ at *m/z* 548, 576, and 604, and analysis of acetylated TL-2 showed ions at *m/z* 674, 702, and 730 (Fig. 3B). The +126 mass shift after peracetylation suggested the presence of three hydroxyl groups. Because TL-2 contained only saturated fatty acids, these results

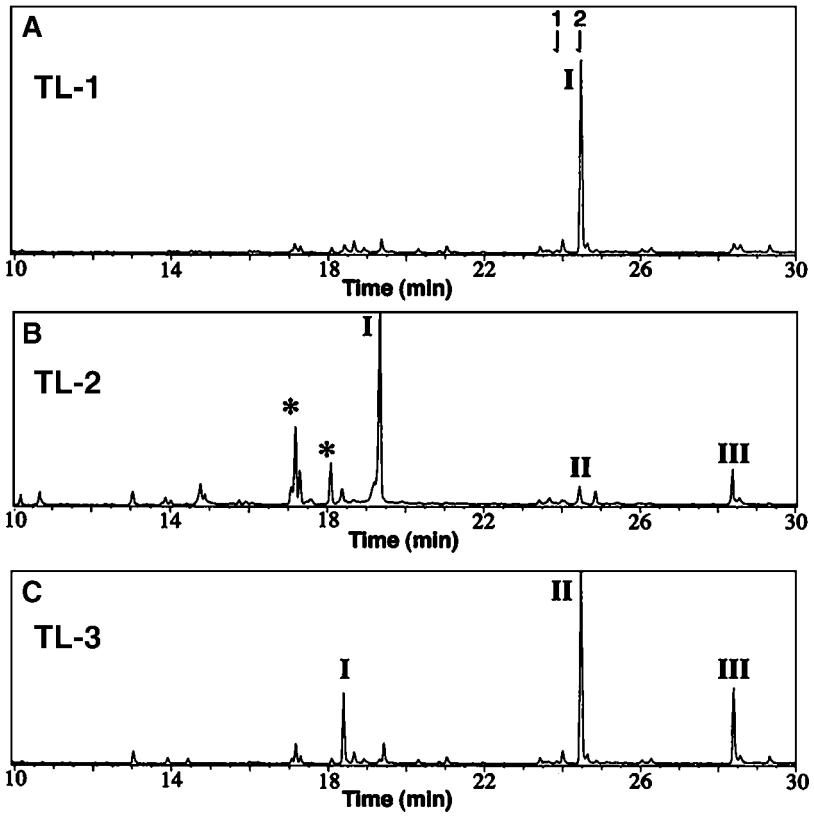

Fig. 4. Total ion chromatograms of trimethylsilyl ether derivatives of long chain bases (LCBs) from the isolated ceramides [TL-1(A), TL-2(B), and TL-3(C)]. GC-MS analysis was run as described in Materials and Methods. Arrows indicate the retention times of standard LCBs: 1, sphingenine (d18:1); 2, sphinganine (d18:0). Peaks denoted by asterisks were found not to be LCBs.

