were polymerized by irradiation at 254 nm. Transmission electron microscopy of the liposomes revealed ellipsoid structures averaging on the order of 40 nm in length and 15 nm in width. The indicated percentage of sialoside presented at the liposome surface (0%, 1%, 5%, 10%, 30%, or 60%) represents the mole percentage of lipid monomer **3** used in the liposome preparation.

Liposome preparations I–VI were tested for binding to influenza virus using a standard hemagglutination inhibition (HAI) assay (Table I).[4] To achieve 50% inhibition of viral binding, the α-*O*-methyl glycoside of sialic acid (compound **1**) required a concentration of 2 mM[3] and compound **2b** required a concentration of 10 mM.[8] In sharp contrast, liposome preparations III and IV (5% and 10% of sialoside **3**) required as little as 5.7×10^{-7} and 3.3×10^{-7} M concentrations of the sialoside to achieve complete inhibition of agglutination. This represents an increase in potency of approximately 30 000 times over the corresponding monovalent sialic acid derivatives, making it one of the most potent synthetic inhibitors of hemagglutination reported to date. Interestingly, as the percentage of sialoside **3** is increased from 10% to 30% and 60%, inhibition of hemagglutination is no longer observed at these higher concentrations (compare entry 4 to entries 5 and 6). Liposome preparation II (entry 2), which contains only 1% of sialoside **3**, also showed no inhibition of hemagglutination. A similar trend in which high and low percentages of sialoside diminish the capacity of polyvalent materials to inhibit hemagglutination has been observed in sialoside polymers.[6,13]

We next tested the capability of liposome preparations I–VI to prevent infectivity in cell culture using Madin–Darby canine kidney (MDCK) cells in a standard plaque reduction assay (Table I).[14] To our surprise, liposome preparation II (1% sialoside **3**, entry 2) strongly inhibited viral infectivity (96% inhibition at a concentration of only 3 μM sialoside lipid) even though this liposome preparation did not inhibit hemagglutination. In contrast, liposome preparation IV (10% sialoside **3**, entry 4), which showed potent inhibition of hemagglutination, demonstrated only a modest capability to inhibit infectivity (46% inhibition at a concentration of 30 μM sialoside **3**). Liposome preparation III (5% sialoside **3**, entry 3), which has plaque reduction activity equivalent to liposome preparation II, was also a potent inhibitor of hemagglutination. Liposome preparations I (0% sialoside **3**), V (30% sialoside **3**), and VI (60% sialoside **3**) showed no inhibition of plaque formation and no inhibition of agglutination. Our data indicate that synthetic sialosides which are poor inhibitors of hemagglutination (e.g., liposome preparation II, entry 2) can stop infectivity, while strong inhibitors of hemagglutination (e.g., liposome preparation IV, entry 4) may not effectively stop infectivity. The relationship between inhibiting viral binding to the erythrocyte cell surface and the capacity to prevent infectivity is currently under further investigation.

In summary, we have synthesized a polymerizable sialoside lipid **3** and formed mixed liposome preparations that are potent inhibitors of influenza virus in vitro infectivity. We have also shown that the capacity of a sialoside to inhibit hemagglutination does not necessarily reflect its capacity to inhibit infectivity. Polymerized liposome preparations should serve as important models for understanding pathogen–cell interactions and for developing therapeutic agents based on multivalent carbohydrate structures.

Acknowledgment. This research was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Materials Sciences, by the Division of Energy Biosciences of the U.S. Department of Energy (DE-AC03-76SF0098), and by the National Institutes of Health (Award No. R29 GM43037-02). M.B. thanks the American Cancer Society for a Junior Faculty Award and Eli Lilly for a Young Investigator Grant. W.S. thanks the NIH for a predoctoral training grant.

Note Added in Proof. During the preparation of this manuscript, results similar to those reported here were disclosed by Whitesides and co-workers.[13]

(14) Tobita, K.; Sugiura, A.; Enomoto, C.; Furuyama, M. *Med. Microbiol. Immunol.* **1975**, *162*, 9–14.

Supplementary Material Available: Listings of experimental data for the synthesis of compounds **3** and **4**, procedure for liposome formation, procedure for HAI assay, and procedure for plaque reduction assay (6 pages). Ordering information is given on any current masthead page.

