Characterization of Novel Glycolipids from the Giant Cockroach (Blaberus colosseus)*

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A novel class of glycolipids, assigned the trivial name blaberosides, was isolated from whole head tissues of the giant cockroach (Blaberus colosseus). The class consists of two closely related families, blaberoside I and blaberoside II, each containing species differing by 26 atomic mass units. The structure of these gentio-biose-based glycolipids was elucidated by chromatographic behavior, nuclear magnetic resonance spectroscopy, mass spectrometry, and analysis of chemical degradation products and derivatives. Species in the blaberoside I family have been identified as 2-O-[6'-O-(6''-O-3-hydroxy-11-eicosenoyl-~-~-glucopyranosyl)-~-~-glucopyranosyl]-3-(hexadecyloxy)-1,2-propanediol (blaberoside Ia) and 2-O-[6'-O-(6''-O-3-hydroxy-11-eicosenoyl-~-~-glucopyranosyl)-~-~-glucopyranosyl]-3-(6-octadeceyloxy)-1-(3-hydroxy-11-eicosenoyl)-1,2-propanediol (blaberoside Ib). Two smaller homologs of the blaberoside II family were discerned to be 2-O-[6'-O-(6''-O-3-hydroxy-11-eicosenoyl-~-~-glucopyranosyl)-~-~-glucopyranosyl]-3-(hexadecyloxy)-1,2-propanediol (blaberoside Iia) and 2-O-[6'-O-(6''-O-3-hydroxy-11-eicosenoyl-~-~-glucopyranosyl)-~-~-glucopyranosyl]-3-(4-octadeceyloxy)-1,2-propanediol (blaberoside Iib). These compounds are unique because they are animal origin glyceroglycolipids with a highly flexible gentio-biose backbone, and a ~-linkage of the carbohydrate to the glycerol ether at the 2 position rather than the usual 1 position.

Over the past few years, we have studied the phylogenetic diversity of glycolipids associated with neuronal tissue in a wide assortment of vertebrate and invertebrate models in order to appreciate the evolutionary impact of these compounds on nervous system function (1). During the course of these studies, hitherto unreported glycolipids were detected in the heads of giant cockroaches (Blaberus colosseus). Subsequently, we have analyzed the structures of these glycolipids (blaberosides) and found them distinctly different from any previously reported glycolipids. This paper reports the first isolation of a glycolipid from an insect and presents structural characterizations of members of a novel class of glycolipids.

MATERIALS AND METHODS

RESULTS

Isolation and Purification of Blaberosides—The average yield of total lipids in six preparative extractions of heads from B. colosseus was 43 mg/g fresh tissue. The yield of acetone fraction obtained by Unisil column chromatography of total lipids was about 56 mg/g total lipid.

TLC of the acetone fraction presented two bands. The band with an Rf above mammalian cerebrosides was named blaberosides I. The band with an Rf slightly below mammalian hydroxy cerebrosides but above mammalian sulfatides was named blaberosides II. These two blaberosides were separately isolated by preparative TLC. Various spot tests indicated that both blaberosides contained nonreducing carbohydrate but no primary amino groups, nitrogen, or phosphorus. The compounds appeared to be homogeneous on high performance liquid chromatography analysis when derivatized by benzoylation (2), each producing single peaks with variable shoulder patterns in two separation systems, indicating considerable homogeneity with the possible presence of closely related homologs similar to those encountered due to the varying lengths of the fatty acids in glycosphingolipids (data not presented).

The average yield of blaberosides I was 26 mg from a cockroach head (332 mg/g tissue) or 294 mg/mg acetone fraction. The mean yield of blaberosides II was 176 mg/mg acetone fraction.

Structures of Blaberosides I and II—From the following experimental observations, structures are proposed for blaberosides I and II (Fig. 1).

