A Monogalactosyl Diacylglycerol from a Cultured Marine Dinoflagellate, Scrippsidla trochoidea

Yoshiteru Oshima, Sho-hei Yamada, Kimihiro Matsunaga, Takahiro Moriya, and Yasushi Ohizumi

J. Nat. Prod., **1994**, 57 (4), 534-536• DOI: 10.1021/np50106a017 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 4, pp. 534-536, April 1994

A MONOGALACTOSYL DIACYLGLYCEROL FROM A CULTURED MARINE DINOFLAGELLATE, *SCRIPPSIELLA TROCHOIDEA*

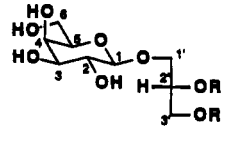
YOSHITERU OSHIMA, SHO-HEI YAMADA, KIMIHIRO MATSUNAGA, TAKAHIRO MORIYA, and YASUSHI OHIZUMI*

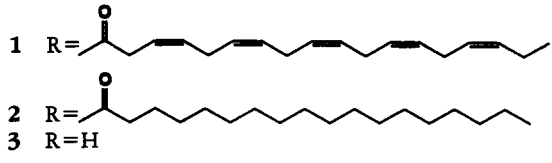
Pharmaceutical Institute, Tohoku University, Aoba-yama, Aoba-ku, Sendai 980, Japan

ABSTRACT.—A novel monogalactosyl diacylglycerol [**1**] has been isolated from a cultured marine dinoflagellate *Scrippsiella trochoidea*, and its structure was elucidated by spectroscopic methods.

Recently, marine microorganisms such as blue-green algae (1) and dinoflagellates (2) have been studied as a valuable new source of pharmacologically useful compounds. In our search for bioactive substances from marine microorganisms, we observed that the EtOAc solubles of a MeOH extract of a cultured dinoflagellate, *Scrippsiella trochoidea*, showed marked cytotoxic action against P-388 and L-1210 leukemia cells in vitro at a concentration of 0.1 mg/ml. The EtOAc solubles were repeatedly chromatographed over Si gel to afford a monogalactosyl diacylglycerol mixture as a bioactive fraction. Further separation of the mixture by hplc using an ODS column yielded the novel monogalactosyl diacylglycerol [**1**].

The monogalactosyl diacylglycerol [**1**], $[\alpha]^{25}D$ $-6.0°$ (c=0.37, $CHCl_3$), showed a quasimolecular ion peak at m/z 789 $[M+Na]^+$ in its fabms. The ir spectrum of **1** displayed absorption bands at 3400 and 1740 cm^{-1}, indicating the presence of hydroxyl and ester functionalities in its molecule. In the ^{1}H-nmr spectrum of **1**, there was a triplet methyl signal at δ 0.98 (6H, t, J=7.5 Hz), a mass of signals between δ 3.50 and 5.27 for oxymethylene and oxymethine hydrogens, and signals between δ 5.30 and 5.64 (20H, m) for olefinic hydrogens. These spectral features are characteristic for glycolipids bearing highly unsaturated fatty acids. Analysis of the ^{1}H-^{1}H COSY spectrum of **1** gave assignments of all the ^{1}H-nmr signals for sugar and glycerol moieties as follows: δ 4.28 (1H, d, J=7.0 Hz, H-1), 3.54 (1H, t, J=7.0 Hz, H-2), 3.54 (1H, m, H-3), 3.90 (1H, br d, J=2.3 Hz, H-4), 3.54 (1H, m, H-5), 3.77 (2H, m, H-6) [sugar part]; 3.76 (1H, dd, J=11.0 and 5.2 Hz, H-1′), 3.97 (1H, dd, J=11.0 and 5.4 Hz, H-1′), 5.25 (1H, m, H-2′), 4.28 (1H, dd, J=11.8 and 6.6 Hz, H-3′), 4.41 (1H, dd, J=11.8 and 3.3 Hz, H-3′) [glycerol part]. The chemical shifts and splitting patterns of the signals clearly pointed out that **1** is a β-galactosyl 2′,3′-diacyl–glycerol. In addition to these, characteristic ^{1}H-nmr signals were observed at δ 3.18 and 3.20 (2H, each d, J=5.8 Hz), the chemical shifts of which matched





