We found for the first time that the Zygomyces species showed resistance to Aureobasidin A, an antifungal agent. A novel family of neutral glycosphingolipids (GSLs) was found in these fungi and isolated from Mucor hiemalis, which is a typical Zygomyces species. Their structures were completely determined by compositional sugar, fatty acid, and sphingoid analyses, methylation analysis, matrix-assisted laser desorption ionization time-of-flight/mass spectrometry, and 1H NMR spectroscopy. They were as follows: Galβ1-6Galβ1-6Galβ1-1Cer (CDS), Galα1-6Galβ1-1Cer (CTS), Galβ1-6Galβ1-6Galβ1-1Cer (CET), and Galβ1-1Cer (CPS). The ceramide moieties of these GSLs consist of 24:0, 25:0, and 26:0 2-hydroxy acids as major fatty acids and 4-hydroxyoctadecasphinganine (phytosphingosine) as the sole sphingoid. However, the glycosylinositolphosphoceramide families that are the major GSLs components in fungi were not detected in Zygomyces at all. This seems to be the reason that Aureobasidin A is not effective for Zygomyces as an antifungal agent. Our results indicate that the biosynthetic pathway for GSLs in Zygomyces is significantly different from those in other fungi and suggest that any inhibitor of this pathway may be effective for mucormycosis, which is a serious pathogenic disease for humans.
and that they contain a novel family of galactose-containing GSLs but not phosphinositol-containing sphingolipids.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Conditions**—Fungal strains **Aspergillus oryzae** (NBRC4075), **Aspergillus niger** (NBRC1125), **Mucor fragilis** (NBRC-6449), **Mucor racemosus** (NBRC581), **Rhizopus oryzae** (NBRC9364), **Rhizopus microsporus** (NBRC30499), **Rhizopus stolonifer** (NBRC-32998), **Rhizomucor pusillus** (NBRC9745), **Penicillium oxalicum** (NBRC5748), and **Absidia corymbifera** (NBRC32279) were obtained from the National Institute of Technology and Evaluation Biological Resources Center (NITE BRC) and identified by our laboratory (11, 12). These strains were cultivated in YPG liquid medium (0.5% yeast extract, 0.5% peptone, 0.5% NaCl, 1% glucose, pH 6.5) at 28°C for 48 h in a 2-liter shaking flask containing 700 ml of the medium on a reciprocal shaker at 120 rpm (12). For assaying the growth inhibition by Aureobasidin A (AbA), fungal strains were grown on YPD plates containing 0.1–10 μg/ml AbA for 48 h at 28°C (13).

**Materials**—**QAE-Sephadex A-25**, **DEAE Sephadex A-25**, and d-[U-14C]glucose were purchased from Amersham Biosciences. Iatrobeds 6RS-8060 was obtained from Iatron Laboratories Inc. Silica gel 60 precoated plates were from Merck, magnesium silicate (Florisil) was from Nakai Tesque, green coffee bean α-galactosidase was from Sigma, and jack bean β-galactosidase was from Seikagaku Co. Aureobasidin A was obtained from Takara Bio Inc. All other reagents used were of best grade available commercially.

**Extraction and Purification of Sphingolipids**—Sphingolipids were prepared from mycelia by consecutive extractions, as described elsewhere (14). Lipid extracts were saponified with 0.5 M KOH in methanol-water (95.5, v/v) at 37°C for 6 h. The hydrolysate was acidified to pH 1.0 with concentrated HCl and then dialyzed against tap water for 2 days followed by concentration and precipitation with acetone. The sphingolipids were fractionated on a QAE-Sephadex A-25 column (20 × 300 mm, OH− form). The neutral fraction was further purified by silica gel chromatography and thin-layer chromatography (TLC), with a linear gradient elution system of chloroform-methanol-water (400 ml of 90:10:0.5 by volume to 400 ml of 40:60:10 by volume). The polar fraction was then applied to a column of DEAE-Sephadex A-25 (20 × 200 mm, acetate form), as described elsewhere (14).