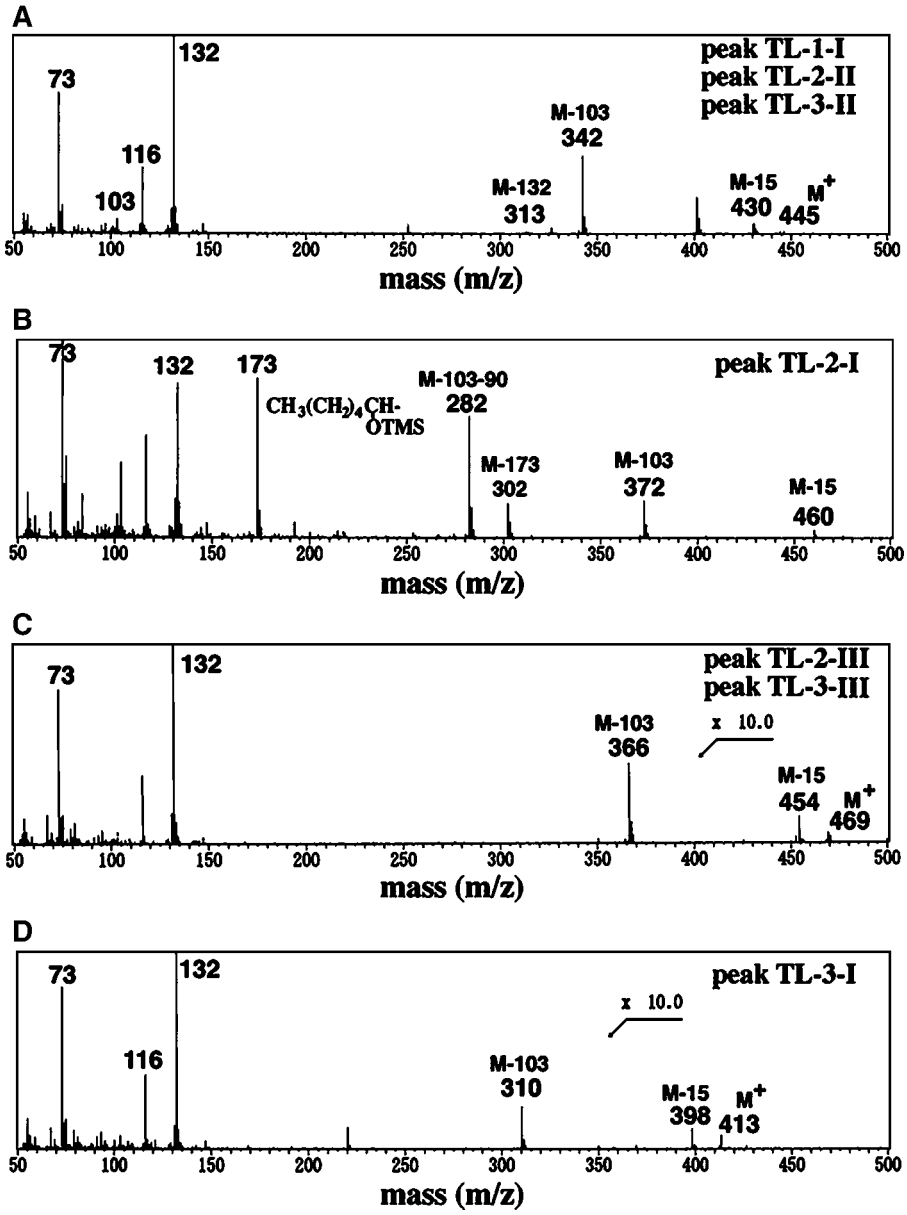

Fig. 5. Mass spectra of trimethylsilyl ether derivatives of LCBs. The peak numbers (A; TL-1-I, TL-2-II, TL-3-II, B; TL-2-I, C; TL-2-III, TL-3-III, D; TL-3-I) refer to those of the total ion chromatogram in Fig. 4. Conditions for mass spectrometry were described in Materials and Methods.

strongly suggested a trihydroxy LCB. The gas chromatogram of TMS-LCBs from TL-2 gave one predominant peak (TL-2-I), as shown in Fig. 4B, which showed ions at *m/z* 73 and 132 characteristic for a TMS-sphingoid base (Fig. 5B). The molecular weight was 475, as deduced from the ions at *m/z* 460 $[M\text{-}15]^+$, 372 $[M\text{-}103]^+$, and 282 $[M\text{-}103\text{-}90]^+$, and a very strong specific fragment ion at *m/z* 173 corresponded to CH_3- CH_2-CH_2-CH_2-CH_2-CH(OTMS-) (Fig. 5B). Therefore, an OTMS residue was present at the ω-5, C-1, and C-3 positions, indicating a C_{14}-trihydroxysphingenine.