Polycavernoside A: A Novel Glycosidic Macrolide from the Red Alga *Polycavernosa tsudai* (*Gracilaria edulis*)

Mari Yotsu-Yamashita,[†] Robert L. Haddock,[‡] and Takeshi Yasumoto*,[†]

Faculty of Agriculture, Tohoku University
1-1 Tsutsumidori-Amamiyamachi
Aoba-ku, Sendai 981, Japan
Health and Social Services, Government of Guam
P.O. Box 2816 Agana, Guam 96910

Received October 20, 1992

Human intoxication resulting from ingestion of the red alga *Polycavernosa tsudai* (formerly *Gracilaria edulis*) occurred in Guam in late April, 1991.[1] Thirteen people became ill, three of whom died. As the alga had been eaten widely with no previous record of potential risk, identification of the toxin was imperative. In this communication, we report the isolation of two toxins, polycavernoside A (**1**) and B (**2**), and the planar structure of **1**, which is a novel macrolide disaccharide.

P. tsudai (2.6 kg) was collected on June 4, 1991, at Tanguisson Beach, Guam, where the causative alga had previously been collected. Toxins were extracted from the alga with acetone, freed of polar contaminants by partition between water and CH_2Cl_2, and purified by column chromatography,[2] guided by mouse bioassays. Both **1** (400 μg, recovery 14%) and **2** (200 μg, recovery 7%) were obtained as colorless solids: LD_{99} in mice (ip) was 200–400 μg/kg for both. ^1H–^1H COSY spectra of **1** and **2** suggested their structural similarity, but further analysis of **2** was hampered by sample size. [**1**: UV_{max} (MeCN) 259 (ϵ 25 000), 270 (32 000), 280 (26 000) nm; IR (film) 1630, 1730, 1738 cm^{-1}; HR-FABMS [M + Na]$^+$ m/z 847.4483 (calcd for $[C_{43}H_{68}O_{15}Na]^+$ m/z 847.4455).] Partial structures H2–H8, H11–H13, H15–H23,28, H1′–H5′, and H1″–H6″ were deduced from detailed analyses of ^1H–^1H COSY and 2D HOHAHA spectra.[3] The conjugated triene (H16–H21) was also supported by the UV maxima; the $^3J_{HH}$ value (15 Hz) determined by the 2D *J* spectrum pointed to *E,E,E* geometry.

The ^{13}C NMR spectrum (CD_3CN) confirmed the presence of a ketone (δ 207.4) and an ester (δ 172.1) suggested by the IR bands. A ^{13}C decoupled HMQC spectrum led to assignments of all ^1H and ^{13}C signals except those of two quaternary carbons, C10 (δ 103.9) and C14 (δ 40.5). HMBC spectra[3] clarified the connectivities around quaternary and carbonyl carbons by giving cross peaks due to $^{2,3}J_{CH}$ between C1/H2a, C1/H15, C9/H8a, C10/H12a, C10/H12b, C10/Me25, C13/Me26, C13/Me27, C14/H15, C14/Me26, C14/Me27, C15/Me26, C15/Me27, C26/Me27, and C27/Me26. The structural features around C14 were supported by NOEs (NOESY 270 MHz, ROESY 400

[†] Tohoku University.
[‡] Government of Guam.

(1) Haddock, R. L.; Cruz, O. L. T. *Lancet* **1991**, *338*, 195–196.

(2) Chromatography was done successively on silica gel with CH_2Cl_2/MeOH [1:0, 99:1(**2**) and 9:1(**1**)], ODS-Q and Develosil ODS-7 with CH_3CN/H_2O (85:15), and Cosmosil 5C18-AR with CH_3CN/H_2O (4:1).

(3) ^1H–^1H COSY spectra were recorded on a JEOL GSX-400 (400 MHz) spectrometer in $CDCl_3$, CD_3CN, or C_5D_5N; 2DHOHAHA and HMQC in CD_3CN; and HMBC in CD_3CN or CD_3CN/D_2O (9:1). In the HMBC experiment (J_{CH} 6.0 Hz), the column width for ^{13}C NMR was reduced to about one-fifth of the conventional width to enhance the digital resolution. The column widths and spectral centers were adjusted so as to avoid overlapping of ^{13}C signals when they were folded back.

0002-7863/93/1515-1147$04.00/0

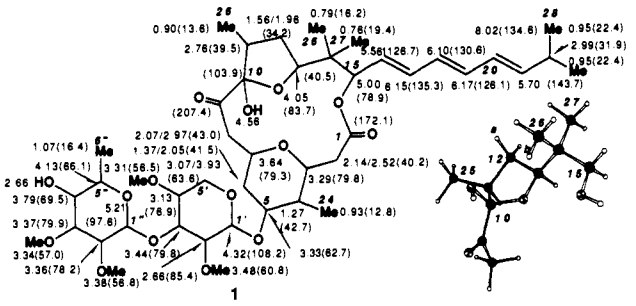

Figure 1. Structure and NMR assignment of polycavernoside A (**1**) and the stereo structure of a cyclohemiketal model corresponding to C8–C15 of **1** deduced from MM2. ^{1}H NMR chemical shifts and ^{13}C NMR chemical shifts (in parentheses) are those in CD_3CN.