**Carbohydrate and Fatty Acid Composition Analyses**—For determination of the compositions of the fatty acids and sugars in GSLs, 100–200 μg of GSLs were methylated in thick glass test tubes with 200 μl of freshly prepared 1 M anhydrous methanolic HCl using a microwave oven (14, 15). After methanolation, the fatty acid methyl esters were extracted three times with 400 μl of n-hexane and then analyzed by capillary gas-liquid chromatography (GLC/MS) (14, 15). The remaining methanolic phase was evaporated to dryness for deacidification under a nitrogen stream. The residue containing methylglycosides was trimethylsilylated and then analyzed by GLC. Sphingolipids prepared from GSLs by methanolation with 1 M aqueous methanolic HCl at 70°C for 18 h were converted to their trimethylsilyl (TMS) derivatives and then analyzed by GLC/MS (14, 15).

**Methylation for Sugar Linkage Analysis**—For determination of the sugar linkages of oligosaccharides in GSLs, 300 μg of a purified GSL was partially methylated with NaOH and CH3I in Me2SO (16). The permethylated GSL was acetylated and hydrolyzed with 300 μl of a mixture of HCl-water-acetic acid (0.5:1.5:8 by volume) by exposure to 70°C for 1 h. After hydrolysis, the mixture was acidified with concentrated HCl and then dialyzed against tap water for 2 days. The dialyzed neutral fraction was further purified by silica gel chromatography and TLC. The partially methylated alditol acetates were analyzed with GLC and GLC/MS equipped with a Shimadzu GC-14B gas chromatograph.

**Matrix-assisted Laser-desorption Ionization Time-of-Flight MS (MALDI-TOF-MS)**—MALDI-TOF-MS analyses of the purified neutral GSLs were performed with a Shimadzu/KRATOS COMPACT MALDI I mass spectrometer equipped with a Workstation SPARC station, operating in the positive-ion linear mode (14). Ions were formed by a pulsed ultraviolet laser beam (N2 laser, 337 nm; 3-ns wide pulses). The matrix used was 7-amino-4-methylcoumarin (Sigma) (17).

**31P NMR Spectroscopy**—NMR spectra of the purified neutral GSLs were obtained with a JEOL A-500 500 MHz 1H NMR spectrometer at 60°C as the operating temperature. Each purified GSL was dissolved in 0.6 ml of dimethyl sulfoxide-d6 containing 2% D2O with the chemical shift being referenced to the solvent signals (δH = 2.49 ppm) in Me2SO-d6 as the internal standard.

**Matrix-assisted Laser-desorption Ionization Time-of-Flight MS (MALDI-TOF-MS)**—MALDI-TOF-MS analyses of the purified neutral GSLs were performed with a Shimadzu/KRATOS COMPACT MALDI I mass spectrometer equipped with a Workstation SPARC station, operating in the positive-ion linear mode (14). Ions were formed by a pulsed ultraviolet laser beam (N2 laser, 337 nm; 3-ns wide pulses). The matrix used was 7-amino-4-methylcoumarin (Sigma) (17).
**Newly Discovered Glycolipids in Zygomycetes**

**RESULTS**

**Growth of Fungi Resistant to AbA**—During studies on GSLs of fungi, we found that all *Zygomycetes* species examined were resistant to AbA and grew in the medium containing AbA (0.1–10 μg/ml). As shown in Fig. 1A, AbA strongly inhibits the growth of filamentous fungi such as *A. oryzae*, *P. oxalicum*, and *Acremonium* sp., previously reported for the yeast *Saccharomyces cerevisiae* (W303-1A) and other fungi (7–10, 13). However, growth inhibition by AbA was not observed for any *Zygomycetes* species such as *M. hiemalis*, *R. microsporus*, *R. pusillus*, and *A. corymbifera* (Fig. 1B). These results suggested that the GSLs in *Zygomycetes* species were very different from those in other fungi.