The LCB fraction from TL-2 was oxidized at vicinal hydroxyls to aldehydes with sodium metaperiodate and analyzed by GC-MS after TMS derivatization. The aldehydes were reduced with sodium borohydride to corresponding alcohols and analyzed by GC-MS as TMS derivatives. The most predominant TMS-aldehyde gave an $[M\text{-}15]^+$ ion at *m/z* 255 and the TMS-alcohol at *m/z* 329 (data not shown). From these results, both aldehyde and alcohol were C12 with one double bond and one hydroxy residue. These spectra of TMS-aldehyde and TMS-alcohol gave a characteristic ion at *m/z* 173, corresponding to CH_3-CH_2-CH_2-CH_2-CH_2-CH(OTMS-) indicating that both aldehyde and alcohol had a hydroxyl group at the ω-5 position. To confirm the double bond position, the LCB alcohol was oxidized by OsO_4 and the TMS polyhydroxylated alcohol was analyzed by GC-MS. The molecular weight was 522 from fragment ions at *m/z* 432 $[M\text{-}90]^+$ and *m/z* 417 $[M\text{-}105]^+$ (**Fig. 6**), and the positions of vicinal OTMS groups were confirmed at C-4 and C-5 from the intense ions at *m/z* 233 $[b]^+$ and 143

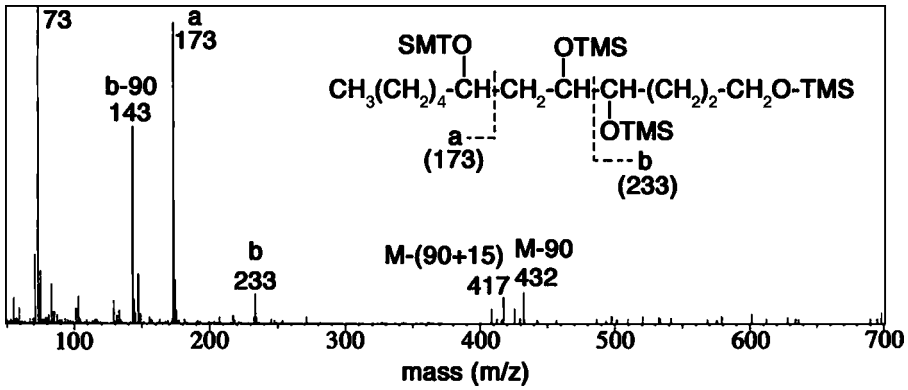

Fig. 6. Mass spectrum of a trimethylsilyl ether derivative of a polyhydroxylated alcohol from LCB of TL-2. The conditions of GC-MS analysis were the same as for Fig. 4.

$[b\text{-}90]^+$. Therefore, the double bond position in the original LCB was at C-6.

From these results, the structure of the LCB in TL-2-I was established to be a novel sphingosine: 1,3,9-trihydroxy-2-amino-6-tetradecene. Of minor components in the TMS-LCBs from TL-2, TL-2-II eluted at the same position as TL-1-I and gave the same mass spectral profile. The LCB in peak TL-2-III was the same as in TL-3-V, as described below.

In MALDI-TOF MS analysis of TL-3, pseudomolecular ions $[M+Na]^+$ were detected at *m/z* 602, 606, 630, 634, 658, and 662 as the major species (Fig. 3C). Peracetylation of TL-3 gave a +126 mass shift, indicating the presence of three OH groups. Because TL-3 contained only 2-hydroxy fatty acids, the LCB must be dihydroxy-. The gas chromatogram of TMS-LCBs from TL-3 gave three peaks (TL-3-I, TL-3-II, and TL-3-III), as shown in Fig. 4C. The mass spectra showed characteristic ions for a TMS-sphingoid base at *m/z* 73 and 132 (Fig. 5A, C, D). The $[M]^+$, $[M\text{-}15]^+$, and $[M\text{-}103]^+$ ions for the LCB(s) were detected at *m/z* 413, 398, and 310 for peak TL-3-I (Fig. 5D), at *m/z* 445, 430, and 342 for peak TL-3-II (Fig. 5A), and at *m/z* 469, 454 and 366 for peak TL-3-III (Fig. 5C), respectively. This suggested that TL-3-I was a C_{16}-sphingadienine, peak TL-3-II was a C_{18}-sphinganine, and peak TL-3-III was a C_{20}-sphingadienine.