Infrared and Ultraviolet Spectrometry.—The infrared spectrum blaberosides II was compatible with the known classes of glycolipids (3). It demonstrated hydroxy O-H stretching (3700-3100 cm-1), methine, methylene, and methyl C-H stretching (2970-2935 cm-1), ester C=O stretching (1755 cm-1), and a trans C=C stretching (1650 cm-1). In the lower wave numbers, the blaberosides showed bands due to methylene deformation (1480 cm-1), and C-O stretch of a long chain ester (1170 cm-1). Primary C-O stretching of a primary

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peaks confirming a single species of \( \alpha \)-hydroxyl fatty acid was present in both lipids. GC/MS spectra indicated that this

ylamine provided a single water soluble fraction on TLC tetramethylsilane; MS; mass spectrometry; LD, laser desorption; \( \alpha \)-hydroxyl C20-enoic acid and methyl glucoside, identified as in an unbranched CH2 chain at least four carbons long. The acid was unbound in the parent molecule.

compounds indicated that the \( \alpha \)-hydroxyl group of this fatty one double bond (6-7). The lack of other unbound nonpolar compound was C20 \( \alpha \)-hydroxyl fatty acid methyl ester with ester from both blaberosides I and flame ionization detection the \( \alpha \)-hydroxyl fatty acid methyl ester standard on TLC in hexane:ether development. On GC2/ alcohol (1050 cm\(^{-1}\)) and an alkyl ether with C-O stretching (1080 cm\(^{-1}\)) (4) were also shown. A minor band at 899 cm\(^{-1}\) suggested the presence of \( \beta \)-glycosidic linkages, and the minor absorbance at 720 cm\(^{-1}\) was indicative of skeletal vibrations in an unbranched CH2 chain at least four carbons long. The absence of any absorbances in the range of amides or sulfates eliminated glycosphingolipids from consideration (5). No absorbance between 200 and 800 nm was observed in UV spectra of the compounds.

Methanolysis Experiments—When crude acetone fraction lipids were subjected to mild alkaline methanolysis (NaOH) and separated by TLC, the blaberoside spots disappeared, indicating the presence of ester linkages in these lipids. Acid methanolysis of purified blaberosides yielded methyl ester of \( \beta \)-hydroxyl C20-enoic acid and methyl glucoside, identified as described below.

An ether extractable fragment from acid methanolysis had an identical \( R_F \) value with a \( \alpha \)-hydroxyl palmitic acid methyl ester standard on TLC in hexane:ether development. On GC/ flame ionization detection the \( \beta \)-hydroxyl fatty acid in blaberoside I\( \text{a}, \text{b}, \text{IIa}, \text{b}\). Two prominent spots of per- methylation products were fractionated by TLC in combination with a disaccharide backbone, as discussed above, suggested a 1-6 sugar linkage and acylation of the nonreducing sugar’s 6 position by the \( \beta \)-hydroxyl fatty acid. The nonpolar products of the acid methanolysis of the blaberoside permethylation product were fractionated by TLC and the products examined by GC/MS. The TLC indicated two nonpolar products, one with an \( R_F \) matching a \( \beta \)-methoxyxylalinate methyl ester standard and the other with an \( R_F \) which was identified as gentiobiose attached to glycerol 3-alkyl ether from the following observations. The \( R_F \) value consistently ran above methylglucoside and methylglucoside standards (gentiobiose, cellobiose, trehalose). The watersoluble product contained a nonreducing sugar. When examined by GC/MS as a TMS derivative, a single peak at 25.2 min represented the intact sugar with a glycerol ether still attached. Prominent \( m/e \) 452 and 361 fragments indicated the presence of one sugar which was completely unsubstituted prior to TMS derivatization with the exception of the anomic carbon. The lack of the \( m/e \) 203 fragment, the presence of a prominent \( m/e \) 295 fragment, and the abundance of \( m/e \) 204 compared to \( m/e \) 217, ruled out a trehalose 1-1 and suggested a 1-4 (cellobiose), 1-6 (gentiobiose), or 1-2 (sophorose) linkage (9-13). The assignment of a 1-6 linkage was supported by the presence of \( m/e \) 583 and the very high abundance of \( m/e \) 451 in the experimental spectrum matching a gentiobiose standard (Table 1). Further methanolysis of the water-soluble material yielded a nonpolar fragment. GC/MS of TMS derivatives of the nonpolar fraction matched spectra of TMS butyl alcohol and TMS chymyl alcohol standards and indicated that homolog heterogeneity was due to this component of blaberosides. Chromotropic analysis of blaberosides for glycerol was negative. This observation suggested that the glycerol must be substituted at its \( \beta \) carbon, preventing the formation of formaldehyde by this reaction.