MHz)[4] between H12a/Me26, H13/Me27, H15/Me26, and H16/Me27. The connectivities of C1/C2 and C8/C9 were further supported by the chemical shifts of H_2-2 (δ 2.14, 2.52) and H_2-8 (δ 2.07, 2.97) typical for methylenes α to carbonyl. The deuterium-exchangeable signals at δ 4.58 and 2.66 in the ^{1}H NMR spectrum (CD_3CN) were assigned to 10-OH and 4″-OH, respectively, on the basis of the cross peaks due to $^2J_{CH}$ between C10/10-OH and C4″/4″-OH in HMBC spectra. The connectivities of two remaining quaternary carbons, C9 and C10, were deduced from the NOE between H8b/H11.[4] The adjacent carbonyl (C9) caused a significant downfield shift of 10-OH (δ 4.58) by an anisotropic effect and formation of a hydrogen bond. The ether linkage between C3/C7 was evident from the NOE between H3/H7. The remaining hemiketal carbon (C10) and an oxycarbon (C13) were linked to form a tetrahydrofuran ring; $^3J_{HH}$ of H11–H13 agreed with those expected from MM2 energy calculations.[5,6]

The glycosidic residue, *O*-2,3-di-*O*-methylfucopyranosyl-(1″–3′)-*O*-2,4-di-*O*-methylxylopyranosyl-(1′–5), was deduced from the cross peaks in HMBC spectra due to $^3J_{CH}$ between C2′/OMe2′, C4′/OMe4′, C2″/OMe2″, C3″/OMe3″, C5/H1′, and C3′/H1″, those in NOESYs (400 and 600 MHz) due to NOEs between H1′/H3′, H1′/H5a′, H5/H1′, and H3′/H1″, and from $^3J_{HH}$ of H1′–H5′ and H1″–H6″. The positive FABMS supported this struture by showing prominent fragment ions (m/z 651, 633, 491, and 473) corresponding to sequential loss of each residue.

The above results led to **1** as the planar structure of polycavernoside A and allowed assignment of all ^{1}H and ^{13}C signals (Figure 1). The carbon backbone of **1**, a 3,5,7,13,15-pentahydroxy-9,10-dioxotricosanoic acid, is a new molecular entity. A smaller macrocycle, a trioxatridecane, is reminescent of the aplysiatoxins, which contain trioxadodecane.[7] The similarity of observed symptoms in experimental animals and human patients supports the belief that **1** and **2** caused the intoxication.[8] Algal toxicity likely was much higher in April than in June, as indicated by a rapid decrease of toxicity in samples collected afterward. Although the unique molecular structure of the aglycone offers no hint, the methylated fucose of **1** suggests its algal origin. The sudden and transient occurrence of the toxins in the alga remains unexplained, but may provide a clue to previous outbreaks of fatal food poisoning caused by two other *Gracilaria*, *G. chorda*[9] and *G. verrucosa*.[10,11]

Acknowledgment. We thank Drs. M. Murata, Tohoku University, and M. Isobe, Nagoya University, for discussions; S. Matsunaga, Tokyo University, for measuring 600-MHz NOESY; and P. J. Scheuer, University of Hawaii, for reading the manuscript. This work was supported by a grant from the Ministry of Education, Science, and Culture, Japan.

Supplementary Material Available: Table of ^{13}C and ^{1}H NMR assignments and ^{1}H and ^{13}C NMR, 2D HOHAHA, ^{1}H–^{1}H COSY, HMQC, HMBC, 2D *J*, ROESY, and NOESY spectra of **1** (14 pages). Ordering information is given on any current masthead page.

(4) NOESY spectra were recorded at 270 MHz (200 ms) at 27 °C in CD_3CN (positive and negative NOEs), at 400 MHz (200 ms) at −27 °C, and at 600 MHz (700 ms) at 27 °C in C_5D_5N (all negative NOEs). The ROESY spectrum was recorded at 400 MHz (200 ms) at 27 °C in CD_3CN. NOE difference spectra were measured at 400 MHz at −27 °C in C_5D_5N.