For the double bond position of TL-3-I and TL-3-III, the LCB from TL-3 was oxidized with sodium metaperiodate followed by osmium tetroxide, and the TMS derivatives of the polyhydroxyl alcohols were analyzed by GC-MS (**Fig. 7A**). The mass spectra of the TMS-alcohols, peak 1, peak 2, and peak 3, are shown in Fig. 7B–D. The $[M\text{-}15]^+$ ion, which determines the chain length of TMS-alcohols, appeared at *m/z* 299 for peak 1 (Fig. 7B). This result indicated that the alcohol was hexadecanol derived from the putative C_{18}-sphinganine (peak TL-3-II). Peak 2 corresponded to a tetrahydroxy C_{14}-alcohol and peak 3 to a tetrahydroxy C_{18}-alcohol, deduced from the $[M\text{-}105]^+$ ions at *m/z* 533 and 589, respectively (Fig. 7C, D). These results indicated the introduction of four OH groups by OsO_4 oxidation and the presence of two double bonds. In Fig. 7C, the positions of vicinal OTMS groups were confirmed to be located at C-8 and C-9 from the intense ions at *m/z* 173 $[a]^+$ and *m/z* 375 $[b\text{-}90]^+$ accompanying the ions at *m/z* 285 $[b\text{-}90\text{-}90]^+$ and *m/z* 195 $[b\text{-}90\text{-}90\text{-}90]^+$. Another set of ions at *m/z* 301 $[c\text{-}90]^+$, 211 $[c\text{-}90\text{-}90]^+$, and 247 $[d]^+$ indicated that another pair of OTMS groups was located at C-5 and C-6. Therefore, the double bond positions of the original LCB (C16:2) were at C-7 and C-10.

Fragment ions in the mass spectrum shown in Fig. 7D were interpreted in a similar manner. The location of one pair of vicinal OTMS groups was confirmed to be C-9 and C-10 from the intense ions at *m/z* 303 $[d]^+$ and 301 $[c\text{-}90]^+$ accompanying the ion at *m/z* 211 $[c\text{-}90\text{-}90]^+$. The location of another pair of vicinal OTMS groups was determined to be C-12 and C-13, based on fragment ions at *m/z* 173 $[a]^+$, 431 $[b\text{-}90]^+$, and 341 $[b\text{-}90\text{-}90]^+$. Therefore, the double bond positions of the original LCB (d20:2) were at C-11 and C-14.

Based on these results, the compounds in TL-1, TL-2, and TL-3 from the frontal gland secretions of Formosan subterranean termite soldiers were identified as novel ceramides composed of a variety of fatty acids and novel LCBs, as shown in **Table 1**.

DISCUSSION

Lipid components from the frontal gland secretions of Formosan subterranean termite soldiers appear to be unique among already studied defense secretions of termites. It is known that most termite soldiers with slashing mandibles produce irritants such as hydrocarbons derived from fatty acids and volatile C12-C20 fatty acids (11). However, free ceramides have not been reported previously in the soldier frontal gland secretion of any termite species. It is well recognized that free ceramide acts as a signal to induce apoptosis and cell differentiation (16, 17). Although the function of these unusual free ceramides as a defense chemical is not investigated here, we speculate that they may play a role as a chemical effector on predatory ants, or as colony signals, such as common nest signature and alarm pheromones. We have found that the defense secretion, which would certainly signal the loss of some soldiers, stimulates the transformation of workers to