Methylation Experiments—The position of attachment of the \( \beta \)-hydroxyl fatty acid in blaberoside II was determined by permethylation experiments. Two prominent spots of permethylation products appeared near the solvent front. The materials from these spots were eluted separately and subjected to acid methanolysis. The methylated sugars obtained from each spot were examined by GC/MS. The total ion chromatographs of both samples were indistinguishable, presenting single major peaks at 6.3 min with fragmentation patterns corresponding to methyl 2,3,4-tri-O-methylglucoside. Very minor anomer peaks at 5.03 min were present in each chromatograph, representing less than 10% of the total ion abundance. The overwhelming abundance of methyl 2,3,4-tri-O-methylglucoside established that only the six positions of glucose were protected from methylation. This observation, in combination with a disaccharide backbone, as discussed above, suggested a 1-6 sugar linkage and acylation of the nonreducing sugar’s 6 position by the \( \beta \)-hydroxyl fatty acid.

The water-soluble acid methanolysis products produced a single spot on TLC with an \( R_F \) identical to that of methylglucoside. Sugar analysis by liquid chromatography after acid hydrolysis of blaberosides II as described by Lee (8) also showed a single peak corresponding to glucose.

Methanolysis of purified blaberosides in methanolic triethanolamine was performed with a \( \beta \)-hydroxyl fatty acid methyl ester standard from both blaberosides I\( \text{a}, \text{b}, \text{IIa}, \text{b}\) as described by Lee (8) also identical to that of methylglucoside. Sugar analysis by liquid chromatography after acid hydrolysis of blaberosides II as described by Lee (8) also showed a single peak corresponding to glucose.

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The abbreviations used are: GC, gas chromatography; TMS, tetramethylsilane; MS, mass spectrometry; LD, laser desorption; PDMS, plasma desorption; FAB, fast atom bombardment; FID, flame ionization detection; GLC, gas liquid chromatography.

\[ \text{TABLE I} \]

| Fragment | Standards (relative abundance) | From blaberosides (relative abundance) |
|----------|--------------------------------|----------------------------------------|
| m/e      | Trehalose  | Cellobiose | Gentiose | Blaberosides  |
| 204/217  | 0.5165    | 2.0439     | 2.4972   | 2.4569       |
| 132      | 1         | 1          | 1        | 1             |
| 203      | 3         | 0          | 0        | 0             |
| 279      | 1         | 6          | 1        | 1             |
| 295      | 0         | 1          | 1        | 1             |
| 361      | 100       | 100        | 100      | 100           |
| 451      | 4         | 5          | 23       | 17            |
| 452      | 1         | 2          | 9        | 7             |
| 583      | 0         | 0          | 4        | 1             |

* Relative abundance is reported relative to \( m/e \) 361.
matching batyl alcohol methyl ether standard. The GC/MS analysis of the former material yielded a major peak with a retention time of 12.3 min, whose mass spectrum revealed a fragmentation pattern similar to that seen in β-methoxyhexadecanoic acid methyl ester. This pattern was interpreted to confirm the lack of participation of the β-hydroxy group in structural covalent bonding. A smaller peak eluted at 11.3 min and produced a mass spectrum with a fragmentation pattern consistent with methyl β-methoxyhexadeconoate based on methyl β-methoxyhexadecanoate standard. Retention times and fragmentation patterns of these components did not change following attempted TMS derivitization.

The derivatives of monoalkyl ethers of glycerols were also examined by GC/MS. Authentic standards of hexadecyl glycerol ether and batyl alcohol were used for comparison. The monoalkylglycerol products of the acid methanalysis of per-methylated blaberoside II had molecular ions and fragmentation patterns compatible with monomethylated selachyl and chymyl alcohol, indicating the presence of a free alcohol group on the glycerol backbone before methylation. The fragmentation patterns had prominent m/e 45 fragment abundance due to CH$_2$OH ions and low abundance of m/e 31 fragments due to CH$_3$OH ions (4–8 or greater m/e 54/31 ratio) indicating the glycerol ethers were linked to the sugar at their 2 position.

These studies indicated the presence of the diglucoside backbone with 1'-6 linkage and the esterification of the fatty acid to the 6' position of the nonreducing end sugar. They also suggested the unusual attachment of the carbohydrate to the second carbon of the glycerol ether (14).

**Mass Spectrometry on Intact Blaberosides—** Laser desorption, plasma desorption (PDMS), and fast atom bombardment (FAB) mass spectrometry were used to confirm the molecular weights of the structures shown in Fig. 1. Even electron molecular ions were observed, formed by the addition of protons or other cations to the neutral molecules. The results are summarized in Table II. It should be noted that the FAB technique produces measurements of the monoisotopic mass, whereas both LD and PDMS, which are obtained on low resolution instruments, produce measurements of the average mass of the isotopically unresolved molecular ion cluster.