(5) MM2 energy calculations done on a cyclohemiketal model (Figure 1) constructed on the basis of the observed NOE (H8b/H11), $^3J_{H(11)/H(12a)}$ (11.6 Hz), and $^3J_{H(12a)/H(13)}$ (11.6 Hz) led to dihedral angles of H11/H12a, 165.7°; H11/H12b, 44.6°; H12a/H13, 161.0°; and H12b/H13, 38.2° for a stable conformer. Coupling constants of H11/H12a, H11/H12b, H12a/H13, and H12b/H13, calculated by the modified Karplus equation,[6] were 12.1, 5.6, 11.4, and 4.1, respectively, and agreed with the observed values (11.6, 6.7, 11.6, and 5.1 Hz).

(6) Imai, K.; Osawa, E. *Tetrahedron Lett.* **1989**, *30*, 4251–4252.

(7) Kato, Y.; Scheuer, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 2245–2246.

(8) Both **1** and **2** caused diarrhea, hypersalivation, lacrymation, muscle spasms, and cyanosis. According to Dr. R. Roos, Guam Memorial Hospital, these symptoms were comparable with those observed in his patients.

(9) Aikawa, S.; Suzuki, M.; Ono, K. *Rep. Yamagata Prefectural Inst. Public Health* **1981**, *13*, 81–82.

(10) Sonoda, T. *J. Food Hyg. Soc. Jpn.* **1983**, *24*, 507–508.

(11) Fusetani, N.; Hashimoto, K. *Bull. Jpn. Soc. Sci. Fish.* **1984**, *50*, 465–469.

Addition of Azides to C_{60}: Synthesis of Azafulleroids

M. Prato,† Q. Chan Li, and F. Wudl*

Institute for Polymers and Organic Solids
Department of Chemistry and Physics
University of California at Santa Barbara
Santa Barbara, California 93106

V. Lucchini

Dipartimento di Scienze Ambientali
Universita di Venezia, Dorsoduro 2137
30133 Venezia, Italy

Received October 5, 1991

Although macroscopic amounts of buckminsterfullerene C_{60}[1] are becoming increasingly accessible through easier and more economic methods of synthesis,[2] the functionalization of fullerenes is not yet a trivial task due to the multifunctionality of C_{60} which usually results in the formation of numerous inseparable products.[3] We have recently demonstrated that stable, pure fulleroids can be obtained by allowing C_{60} to react with substituted diazomethanes.[4] In this communication, we report our preliminary results on the reaction of C_{60} with organic azides[5] which provides an excellent method for the preparation of "azafulleroids".

Refluxing an equimolar solution of C_{60} and [(trimethylsilyl)-ethoxy]methyl azide ($SEMN_3$) (**1a**) in chlorobenzene overnight produced two major products (24% and 30%, respectively, based on C_{60} conversion), which were purified by column chromatography (silica gel, mixtures of hexanes/toluene). A more polar compound (**A**, see structures below) was relatively stable at room temperature, but was transformed to a less polar product when heated in refluxing chlorobenzene for a few hours or for a few

† On leave from CMRO-CNR and Department of Organic Chemistry, University of Padova, Italy.

(1) Krätschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. *Nature* **1990**, *347*, 354. Haufler, R. E.; Conceicao, J.; Chibante, L. P. F.; Chai, Y.; Byrne, N. E.; Flanagan, S.; Haley, M. M.; O'Brien, S. C.; Pan, C.; Xiao, Z.; Billups, W. E.; Ciufolini, M. A.; Hauge, R. H.; Margrave, J. L.; Wilson, L. J.; Curl, R. F.; Smalley, R. E. *J. Phys. Chem.* **1990**, *94*, 8634; Smalley, R. E. *Acc. Chem. Res.* **1992**, *25*, 98. Hare, J. P.; Kroto, H. W. *Acc. Chem. Res.* **1992**, *25*, 106.

(2) MER Corp., Tucson, AZ; Texas Fullerenes, Houston, TX; Ulvick Industries, Houston, TX; SES Research, Houston, TX; Polygon, Waco, TX; Research Materials Inc., Littleton, CO; Syncom, Groningen, The Netherlands; Idea, Sydney, Australia.

(3) *Fullerenes Synthesis, Properties, and Chemistry of Large Carbon Clusters*; Hammond, G., Kuck, V. J., Eds.; ACS Symposium Series 481; American Chemical Society: Washington, DC, 1992.

(4) Suzuki, T.; Li, Q.; Khemani, K. C.; Wudl, F.; Almarsson, Ö. *Science* **1991**, *254*, 1186. (b) Wudl, F. *Acc. Chem. Res.* **1992**, *25*, 157.

(5) A very preliminary account of C_{60}'s reactivity toward *p*-nitrophenyl azide has appeared in ref 3.

0002-7863/93/1515-1148$04.00/0