**TLC analyses of glycolipids from various fungi.** TLC was performed with chloroform-methanol-water (60:35:8, by volume, neutral solvent), with visualization with orcinol-H₂SO₄ reagent. A, TLC of polar GSLs from various fungi. B, TLC of NGLs from various fungi. MIPC was purified from *S. cerevisiae* (W303-1A). Lanes **An**: MIPC, **Po**: A. Po, **Ac**: A. Ac, **Mh**: M. Hi, **Mr**: M. Mr, **Rs**: R. Rs, and **Aco**: A. Aco are polar GSLs isolated from *A. oryzae* (NBRC31125), *P. oxalicum* (NBRC5748), *A. oryzae* (NBRC4075), *M. hiemalis* number 314, *M. fragilis* (NBRC6449), *M. racemosus* (NBRC5748), *R. stolonifer* (NBRC32279), and *A. corymbifera* (NBRC9364), respectively. C, TLC of purified NGLs from *M. hiemalis* number 314. Lane **Mt**: total NGL fraction of *M. hiemalis* number 314; lanes **1–5**: purified GSLs isolated from NGLs: CMS, CDS, CTS, CTeS, and CPS, respectively.

**Ceramide Compositions of NGLs of *M. hiemalis***—The aliphatic components of the NGLs of *M. hiemalis* were determined by GLC and GLC/MS and summarized in Table I. The fatty acid composition of CMS comprised of C14h:0 (10.5%) and C16h:0 (89.5%) fatty acids, but CDS–CPS were composed of long chain fatty acids such as C24h:0, C25h:0, and C26h:0. The sphingoid components of CMS were 9-methyl-octadeca-4,8-sphingadienine (d19:2), eicosasphingenine (d20:1), and the unknown (18), whereas those of CDS–CPS were entirely 4-hydroxyoctadecasphinganine (phytosphingosine, t18:0). Because the ceramide compositions of CDS–CPS were entirely the same, these NGLs were supposed to be a series of intermediates of GSL biosynthesis. Such phytoceramides consisting of a 2-hydroxy fatty acid and phytosphingosine are generally found in fungal cells as the major aliphatic component of glycosylinositolphosphoceramides (1–3). Surprisingly, they were not found in *Zygomycetes* species (Fig. 2A), and only neutral GSL (NGLs) being present. A TLC of the NGLs of all *Zygomycetes* species gave the same pattern but they were apparently different from those of other fungi (Fig. 2B). The NGLs of all *Zygomycetes* species comprised five components, which were identified to be ceramide mono-, di-, tri-, tetra-, and pentasaccharides (tentatively named as CMS, CDS, CTS, CTeS, and CPS, respectively). On the other hand, for the other fungi, only ceramide monosaccharide was found in the neutral fraction. Then these NSLs of *M. hiemalis* number 314 were further purified by silica gel column chromatography and confirmed by TLC, as shown in Fig. 2C. The yields were CMS, 20.8 mg; CDS, 6.3 mg; CTS, 16.1 mg; CTeS, 14.0 mg; and CPS, 2.3 mg, respectively. The purified GSLs were then analyzed by MALDI-TOF/MS and quantified by carbohydrate constituent analysis.

**Structural Characterization of Oligosaccharides of NGLs**—The sugar constituents of the purified GSLs were identified by GLC/MS, which revealed the presence of Glc in CMS and only Gal in all the other NGLs. MALDI-TOF/MS spectra of the NGLs showed the presence of pseudomolecular ions [M + H]+ in

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**Table I**

| Sugar | CMS | CDS | CTS | CTeS | CPS |
|-------|-----|-----|-----|------|-----|
| Glc   | +   | +   | +   | +    |     |
| Gal   |     | +   |     | +    | +   |

Fatty acid (%)

| Af | CMS | CDS | CTS | CTeS | CPS |
|----|-----|-----|-----|------|-----|
| h14:0 | 10.5 |     |     | +    |     |
| h16:0 | 89.5 |     |     |     |     |
| h24:0 | 65.2 | 63.2 | 63.8 | 67.9 |     |
| h25:0 | 10.8 | 17.3 | 17.2 | 15.4 |     |
| h26:0 | 24.0 | 19.5 | 19.0 | 16.7 |     |

Sphingoid (%)

| Af | CMS | CDS | CTS | CTeS | CPS |
|----|-----|-----|-----|------|-----|
| d19:2 | 53.6 |     |     |     |     |
| d20:1 | 27.5 |     |     |     |     |
| Unknown | 18.9 |     |     |     |     |
| t18:0 |     | >99 | >99 | >99 | >99 |

* h, 2-hydroxy fatty acid; d, dihydroxy; t, trihydroxy sphingoid.

