Iotroridoside-A, a Novel Cytotoxic Glycosphingolipid from the Marine Sponge *Iotrochota ridley*

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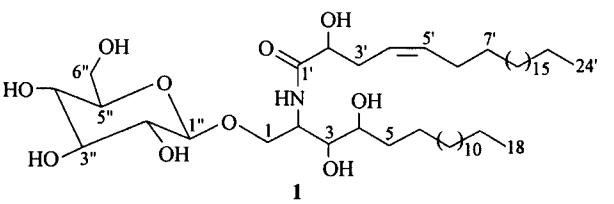
A new cytotoxic glycosphingolipid, Iotroridoside-A, was isolated from the marine sponge *Iotrochota ridley* collected from the South China Sea near Hainan Island, China. On the basis of chemical degradation method and IR, MS, ^{1}H NMR, ^{13}C NMR and 2D NMR spectrometry, its structure was assigned as 1-*O*-β-*D*-glucopyranosyl-2-[(4′*Z*)-2′-hydroxytetracosene amido]-4-tetradecyl-1, 3, 4-butantriol. The new compound exhibits strong cytotoxicity against L1210 murine leukemia cells *in vitro* (ED_{50} = 0.08 μg/mL).

Keywords Sponge, *Iotrochota ridley*, glycosphingolipid, isolation, structure determination

Introduction

Marine sponges have yielded unusual sphingolipids and glycosphingolipids, some of which have promising antitumor activity.[1-3] In the course of our study on biological active substances in Chinese marine organisms, we obtained a new cytotoxic glycosphingolipid—Iotroridoside-A (**1**) from the marine sponge *Iotrochota ridley*. It is firstly reported that the amide unit of sphingolipid from marine organisms contains a double bond ($\Delta^{4,5}$), although analogous glycolipids containing the segment of monounsaturated 2-hydroxy fatty acids were identified from terrestrial plants many years ago.[4] Iotroridoside-A (**1**) exhibits strong cytotoxicity against L_{1210} murine leukemia cells *in vitro* (ED_{50} = 0.08 μg/mL). We herein describe the isolation and the structure elucidation of the novel compound.



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Experimental

Apparatus

IR spectra were recorded on an Analect RFX-65 spectrophotometer. Optical rotation was measured on a Perkin-Elmer 241 MC polarimeter. NMR spectra were recorded on Bruker AMX-600 MHz and DRX-400MHz instruments in C_5D_5N and $CDCl_3$ with TMS as an internal standard. EIMS and SIMS data were obtained on HP5989A and ATEX Ⅱ mass spectrometers, respectively.

Animal material

The dried dark sponge *Iotrochota ridley* was collected from the South China Sea around Hainan Island, China, in April 1992. The voucher specimen (No. SS302) was deposited at the Guangzhou Institute of Chemistry, Chinese Academy of Sciences.

Extraction and isolation

Chopped dried sponge (2.2 kg) was extracted with

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95% ethanol. Concentration of the extract under reduced pressure gave 168 g of dark brown gum. The gum was partitioned between ethyl acetate and water, *n*-butanol and water, respectively. Butanol extract was concentrated under reduced pressure to give a crude residue (23.0 g). The crude residue was chromatographed on a Si gel column with $CHCl_3$-MeOH (100:0—70:30). The fraction of $CHCl_3$-MeOH (85:15) was evaporated under reduced ressure to give a residue (1.0 g). The residue was separated by repeated Si gel column chromatography with $CHCl_3$-MeOH (90:10) and Sephadex LH-20 column chromatography with $CHCl_3$-MeOH (30:70) to obtain a pure compound **1** as amorphous solid (29.6 mg). $[\alpha]_D^{25}$ - 7.2° (*c* 0.00305, C_5H_5N). HRESIMS *m/z*: 866.6665 $[M+Na]^+$ (calcd. for $C_{48}H_{93}NO_{10}$ + Na, 866.6662), 664.6294 $[M+H-Glc]^+$. ν_{max}(KBr): 3363, 2919, 2850, 2287, 1629, 1536, 1465, 1376, 1168, 1035, 898, 721, 605 cm^{-1}. ^1H NMR and ^{13}C NMR see Table 1.

Hydrolysis of **1**

A sample of **1** (5.0 mg) was refluxed in 6.5 mL of 1 mol/L HCl in 82% MeOH for 12 h. The reaction mixture was extracted with petroleum ether (5 mL × 3) and the petroleum ether layer was washed with water, dried (Na_2SO_4) and evaporated to give a crude product (3.5 mg). The crude product was chromatographed on Si gel column with petroleum ether-EtOAc (80:20) to give compound **2** (2.0 mg). $[\alpha]_D^{25}$ - 13.2° (*c* 0.00325, $CHCl_3$). δ_H($CDCl_3$, 400 MHz): 0.88(t, *J* = 6.6 Hz, CH_3), 1.24—1.50(m, 17CH_2), 2.01—2.02(m, 6′-H), 2.44—2.60(m, 3′-H), 3.78(s, OCH_3), 4.26 (t, *J* = 5.4 Hz, 2′-H), 5.34—5.40(m, 4′-H), 5.55—5.61(m, 5′-H). *m/z*(%): 396(M^+), 378 (M - H_2O), 337(M - $COOCH_3$), 304, 139, 125, 111, 103, 90(100), 83, 69, 57, 55, 43.

Results and discussion

The ethanol extract of the dried sponge was partitioned between ethyl acetate and water, *n*-butanol and water, respectively. Silic gel chromatography of the butanol extract followed by Sephadex LH-20 column chromatography afforded Iotroridoside-A (**1**).