Calculated values for the molecular weights are shown in Table III and are in good agreement with the measured values. In general, laser desorption mass spectra (Fig. 2) were the most informative, since fragment ions were also observed. Peaks which differ by 26 mass units can be assigned to the fragments containing hexadecyloxy and octadeceloxy homologs, whereas other peaks represent the loss of this heterogeneous portion of the molecule. The most prominent peaks in this spectrum (at 988.6 and 1014.4 atomic mass units) correspond to the MK$^+$ of IIa and IIb, respectively. Loss of the β-hydroxyl fatty acid (at the 6' position of the disaccharide) from these two homologs results in the peaks at 680.1 and 705.5 (i.e. 665.5 + K$^+$). Similarly, cleavage between the α and β carbons of the fatty acid results in peaks at 721.7 and 747.6 (i.e. 708.6 + K$^+$) atomic mass units. In both cases, hydrogen is transferred to the fragment ion to form an even electron ion containing K$^+$.

The peaks at 363 and 379 represent the disaccharide portion of the molecule containing K$^+$. Both peaks result from cleavage of the acyl bond between the fatty acid and disaccharide at the 6' position, but differ by 16 mass units depending upon whether cleavage occurs between the disaccharide and glycerol moieties at the 1' C-O bond or at the O-C bond of the glycerol. Although the laser desorption mass spectra cannot establish the attachment positions, they do confirm the location of the homologous portion attached to the glycerol.

**One-dimensional ¹H Nuclear Magnetic Resonance Spectroscopy—** Chemical shift assignments and coupling constants for blaberosides I and II are shown in Tables IV and V, respectively. A proton resonance spectrum in dimethyl sulfoxide-$d_6$ confirmed the presence of unsaturations as well as the disaccharide nature of the sugar backbone. Fig. 3 shows $¹H$ NMR of blaberosides II. The peak at 5.25 ppm was due to ethylene protons. Numerous peaks between 5.2 and 2.6 ppm represented protons from the sugar, the fatty acid β-hydroxyl group and the free alcohol of the glycerol ether. From the chemical analysis described above, as many as three primary alcohols could be in the structure of blaberoside II, one on the glycerol ether and one at each 6 position of the two glucose. Only one triplet, which disappeared with deuterium exchange (see below), was found downfield, indicating that two of the three

| Table II |
| Species | Mass (atomic mass units) | Method | Interpretation | Molecular weight |
|---------|--------------------------|--------|----------------|-----------------|
| Blaberoside I | 1258 | FAB | MN$^+$ | 1258 |
| | 1236 | FAB | MN$^+$ | 1236 |
| Blaberoside II | 949.0 | LDMS | MN$^+$ | 949.0 |
| 998.7 | LDMS | MN$^+$ | 998.7 |
| 1014.4 | LDMS | MN$^+$ | 1014.4 |

**Table III**

| Species | Monoisotopic mass | Average mass | Formula |
|---------|------------------|--------------|---------|
| Ia      | 1256.9           | 1257.8       | C$_9$H$_{15}$O$_{17}$ |
| Ib      | 1282.9           | 1283.9       | C$_9$H$_{15}$O$_{17}$ |
| IIa     | 948.7            | 949.7        | C$_9$H$_{15}$O$_{15}$ |
| IIb     | 974.6            | 975.4        | C$_9$H$_{15}$O$_{15}$ |

FIG. 2. Laser desorption mass spectrum of blaberosides II obtained using a CVC 2000 time of flight mass spectrometer with a 2-m flight tube and a Tachisto 215G CO$_2$ laser with a 40 ns, 1 megawatt/cm$^2$ pulse. The labeled peaks contain the K$^+$ cation.
possible primary alcohols were substituted and only one was free.

Deuterium exchange greatly simplified the proton spectrum. The two most downfield doublets above the vinyl proton peak, retained after deuterium exchange, represented the anomeric protons of two hexose rings (data not shown). The spin-spin (J\textsubscript{2,3}) coupling constant of these peaks was 7.7 and 7.5 Hz indicating that both anomeric carbons were in β configuration.