Therefore, we analyzed the GSLs in *Zygomycetes* species to investigate the AbA resistance mechanism.

**GSLs of Various Fungi Zygomycetes Species**—GSLs of fungi were separated into neutral, acidic, and zwitterionic fractions by ion-exchange column chromatography based on their polarities. Each fraction was analyzed by TLC with a chloroform-methanol-water system. The GSLs of all *Zygomycetes* species examined were recovered in the neutral fraction but not in the acidic and zwitterionic fractions. In general, acidic GSLs are found in all fungal cells and have been reported to be inositol-phosphate-containing sphingolipids such as glycosylinositolphosphoceramides (1–3). Surprisingly, they were not found in *Zygomycetes* species (Fig. 2A), and only neutral GSL (NGLs) being present. A TLC of the NGLs of all *Zygomycetes* species gave the same pattern but they were apparently different from those of other fungi (Fig. 2B). The NGLs of all *Zygomycetes* species comprised five components, which were identified to be ceramide mono-, di-, tri-, tetra-, and pentasaccharides (tentatively named as CMS, CDS, CTS, CTeS, and CPS, respectively). On the other hand, for the other fungi, only ceramide monosaccharide was found in the neutral fraction. Then these NSLs of *M. hiemalis* number 314 were further purified by silica gel column chromatography and confirmed by TLC, as shown in Fig. 2C. The yields were CMS, 20.8 mg; CDS, 6.3 mg; CTS, 16.1 mg; CTeS, 14.0 mg; and CPS, 2.3 mg, respectively. The purified GSLs were then analyzed by MALDI-TOF/MS and quantified by carbohydrate constituent analysis.

**Structural Characterization of Oligosaccharides of NGLs**—The sugar constituents of the purified GSLs were identified by GLC/MS, which revealed the presence of Glc in CMS and only Gal in all the other NGLs. MALDI-TOF/MS spectra of the NGLs showed the presence of pseudomolecular ions [M + H]+ in...
the positive ion mode, as shown in Fig. 3. They had different pseudomolecular ions because of different ceramide species, which were in agreement with the mass values calculated from the proposed structures; the major \([M + H]^+\) ions of CMS at \(m/z\) 698.7 and 726.3 (Fig. 3A) coincided with the mass value of 1 mol each of Glc, fatty acid (2-hydroxy C14:0 or C16:0), and sphingoid (d19:2 or d20:1) (see Table I), those of CDS at \(m/z\) 1007.1 and 1035.1 (Fig. 3B) coincided with the mass value of 2 mol of Gal and phytoceramide (t18:0, and 2-hydroxy C24:0 and C26:0), those of CTS at \(m/z\) 1169.5 and 1197.4 (Fig. 3C) coincided with the mass value of 3 mol of Gal and phytoceramide (t18:0, and 2-hydroxy C24:0 and C26:0), those of CTeS at \(m/z\) 1332.9 and 1361.0 (Fig. 3D) coincided with the mass value of 4 mol of Gal and phytoceramide (t18:0, and 2-hydroxy C24:0 and C26:0), and those of CPS at \(m/z\) 1493.5 and 1522.0 (Fig. 3E) coincided with the mass value of 5 mol of Gal and phytoceramide (t18:0, and 2-hydroxy C24:0 and C26:0), respectively.

Subsequently, to determine the sugar linkages, the partially methylated alditol acetate derivatives of NGLs were analyzed by GLC and GLC/MS, and the results are shown in Fig. 4. The methylation analysis demonstrated the presence of one substituted Glc (1,5-di-(O-acetyl)-2,3,4,6-tetra-(O-methyl)glucitol, 1Glc) for CMS; on the other hand, one substituted Gal (1,5-di-(O-acetyl)-2,3,4,6-tetra-(O-methyl)galactitol, 1Gal) and 1,6 substituted Gal (1,5,6-tri-(O-acetyl)-2,3,4,6-tetra-(O-methyl)galactitol, 1,6Gal) were detected from CDS–CPS. The peak area of 1,6Gal increased depending on the number of Gal residues; the ratio of 1,6Gal to each 1Gal (\(n = 1.00\)) in NGLs were 0.92 for

- A, CMS; B, CDS; C, CTS; D, CTeS; and E, CPS.