well with those of methylene hydrogens lying between double bond and carbonyl functionalities. The methylene signals, together with the integration of the olefinic hydrogen signals and the fragment ion peak at *m/z* 257 produced by cleavage α- to the carbonyl group, indicated the presence of two moles of octadeca-3,6,9,12,15-pentaenoic acid in the molecule, and this was substantiated by catalytic hydrogenation of **1** affording monogalactosyl 2′,3′-distearylglycerol [**2**] {fabms: *m/z* 809 $[M+Na]^+$, 267 $[C_{17}H_{35}CO]^+$; ^1H nmr: δ 0.95 (6H, t, J=6.0 Hz), 1.22–2.00 (60H, br s) and 2.15 (4H, t, J=7.0 Hz)}. The absence of significant ir absorption at 965 cm^{-1} indicated that all the double bonds of the octadeca-3,6,9,12,15-pentaenoic acid unit in **1** have cis- geometry (3). Alkaline hydrolysis of **2** with NaOMe in MeOH yielded methyl stearate and a monogalactosyl glycerol [**3**] which was identical in all respects with (2′*R*)-1-*O*-glyceryl-β-D-galactopyranoside (4), confirming the stereochemistry of both the sugar and glycerol parts in **1**. The structure of the new monogalactosyl diacylglycerol was therefore represented as formula **1**.

Although compound **1** was not cytotoxic against P-388 and L-1210 leukemia cells in vitro at a concentration of 50 μg/ml, a preliminary physiological examination showed that an acetyl mixture of the monogalactosyl diacylglycerol fraction had strong inhibitory activity against Ca^{++} ion-influx in rabbit platelet cells (Y. Oshima, K. Furukawa, S. Yamada, and Y. Ohizumi, unpublished data). The physiological functions of this class of compounds is of great interest for future investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The ir spectrum was taken on a Shimadzu IR-408 spectrometer. Ei and fab mass spectra were obtained on a JEOL JMS DX-303 spectrometer. ^1H-Nmr spectra were recorded on a JEOL JNM GX-500 spectrometer using TMS as an internal standard.

CULTIVATION.—The dinoflagellate, *Scrippsiella trochoidea*, was supplied by the National Institute for Environmental Studies, Environment Agency, Japan. Uni-algal cultures of *S. trochoidea* were grown in 3-liter glass bottles containing 2 liters of sea water medium enriched with modified ESM supplement (5) which consisted of the following elements in 1 liter of sea water: $NaNO_3$, 120 mg; K_2HPO_4, 5 mg; Fe-EDTA, 259 μg; Mn-EDTA, 332 μg; vitamin B_{12}, 1 μg; thiamine hydrochloride (vitamin B_1), 100 μg; D-biotin (vitamin H), 1 μg; tris(hydroxymethyl)aminomethane, 1 g. The pH of the supplement was adjusted to 8.0 with 3 M HCl, prior to mixing with sea water which was sterilized by autoclaving. Cultures were incubated statically at 25° in an apparatus where illumination from a fluorescent light source was supplied in a cycle of 16 h light and 8 h darkness. After 10–14 days the cultured cells were harvested with a glass filter (GF/F, Whatmann), by suction, to yield the cells.

ISOLATION OF A MONOGALACTOSYL 2′,3′-DIACYL GLYCEROL [**1**].—The harvested cells from 500 liters of culture were extracted with MeOH (300 ml×3) to give a MeOH extract that was partitioned with EtOAc/H_2O. The EtOAc solubles (993 mg) were subjected to Si gel cc (Kieselgel 60, Merck) eluted with $CHCl_3$/MeOH to give a monogalactosyl diacylglycerol mixture. The fraction was separated by hplc [column: Tosoh TSKgel ODS-120A (2.54 cm i.d.×30 cm); flow rate: 4 ml/min; solvent: H_2O-MeOH (5:95); detection: uv (215 nm)] to give a monogalactosyl diacylglycerol [**1**] (8 mg) as an amorphous powder, $[\alpha]^{25}D$ −6.0° (c=0.37, $CHCl_3$); fabms *m/z* 789 $[M+Na]^+$, 257; ir (nujol) ν max 3400, 1740 cm^{-1}; ^1H nmr (Me_2CO-d_6) δ 0.98 (6H, t, J=7.5 Hz), 3.18, 3.20 (2H each, d, J=5.8 Hz), 3.54 (1H, t, J=7.0 Hz), 3.54 (2H, m), 3.76 (1H, dd, J=11.0 and 5.2 Hz), 3.77 (2H, m), 3.90 (1H, br d, J=2.3 Hz), 3.97 (1H, dd, J=11.0 and 5.4 Hz), 4.28 (1H, d, J=7.0 Hz), 4.28 (1H, dd, J=11.8 and 6.6 Hz), 4.41 (1H, dd, J=11.8 and 3.3 Hz), 5.25 (1H, m).