\textsuperscript{1}H NMR of the polar triethanolamine methanolysis products, blaberosides IIa and IIb without their β-hydroxylated fatty acids, showed a loss of the doublet centered on 4.6 ppm corresponding to the β-hydroxyl group, the observation which confirmed that the hydroxyl group was free in the parent compound. The 5.25-ppm vinyl proton peak was also reduced about in half, confirming the presence of an unsaturation on the fatty acid.

\textsuperscript{13}C NMR was useful in establishing the position of substitutions in blaberosides (Fig. 4). The shift of the sixth carbon peak of the reducing end sugar from 60.6 to 69.8 ppm confirmed a 6 linkage and the presence of a gentiobiose (Glul-Glu') sugar backbone, accounting for one of the substituted primary alcohols in the structure. It also further supported the glycosidic bond to the glycerol by eliminating any possible binding to sugar secondary alcohols, leaving only primary alcohol binding sites as alternatives to the anomeric position. The possibility of substitution as alternatives to the anomeric position. The possi-

### Table IV

| Blaberoside I | Chemical shift (ppm) | Coupling constants (Hz) | J(gem) |
|---------------|---------------------|------------------------|--------|
| Alkyl groups\textsuperscript{a} | 26\textsuperscript{a} | 0.8-0.9 | 2.6 |
| 11′-15′ | 1.2-1.5 |
| 14-18, 23′-28 | 1.2-1.5 |
| 38-46 | 1.2-1.5 |
| 10′ | 1.9-2.1 |
| 8′, 11′, 13 | 2.3-2.6 |
| 7, 8, 9 | 3.0-3.4 |
| 9′, 12 | 3.8 |
| 17′, 18′, 20, 21, 35, 36 | 5.3 |

### Table V

| Blaberoside II | Chemical shift (ppm) | Coupling constants (Hz) |
|---------------|---------------------|------------------------|
| Alkyl groups\textsuperscript{a} | 26′ | 13.7 | 0.8-0.9 |
| 27′ | 13.7 | 0.8-0.9 |
| 26′ | 21.9 | 1.2-1.5 |
| 26′ | 21.9 | 1.2-1.5 |
| 13 | 24.9 | 1.2-1.5 |
| 15 | 24.9 | 5.3 |
| 16′ | 25.5 | 1.9-2.1 |
| 19′ | 25.5 | 1.9-2.1 |
| 16 | 26.5 | 1.9-2.1 |
| 23 | 26.5 | 1.2-1.5 |
| 11′-12, 17′-22 | 28.5-28.6 | 1.2-1.5 |
| 24′ | 31.1 | 1.2-1.5 |
| 25 | 31.1 | 1.2-1.5 |
| 23′ | 36.7 | 1.2-1.5 |
| 24 | 42.4 | 1.2-1.5 |
| 10 | 61.0 | 1.9-2.1 |
| 9′ | 66.9 | 3.8 |
| 8′ | 70.0 | 3.0-3.4 |
| 8′ | 73.8 | 3.0-3.4 |
| 14 | 127.6 | 1.9-2.1 |
| 17′-18′ | 129.5 | 5.3 |
| 7′ | 176.1 |
| 11′-15′, 20′-22′ | 1.2-1.5 |
| 8′, 10′ | 2.3-2.6 |

### Sugar ring protons

| H | 102.3 | 4.3 |
| 2H | 73.4 | 2.9 |
| 3H | 76.3 | 2.9 |
| 4H | 70.0 | 3.1 |
| 5H | 75.9 | 3.5 |
| 6H | 68.0 | 4.3 |
| 6H | 10.5 | J\textsubscript{6,5} = 11.8; J\textsubscript{6,5} = 6.7 |

### Sugar ring protons

| H | 103.0 | 4.2 |
| 2H | 73.2 | 3.0 |
| 3H | 76.7 | 2.9 |
| 4H | 70.5 | 3.1 |
| 5H | 76.7 | 3.9 |
| 6H | 69.8 | 3.8 |
| 6H | 10.5; J\textsubscript{6,5} = 4.8 |
| 6H | 10.5; J\textsubscript{6,5} = 5.3 |

### Hydroxyl groups

| 2-OH (sugar) | 73.4 | 5.1 |
| 2′-OH (sugar) | 73.2 | 4.9 |
| 3-OH (sugar) | 76.3 | 5.0 |
| 3′-OH (sugar) | 76.7 | 4.8 |
| 4-OH (sugar) | 70.0 | 4.9 |
| 4′-OH (sugar) | 70.5 | 3.5 |
| 7-OH (glycerol) | 63.4 | 4.4 |
| 9″-OH (fatty acid) | 66.9 | 4.6 |

\textsuperscript{a} Multiplet, peaks are superimposed, cannot measure accurately.
\textsuperscript{b} Too close to HDO signal to measure accurately.
\textsuperscript{c} Too close to dimethyl sulfoxide-de signal to measure accurately.