Fig. 3. MALDI-TOF/MS spectra of purified GSLs. Analyses were performed in the positive-ion linear mode. Pseudomolecular ions are given in average masses. The details are given in the text. The open hexagon is glucose and the gray hexagons are galactose. A, CMS; B, CDS; C, CTS; D, CTeS; and E, CPS.
demonstrating 2 mol of \(\text{Gal}^1\)-methylglucitol; 1Gal, 1,5-di-(O-acetyl)-2,3,4,6-tetra-(O-methyl)galactitol; and 1,6Gal, 1,5,6-tri-(O-acetyl)-2,3,4,tri-(O-methyl)galactitol.

CDS, 1.98 for CTS, 3.15 for CTeS, and 4.31 for CPS, respectively. This revealed that the Gal residues of CDS–CPS were linked to the C-6 position of their glycans.

Analysis of Sugar Components of NGLs—For determination of the anomeric configurations of the sugar residues, CDS–CPS were subjected to proton NMR spectroscopy (Fig. 5). In the anomeric region of the spectrum of each glycolipid, the following anomeric proton resonances were observed: at 4.12 \((J = 7.3 \text{ Hz})\) and 4.15 ppm \((J = 7.3 \text{ Hz})\) demonstrating 2 mol of \(\beta\)-galactose for CDS (Fig. 5A), at 4.11 \((J = 7.3 \text{ Hz})\), 4.16 \((J = 7.3 \text{ Hz})\), and 4.69 ppm \((J = 3.1 \text{ Hz})\) demonstrating 2 mol of \(\beta\)-galactose and 1 mol of \(\alpha\)-galactose for CTS (Fig. 5B), at 4.12 \((J = 7.3 \text{ Hz})\), 4.17 \((J = 7.3 \text{ Hz})\), 4.70 \((J = 3.0 \text{ Hz})\), and 4.72 ppm \((J = 3.0 \text{ Hz})\) demonstrating 2 mol of \(\beta\)-galactose and 2 mol of \(\alpha\)-galactose for CTeS (Fig. 5C), and at 4.12 \((J = 7.3 \text{ Hz})\), 4.18 \((J = 7.3 \text{ Hz})\), 4.71 \((J = 3.1 \text{ Hz}, 2H)\), and 4.72 ppm \((J = 3.7 \text{ Hz})\) demonstrating 2 mol of \(\beta\)-galactose and 3 mol of \(\alpha\)-galactose for CPS (Fig. 5D), respectively. Enzymatic hydrolysis of the above NGLs with \(\alpha\)- and \(\beta\)-galactosidase also revealed the presence of \(\alpha\)- and \(\beta\)-galactose residues. As a result, CDS was degraded to ceramide monosaccharide (galactosylceramide (GalCer)) by \(\beta\)-galactosidase, and CTS, CTeS, and CPS were also hydrolyzed to GalCer through the sequential actions of \(\alpha\)- and \(\beta\)-galactosidase (data not shown).

From these findings, the following structures for NGLs were suggested, Glc\(\beta\)-1-Cer for CMS, Gal\(\beta\)-1-6Gal\(\beta\)-1-Cer for CDS, Gal\(\beta\)-1-6Gal\(\beta\)-1-6Gal\(\beta\)-1-Cer for CTS, Gal\(\alpha\)-1-6Gal\(\beta\)-1-6Gal\(\beta\)-1-1Cer for CTeS, and Gal\(\alpha\)-1-6Gal\(\alpha\)-1-6Gal\(\alpha\)-1-6Gal\(\beta\)-1-1Cer for CPS, respectively. This is the first finding of novel glycan chains with Gal\(\beta\)-1-6Gal and/or Gal\(\alpha\)-1-6Gal in glycolipids from fungi.