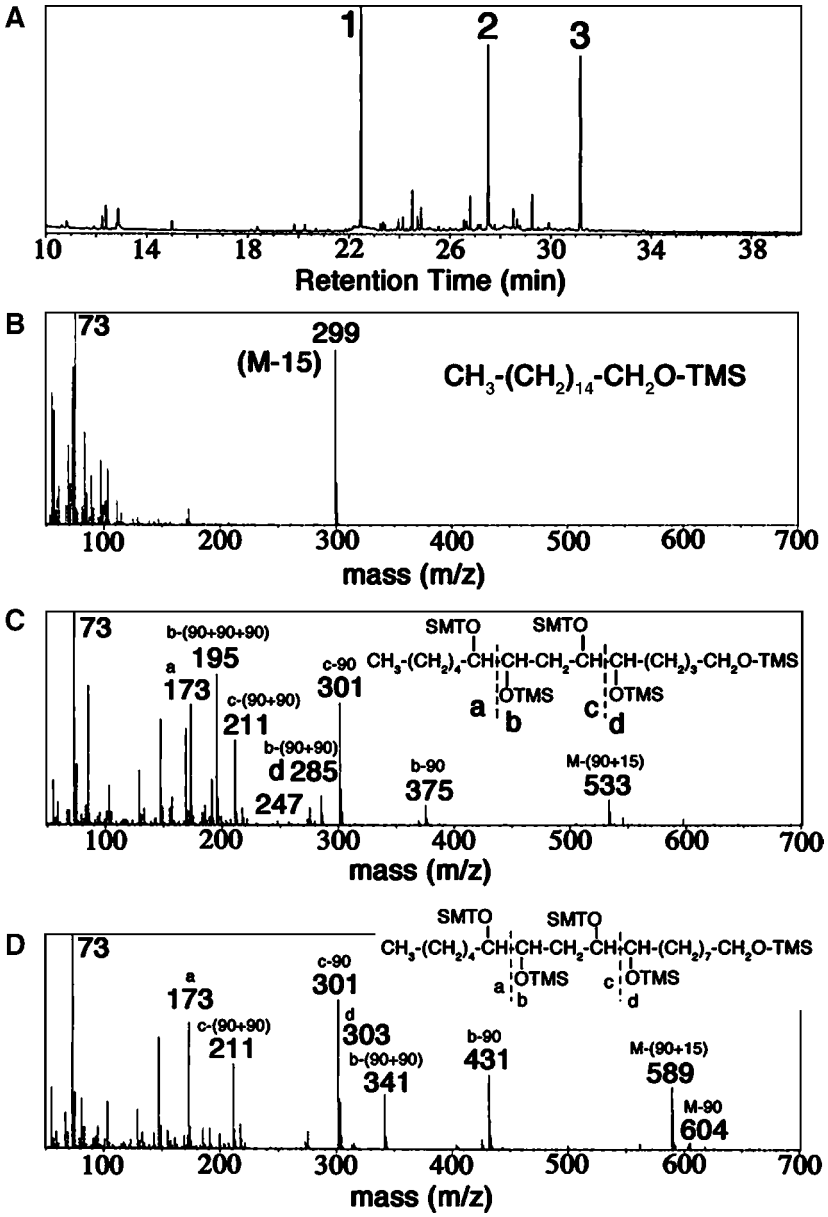

Fig. 7. GC-MS analysis of trimethylsilyl ether derivatives of polyhydroxylated alcohols from LCBs of TL-3. A: Total ion chromatogram of polyhydroxylated alcohols from LCBs of TL-3. B–D: Mass spectra of peaks 1 (B), 2 (C), and 3 (D). The conditions of GC-MS analysis were the same as for Fig. 4.

soldiers in subsequent molts (unpublished results). It is interesting that the unusual free ceramides could play a role in this transformation. In this study, we analyzed only the chloroform-methanol fraction, although unknown simple lipid compounds were also detected in the ether-extracted fractions, which are under investigation.