Hydrogenation of **1**.—A solution of **1** (5 mg) in MeOH (2 ml) was hydrogenated with PtO_2 (5 mg) catalyst for 24 h at atmospheric pressure. Filtration and removal of the solvent at reduced pressure gave a quantitive yield of monogalactosyl 2′,3′-distearylglycerol [**2**] (5 mg) as an amorphous powder, $[\alpha]^{25}D$ −9.5° (c=0.40, $CHCl_3$); fabms *m/z* 809 $[M+Na]^+$, 267; ^1H nmr ($CDCl_3$) δ 0.95 (6H, t, J=6.0 Hz), 1.22–2.00 (60H, br s), 2.15 (4H, t, J=7.0 Hz), 3.61 (1H, br t, J=5.6 Hz, H-5), 3.67 (1H, dd, J=9.5 and 3.1 Hz, H-3), 3.75 (1H, m, H-1′), 3.75 (1H, m, H-2), 4.06 (1H, br d, J=3.1 Hz, H-4), 3.90 (1H, dd, J=11.0 and 5.6 Hz, H-6), 3.93 (1H, dd, J=10.9 and 5.0 Hz, H-1′), 4.04 (1H, dd, J=11.0 and 5.6 Hz, H-6), 4.20 (1H, dd, J=11.3 and 6.3 Hz, H-3′), 4.34 (1H, d,

J=7.5 Hz, H-1), 4.34 (1H, dd, J=11.3 and 3.1 Hz, H-3′), 5.29 (1H, m, H-2′).

Alkaline hydrolysis of **2**.—A solution of **2** (5 mg) in MeOH (0.5 ml) was treated with 3% NaOMe/MeOH (0.5 ml) at room temperature for 20 min. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and partitioned between *n*-hexane and MeOH. Evaporation of the solvent at reduced pressure from the MeOH solubles yielded a residue, which was purified by Si gel cc ($CHCl_3$/MeOH) to furnish (2′*R*)-1-*O*-glyceryl β-D-galactopyranoside [**3**] (2 mg) as an amorphous powder, $[\alpha]^{25}D$ −9.0° (c=0.20, H_2O); fabms *m/z* 277 $[M+Na]^+$; ^1H nmr (C_5D_5N+1 drop of D_2O) δ 4.07 (1H, dd, J=6.4 and 5.6 Hz, H-5), 4.12 (1H, d, J=5.5 Hz, H-3′), 4.15 (1H, d, J=4.7 Hz, H-3′), 4.15 (1H, dd, J=9.4 and 3.5 Hz, H-3), 4.26 (1H, dd, J=10.0 and 4.2 Hz, H-1′), 4.45 (2H, m, H-6), 4.45 (1H, m, H-2′), 4.46 (1H, dd, J=10.0 and 5.9 Hz, H-1′), 4.50 (1H, dd, J=9.4 and 7.5 Hz, H-2), 4.55 (1H, d, J=3.5 Hz, H-4), 4.92 (1H, d, J=7.5 Hz, H-1). The *n*-hexane solubles were evaporated at reduced pressure to give methyl stearate as a colorless oil, which showed a single peak on gc (SS-4, 40 m; column temp., 185°; N_2 flow rate, 1.4 ml/min), eims: *m/z* 298 $[M]^+$.

ACKNOWLEDGMENTS

We thank the National Institute for Environmental Studies, Environmental Agency, Japan, for providing the dinoflagellate. This work was supported in part by a Grant-in-Aid (05152011) from the Ministry of Education, Science and Culture of Japan. Financial support from the Sapporo Foundation and the Tokyo Biochemical Research Foundation are also acknowledged.

LITERATURE CITED

1. J.S. Mynderse, R.E. Moore, M. Kashiwagi, and T.R. Norton, *Science*, **196**, 538 (1977).
2. Y. Murakami, Y. Oshima, and T. Yasumoto, *Bull. Jpn. Soc. Sci. Fish.*, **48**, 69 (1982).
3. J.D. Joseph, *Lipids*, **10**, 395 (1975).
4. M. Kobayashi, K. Hayashi, K. Kawazoe, and I. Kitagawa, *Chem. Pharm. Bull.*, **40**, 1404 (1992).
5. T. Okaichi, S. Nishio, and Y. Imatomi, in: "Yudoku-Plankton-Hassei, Sayo-kiko, Doku-seibun (Toxic Phytoplanktons-Occurrence, Mode of Action, and Toxins)." Ed. by the Japanese Society of Scientific Fisheries, Koseisha-Koseikaku, Tokyo, 1982, p. 23.

Received 6 *October* 1993