Analysis of GSL Synthesis—To investigate the biosynthetic pathway for GSLs, fungal cells were incubated with \(^{14}\text{C\text{glucose}}, \text{and then lipids extracted from mycelia were analyzed by TLC (Fig. 6). Our preliminary results showed that CMS–CPS were found to be labeled by }^{14}\text{C on incubation within }1 \text{ h (Fig. 6A). However, GalCer was not detected on TLC even after incubation for }12 \text{ h (Fig. 6B). We supposed that the metabolic process yielding a digalactosylceramide from a phytoceramide might be rapid.}

DISCUSSION

Sphingolipids have been established to be essential components of eukaryotic cells, where they are predominantly found on the plasma membrane. Inhibitors of specific steps of sphingolipid biosynthesis have proven useful for understanding sphingolipid functions, and some of them have been used as fungicidal agents. One of the more useful agents for blocking sphingolipid synthesis in fungi is AbA, which inhibits IPC synthase in fungi cells. As all fungi and plants seem to synthesize IPC as an intermediate of the biosynthetic pathway for GSLs (Scheme 1), AbA exhibits strong fungicidal activity on the plasma membrane. Inhibitors of specific steps of sphingolipid functions, and some of them have been used as fungicidal agents. One of the more useful agents for blocking sphingolipid synthesis in fungi is AbA, which inhibits IPC synthase in fungi cells. As all fungi and plants seem to synthesize IPC as an intermediate of the biosynthetic pathway for GSLs (Scheme 1), AbA exhibits strong fungicidal activity on the plasma membrane.
against many pathogenic fungi. However, we found that this agent was not entirely effective for the *Zygomycetes* species examined. We also found that *Zygomycetes* species did not have IPC but did have a novel family of neutral GSLs that are not found in other fungi. These GSLs isolated from *M. hiemalis*, which is a typical *Zygomycetes* species, were determined as $\text{Gal} / \text{H}9252 \text{1-6Gal} / \text{H}9252 \text{1-1Cer}$ (CDS), $\text{Gal} / \text{H}9251 \text{1-6Gal} / \text{H}9252 \text{1-6Gal} / \text{H}9252 \text{1-1Cer}$ (CTS), $\text{Gal} / \text{H}9251 \text{1-6Gal} / \text{H}9251 \text{1-6Gal} / \text{H}9252 \text{1-6Gal} / \text{H}9252 \text{1-1Cer}$ (CTeS), and $\text{Gal} / \text{H}9251 \text{1-6Gal}-/\text{H}9251 \text{1-6Gal}/\text{H}9251 \text{1-6Gal}/\text{H}9252 \text{1-6Gal}/\text{H}9252 \text{1-1Cer}$ (CPS). Their aliphatic components were the same phytoceramides consisting of phytosphingosine and C24–C26 2-hydroxy fatty acids, which were bound through amide linkages. These ceramide moieties substantially differed from that of glucosylceramide (CMS) (Table I). The only glucosylceramide detected was ceramide monosaccharide, i.e. we did not identify a galactosylceramide with phytoceramide, which is supposed to be the precursor of a series of galactose-containing glycosphingolipids (CMS–CPS). It seemed that the enzymatic reaction to form CDS from galactosylceramide might proceed rapidly. In fact, there are some preliminary data about the existence of a very little amount of GalCer, which was found by means of sensible method using borated thin layer plate (data not shown). However, we could not know whether this GalCer is an intermediate of metabolic process of these novel NGLs or a degraded product from digalactosylceramide produced. Moreover, it could be speculated that digalactosylceramide is directly formed from phytoceramide by the addition of disaccharide from nucleotide diphosphate sugars. Such investigation is carrying out at present.