Detailed chemical constituents of insect sphingolipids were examined with glycosphingolipids from two species, *Calliphora vicina* (25–27) and *Lucilia caesar* (28–30). The structures of the LCBs from the dipteran sphingolipids were C_{14}- and C_{16}-sphingenine in *L. caesar* and C_{14}-sphingenine in *C. vicina*, clearly different from those of the *C. formosanus* soldier frontal gland secretions, and free ceramides were not reported. The major fatty acids of the reported dipteran sphingolipids were saturated C18, C20, and C22 fatty acids, not containing 2-hydroxy fatty acids.

Sphingolipids having the sphingoid LCB are present in bacteria (*Sphingomonas*) as well as in plants and animals. However, a wealth of varieties of this basic structure, including differences in chain length, degree of unsaturation and branching, and introduction of additional hydroxylation, suggests specific functions. Trihydroxy bases in sphingolipids have been detected in bovine kidney sphingomyelins (31) and *Turbo cornutus* sphingophosphonolipids (32) in addition to plant glycophosphosphingolipids (33). These previously reported trihydroxy LCBs were all "phytosphingosines": that is, the 1,3,4-trihydroxy-2-amino series with saturated carbon chains. In contrast to phytosphingosines, trihydroxy LCBs in TL-2 from *C.*

TABLE 1. Composition of ceramides of frontal gland secretions of Formosan subterranean termite soldiers

Fraction	Long-Chain Bases	Fatty Acids
TL-1	1,3-Dihydroxy-2-aminooctadecane (d18:0)	18:0, 20:0, 22:0
TL-2	1,3,9-Trihydroxy-2-amino-6-tetradecene ($t14:1^{6}$)	18:0, 20:0, 22:0
TL-3	1,3-Dihydroxy-2-amino-7,10-hexadecadiene ($d16:2^{7,10}$)	18h:0, 20h:0, 22h:0
	1,3-Dihydroxy-2-aminooctadecane (d18:0)	
	1,3-Dihydroxy-2-amino-11,14-eicosadiene ($d20:2^{11,14}$)	

formosanus had one hydroxy group not located at C-4 but inside the C-9 alkyl chain and had additional unsaturation. The presence of diene-dihydroxy bases has been reported in sphingolipids from various animals and shellfish (34). However, the positions of the double bond in LCBs from *C. formosanus* differed from those of any LCBs found previously.

Ceramide is biosynthesized de novo in all organisms examined, from serine and 2 mol of fatty acyl-CoA (35, 36). The novel structures of termite LCBs suggest that alternative pathways and enzyme systems may exist for ceramide modification, such as desaturation and/or addition of hydroxy residues. Vrkoc and Ubik (37) have reported that *Prorhinotermes* soldiers have a nitrogen-containing defensive compound, (*E*)-1-nitro-1-pentadecene. Prestwich (6) has proposed that the initial precursors of biosynthesis of this nitroalkene are myristic acid and serine. In both subfamilies, Coptotermitinae and Prorhinotermitinae, it is very interesting that serine was used to synthesize different putative defense compounds. Another subfamily, Rhinotermitinae, expresses saturated ketones, vinyl ketones, and β-ketoaldehydes as soldier defense compounds (38). The change from a primitive nitroalkene to all-carbon lipids may have been one key event leading subsequently to the diverse evolution between Prorhinotermitinae and Rhinotermitinae in the Rhinotermitidae. Therefore, *C. formosanus* may differ in the postulated evolutionary relationships among the Rhinotermitidae by use of lipophilic electrophiles as components of defense secretions. The minuscule amounts of the novel free ceramides reported here, obtainable only from Formosan soldier termites, precludes facile testing of biological activity. Therefore, further studies of the functions of these molecules await organic synthesis.

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