### Two-dimensional \textsuperscript{1}H Nuclear Magnetic Resonance Spectroscopy

By establishing homonuclear shift correlated coupling relationships between protons on adjacent carbons, two-dimensional COSY provided adjunctive evidence for the sugar-
bound fatty acid structure in addition to confirming the proton assignments made in the one-dimensional spectra. The assignment of the upfield hydroxyls (4.6 ppm) of the β-hydroxyl fatty acid in blaberoside I (Fig. 5) was aided by the coupling to the 8′, 9′, 10′ and 11, 12, and 13 protons. The absence of a glycerol hydroxyl group at 4.4 ppm was also confirmed. In blaberoside II (spectrum not shown), the coupling between the farthest upfield hydroxyl group (4.4 ppm) and two proton peaks at 3.6 ppm and 3.4 ppm, confirmed the primary alcohol nature of that hydroxyl originally suggested by its triplet appearance in one-dimensional 'H NMR. The lack of any coupling of this hydroxyl proton resonance to the Cα–H or Cα′–H protons ruled out the possibility of a primary alcohol due to an open sixth carbon sugar hydroxyl. This strengthened the assignment of a 1–6 linkage to the disaccharide and attachment of the β-hydroxyl fatty acid to the 6′ position. The remaining possible primary alcohol in the proposed structure was the 7-carbon hydroxyl of the glycerol ether, dictating that sugar be bound to the β- and not the α-hydroxyl of the glycerol ether moiety.

NMR studies, therefore, supported the proposed structure of blaberoside II with the fatty acid esterified to the 6′ position of gentiobiose and a glycosidic bond to the secondary alcohol of the glycerol ether. The loss of the primary alcohol triplet in spectra of blaberoside I and the coupling patterns established the substitution of a second 3-hydroxyeicosenoyl group on the 1 position of the glycerol ether.

A two-dimensional NOESY spectrum of blaberoside I in dimethyl sulfoxide-d₆, was adequate to show coupling between 1 and 5′ and 6 and 6′ sugar hydrogens establishing their relatively near through space proximity. Although more quantitative nuclear Overhauser effect data are required to assign specific through space distances, the coupling of these nuclei in this experiment indicates proximities of approximately 5 Å. This would require the two sugar molecules to be folded over each other at the 1′–6 linkage.

**Location of Double Bonds**—The unsaturated 3-hydroxyeicosenoic acid methyl ester obtained from alkaline methanolyis of either blaberosides I or II was acetylated and then ozonized. The acetylation was necessary to avoid further degradation of the ozonolysis product. GC/MS analysis of the ozonolysis product, after purification by TLC, revealed only methyl esters of an 11 carbon 3-hydroxy dicarboxylic acid and a nine-carbon fatty acid from samples of blaberoside I or II. These results clearly indicated that the double bond in this fatty acid was located between the 11th and 12th carbons.
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Analysis of 2-alkenylbenzoxazolation products by GC/MS confirmed ozonolysis data.

Analysis of the double bond positions in the glycerol ether components was more difficult. On the basis of fatty acid methyl ester formed by ozonolysis and methanol-BF₃ treatment, as identified by GC/MS and quantitated by GC/flame ionization detection, greater than 70% of blaberoside Ib is unsaturated between carbons 35 and 36 of the alkyl chain in the glycerol ether, with minor peaks, indicating possible bond migration or minor homologs with the double bond between C₁₄-35 or C₁₅-36. Blaberoside Iib is predominately (greater than 70%) unsaturated between carbons 13 and 14 of the glycerol ether alkyl chain. Minor peaks (less than 10%) represent minor homologs unsaturated between C₁₅-16, C₁₇-18, or C₁₈-19, although bond migration in the unprotected glycerol ether during ozonolysis cannot be ruled out.

**DISCUSSION**

This report presents two major findings. First, the four blaberoside homologs characterized in this study represent the first identification and characterization of glycolipids from an insect. Second, these novel glycolipids are structurally unique from other known glycolipids of animal or plant origin.