Galactooligosaccharides with similar structures in novel GSLs have been reported for the leech, *Hirudo nipponia* (i.e. $\text{Gal} / \text{H}9251 \text{1-6Gal} / \text{H}9251 \text{1-6Gal} / \text{H}9252 \text{1-1Cer}$) (19), the earthworm, *Pheretima* sp. (i.e. $\text{Gal} / \text{H}9252 \text{1-6Gal} / \text{H}9252 \text{1-6Gal} / \text{H}9252 \text{1-6Gal} / \text{H}9252 \text{1-1Cer}$) (20), and a parasitic cestode, *Echinococcus multilocularis* (i.e. $\text{Fuc} / \text{H}9251 \text{1-3Gal} / \text{H}9252 \text{1-6Gal-Cer}$) (21). But this is the first finding of GSLs with the $\text{Gal} / \text{H}9251 \text{1-6Gal} / \text{H}9252 \text{1-6Gal}$ sequence in any organism. Furthermore, there have been reports that humans and closely related mammals possess natural anti-α-galactosyl (Galα1-3Gal) antibodies (22), which also strongly react with the epitopes of melibiose (Galα1-6Glc) and Galα1-6Gal (23). It has also been reported that sera of alveolar hydatid disease patients recognized the epitope of Galβ1-6Gal residues in GSLs of *E. multilocularis* (21), and the carbohydrate residues of its GSLs with Galβ1-6Gal sequences were inhibitors of human peripheral blood mononuclear cell proliferation (24). These findings suggest that the GSLs of *Zygomycetes* also might be immunogenic in humans.

The biosynthesis of GSLs in *Zygomycetes* species seemed to be different from that described for other fungal species. In most fungi, sphingolipid synthesis begins in the endoplasmic reticulum, where phytoceramide is converted to IPC before transport to the Golgi apparatus for further glycosylation (1–3). Our results indicated that two independent ceramide groups...
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existing in the *Zygomycetes* species, and the fungal cells synthesized neutral GSLs of both glucosylceramide and galactose-containing glycosphingolipids from different ceramide pools, because the ceramide structures of the two types of GSLs were significantly different. Although glycosylinositolphosphoceramides have been detected in many fungi as important constituents of cells, we could not obtain evidence of their presence in *Zygomycetes* species, nor could we detect inositolphosphate-containing sphingolipids. Surprisingly, *Zygomycetes* species showed strong resistance to AbA, and the above fact seems to be the reason why *Zygomycetes* species are resistant to AbA.

The roles of fungi in infections have been considered to be of lesser important, because only 5% of fungi have been found to be infectious. It has already been reported that aspergillosis (55%) is the most common invasive fungal disease, followed by mucormycosis (zygomyces) (15%), fusariosis (15%), and acremoniosis (10%) (25). The pathogenic fungi responsible for these diseases were not considered previously to be important human pathogens but are widely present in soil, plants, and elsewhere in the environment. *Aspergillus* spp. and *Mucor* spp. have been shown recently to be human pathogens (26). In particular, *Mucor* spp. cause many diseases, and other members of the Mucorales family act as opportunistic human pathogens (27). Mucorales infections are observed in a variety of disease states that cause immunosuppression associated with leukemia (28), aplastic anemia (29), organ or bone marrow transplantation (28), renal disease (30), and asthma (31). Therefore, new effective drugs for mucormycosis are required immediately. In this point, an inhibitor of the synthesis of galactose-containing GSLs might be useful. Although the functional roles of these GSLs have not been elucidated, our finding may facilitate the development of new antifungal agents for Mucorales.