Glycolipids are generally classified into two broad major divisions, the glycosphingolipids, containing long chain amino alcohols, and the glycolipids, which contain glycerol (15). The glycolipids, which would include the class blaberosides, are most abundant in plants and bacteria (3, 16), but also occur in a variety of animal tissues including the mammalian brain as minor components (17). Among various glycolipids, blaberosides represent a previously unreported class of glycolipids distinct from the glycosylmonoacylglycerides, glycosyldiacylglycerols, and glycomonoalkylmonoacylglycerides, which occur commonly in nature and have their sugar attached to the tertiary alcohol position of a glycerol monoester and an ester-linked fatty acid at the 2 position.

The unique structure of blaberosides suggests several characteristics of the molecules. These in turn may prove important in the eventual determination of their role in the biology of the cockroach. The strict conservation of the unusual 20:1 structure of the ß-hydroxy fatty acid implies very specific function of that moiety. The similarity in chain length to the alkyl chain of selachyl alcohol may also indicate similar functions, possibly anchoring the molecule in specific membrane environments. If blaberosides are integral membrane glycolipids, they could be anchored by any or all of their aliphatic chains.

The 1-6 linkage of the gentiobiose backbone is the most flexible of all of the disaccharide linkages (18). If blaberosides are anchored at both ends of the sugar backbone, the flexibility of the gentiobiose backbone would allow for the dynamic restructuring of the sugar by lateral adjustment of the positions of the lipophilic anchors providing the potential for "tuned reception" by a blaberoside receptor.

Membrane anchoring by the glycerol ether alone would
leaves the very flexible gentiobiose 1-6 linkage of blaberosides to serve as a bearing capable of considerable positional change for a β-hydroxyl fatty acid which could have important biological function. For example, the β-hydroxyl group and the first 20 carbons of the primary chain of the mycolic acids in lipid A from mycobacteria are thought to be responsible for lipid A's ability to initiate the complement cascade (18).

The composition of the glycerol ethers is, like the β-hydroxyl fatty acids, highly conserved with only two homologs present. These two glycerol ethers, chiniyl and selachyl alcohols, are the predominant monoalkylglycerols found in other animal tissues, including those of humans (20). The alkyl chains of the glycerol ethers could provide preferential stabilization in specific membrane loci with different membrane environments.

The diglucosyl double sugar backbone of the blaberosides is analogous to the digalactosyl backbone in the digalactosyldiacyl and monoalkylmonosaccahydric glycolipids of vertebrates and may represent an evolutionary divergence of hexose metabolism similar to that postulated in the cerebrosides (1). This may have further implications for the presence of sulfoglycolipid in insects if the lack of insect sulfatide metabolism similar to that postulated in the cerebrosides (1).

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Novel Glycolipids: Blaberosides

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Materials and Methods

Isolation and Purification

Isolation and purification of Blaberosides was carried out using a mixed-bed resin (Biotherm) according to the method of Stoneback et al. (1). The mixture was centrifuged at 10,000 g for 10 minutes, the supernatant was removed, and the mixture was extracted with methanol. The methanol mixture was then passed through a silica gel column and eluted with a gradient of methanol-water (1:1). The eluted fractions were analyzed by thin-layer chromatography to ensure purity.

Acylation to Protect Hydroxy Fatty Acid and Esters

Acylation of protected hydroxy fatty acid esters was performed by adding 0.1 mmol of [[H]AcCl] and heating at 60 °C for 2 hours. The mixture was then subjected to thin-layer chromatography to verify the purity of the acylated products.

Gas Chromatography

Gas chromatography was performed using a Varian 3800 Series Gas Chromatograph with a 5% phenyl-1,1,1-trimethylsiloxane column. The carrier gas was helium at a flow rate of 30 mL/min. The detector was a flame ionization detector (FID). The temperature program started at 50 °C and increased to 250 °C at a rate of 4 °C/min.

Results and Discussion

The resulting Blaberosides were acetylated and characterized by thin-layer chromatography and gas chromatography. The Blaberosides were found to be non-polar and were purified by column chromatography. The Blaberosides were then subjected to a variety of chemical and biological assays to determine their potential applications.

Conclusion

The Blaberosides were shown to be promising candidates for future research due to their unique chemical properties and potential biological activities. Further studies are needed to fully understand the structure and function of these novel glycolipids.