REFERENCES

1. Dickson, R. C. (1998) *Annu. Rev. Biochem.* 67, 27–48
2. Dickson, R. C., and Lester, R. L. (2002) *Biochim. Biophys. Acta* 1583, 13–25
3. Lester, R. L., and Dickson R. C. (1993) *Adv. Lipid Res.* 26, 253–274
4. Fischl, A. S., Liu, Y., Browdy, A., and Cremesti, A. E. (2000) *Methods Enzymol.* 311, 123–130
5. Wang, E., Norred, W. P., Bacon, C. W., Riley, R. T., and Merrill, A. H., Jr. (1991) *J. Biol. Chem.* 266, 14486–14490
6. Nagiec, M. M., Nagiec, E. E., Baltisberger, J. A., Wells, G. B., Lester, R. L., and Dickson, R. C. (1997) *J. Biol. Chem.* 272, 9809–9817
7. Zhong, W., Jeffries, M. W., and Georgopapadakou, N. H. (2000) *Antimicrob. Agents Chemother.* 44, 651–653
8. Heidler, S. A., and Radding, J. A. (2000) *Biochim. Biophys. Acta* 1500, 147–152
9. Takesako, K., Kuroda, H., Inoue, T., Haruna, F., Yoshikawa, Y., Kato, I., Uchida, K., Hirata, T., and Yamaguchi, H. (1993) *J. Antibiot. (Tokyo)* 46, 1414–1420
10. Ikai, K., Takesako, K., Shiono, K., Moriyachi, M., Umeda, Y., Yamamoto, J., Kato, I., and Naganawa, H. (1991) *J. Antibiot. (Tokyo)* 44, 925–933
11. Kadowaki, S., Yamamoto, K., Fujisaki, M., Izumi, K., Tochikura, T., and Yokoyama, T. (1990) *Agric. Biol. Chem.* 54, 97–106
12. Kadowaki, S., Ueda, T., Yamamoto, K., Kumagai, H., and Tochikura, T. (1989) *Agric. Biol. Chem.* 53, 111–120
13. Cheng, J., Park, T. S., Fischl, A. S., and Ye, X. S. (2001) *Mol. Cell. Biol.* 21, 6188–6209
14. Aoki, K., Uchiyama, R., Itonori, S., Sugita, M., Che, F. S., Isogai, A., Hada, N., Hada, J., Takeda, T., Kumagai, H., and Yamamoto, K. (2004) *Biochem. J.* 378, 461–472
15. Itonori, S., Takahashi, M., Kitamura, T., Aoki, K., Dulaney, J. T., and Sugita, M. (2004) *J. Lipid Res.* 45, 574–581
16. Ciucanu, I., and Kerek, F. (1984) *Carbohydr. Res.* 137, 209–217
17. Dai, Y., Whittal, R. M., Bridges, C. A., Isogai, Y., Hindegaul, O., and Li, L. (1997) *Carbohydr. Res.* 304, 1–9
18. Fujino, Y., and Ohnishi, M. (1976) *Biochim. Biophys. Acta* 486, 161–171
19. Nozita, N., Tanaka, R., Miyahara, K., and Sukamoto, T. (1996) *Chem. Pharm. Bull. (Tokyo)* 44, 895–899
20. Sugita, M., Ohta, S., Morikawa, A., Dulaney, J. T., Ichikawa, A., Kushida, K., Inagaki, F., Suzuki, M., and Suzuki, A. (1997) *Jpn. Oil Chem. Soc.* 46, 755–766
21. Persat, F., Bouhours, J. F., Mojon, M., and Petavy, A. F. (1992) *J. Biol. Chem.* 267, 8764–8769
22. Rother, R. P., and Squinto, S. P. (1996) *J. Biol. Chem.* 271, 3682–3687
23. Yamazaki, K. I., Robert, P., Debra, D. T., and Ian, T. (2000) *Clin. Diag. Lab. Immunol.* 7, 480–486
24. Florence, P., Claude, V., Daniel, S., and Madeleine, M. (1996) *Infect. Immun.* 64, 3682–3687
25. Kremers, V., Jr., Kunova, E., Jesenak, Z., Trupl, J., Spanik, S., Marschalik, J., Studena, M., and Kukucova, K. (1996) *Support Care Cancer* 4, 39–45
26. Ribes, J. A., Vanover-Sams, C. L., and Baker, D. J. (2000) *Clin. Microbiol. Rev.* 13, 286–301
27. Tobon, A. M., Arango, M., Fernandez, D., and Restrepo, A. (2003) *Clin. Infect. Dis.* 36, 1488–1491
28. Oliver, M. R., Van Voorhis, W. C., Beech, M., Mattson, D., and Bowden, R. A. (1996) *Clin. Infect. Dis.* 22, 521–524
29. Weitzman, I., Della-Latta, P., Housey, G., and Rebbata, G. (1993) *J. Clin. Microbiol.* 31, 2523–2525
30. Caraveo, J., Trowbridge, A. A., Amaral, B. W., Green, J. B., III, Cain, P. T., and Hurley, D. L. (1997) *Am. J. Med.* 62, 404–408
31. Zabel, D. D. (1997) *Dent. Med. J.* 69, 459–463
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