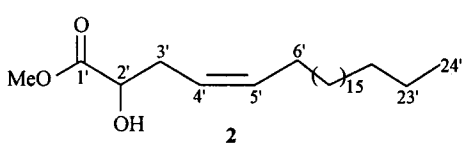
The compound was obtained as amorphous solid, $[\alpha]_D^{25}$ - 7.2°(*c*0.00305, C_5H_5N), and the molecular formula $C_{48}H_{93}NO_{10}$ was established by HRESIMS. Its IR spectrum showed absorption at 3363 and 1035 cm^{-1} (hydroxy), 1629 and 1536 cm^{-1} (secondary amide), and 2919, 2850 and 1465 cm^{-1} (aliphatic). The ^1H NMR spectrum showed the presence of two terminal methyl groups (δ 0.86, t, *J* = 6.6 Hz, 6H), one amide (δ 8.54, d, *J* = 9.0 Hz, 1H), twelve protons of oxygenated methylenes/methines and one proton of NH, including one anomeric proton (δ 4.93, d, *J* = 8.4 Hz, 1H). Two olefinic protons were at δ 5.90 (dt, *J* = 10.2 and 7.2 Hz) and δ 5.60 (dt, *J* = 10.8 and 7.2 Hz) respectively. The configuration of this double bond was found to be *cis*-form, because the coupling constant was less than 12 Hz. Huge methylenes were enveloped at δ 1.20—1.34, indicating the presence of one or two aliphatic linkages. All these spectral signals were reminiscent of a glycosphingolipid. The ^{13}C NMR spectrum exhibited the presence of one carbonyl group (δ 174.98), two olefinic carbons (δ 126.27 and 132.29) and one anomeric carbon (δ 105.53). H-HGCOSY spectrum displayed three coupling systems: ① starting from the anomeric proton signal, a sugar unit was established by tracing and joining the coupling points among C-1″—C-6″. The structure of this unit was recognized as β-*D*-glycopyranoside because their ^{13}C NMR data (105.53, 75.13, 78.52, 72.57, 78.43 and 62.65 by HMQC[5]) were consistent with those of β-*D*-glycopyranoside in relevant literature;[6] ② further analysis of the COSY spectrum resulted in connectivity from C-1 to C-6, which completed the structure of phytosphingosine moiety with an amide group at C-2. The corresponding values of their ^{13}C chemical shifts were determined by HMQC; ③ tracing out the coupling points from two olefinic protons (H-4′ and H-5′) led to assignment of an unsaturated aliphatic linkage (1′→*m*CH_2), which contains one oxygen-bearing methine. The COSY spectrum also displayed that the methine was connected with the carbon of amide group, because a cross peak was observed between an amide proton (δ 8.54) and the methine proton (H-2′, δ 4.62). The ^1H-detected heteronuclear multiple bond connectivity (HMBC) spectrum showed that the amide proton was correlated with an amide carbon at δ 174.98, which was in turn correlated with methine proton (H-2′), and carbon C-1 was correlated with the anomeric proton (see Table 1). Consequently, the above data and deduction completed the sketchy features of structure of **1**.

Table 1 NMR (C_5D_5N, 600 MHz) data of Iotroridoside-A (**1**)

No.	δ_C	δ_H	TOCSY	HMBC(^{1}H)
1	70.42	4.68 dd (4.2, 10.2)*, 4.50 dd (4.2, 10.8)	H-2, H-3, NH	H-3, H-1″
2	51.80	5.21—5.27 m	H-1, H-3, NH, H-4	H-1, H-3
3	75.85	4.27 dd (4.8, 12)	H-1, H-2, H-4, H-5, H-6	H-4
4	71.51	4.14—4.18 m	H-2, H-3, H-5, H-6, H-7	H-3
5	34.22	2.18—2.26 m, 1.84—1.92 m	H-3, H-4, H-6, H-7	H-3
6	26.54	1.84—1.92 m, 1.62—1.70 m	H-3, H-4, H-5, H-7	
16	32.14	1.20—1.28 m		H-17, Me-18
17	22.94	1.20—1.28 m		Me-18
18	14.28	0.86 t (6.6)		H-16, H-17
1′	174.98			NH, H-2′
2′	72.48	4.60—4.64 m	H-3′, H-4′, H-5′	H-3′
3′	33.56	2.98—3.02 m, 2.79—2.85 m	H-2′, H-4′, H-5′, H-6′	H-5
4′	126.27	5.90 dt (10.2, 7.2)	H-2′, H-3′, H-5′, H-6′, H-7′	H-3′, H-6′
5′	132.39	5.60 dt (10.8, 7.2)	H-2′, H-3′, H-4′, H-6′, H-7′	H-3′, H-6′
6′	27.90	2.13—2.18 m	H-3′, H-4′, H-5′, H-7′	H-4′, H-7′
22′	32.14	1.20—1.28 m		H-23′, Me-24′
23′	22.94	1.20—1.28 m		Me-24′
24′	14.28	0.86 t (6.6)		H-22′, H-23′
1″	105.53	4.93 d (8.4)	H-2″, H-3″, H-4″, H-5″	H-2″, H-1
2″	75.13	3.94—4.00 m	H-1″, H-3″, H-4″, H-5″, H-6″	H-3″
3″	78.52	4.13—4.17 m	H-1″, H-2″, H-4″, H-5″, H-6″	H-2″, H-4″
4″	72.57	4.17—4.21 m	H-1″, H-2″, H-3″, H-5″, H-6″	H-6″
5″	78.43	3.82—3.86 m	H-1″, H-2″, H-3″, H-4″, H-6″	H-4″
6″	62.65	4.45 dd (12, 2.4), 4.31 dd (12, 5.4)	H-2″, H-3″, H-4″, H-5″	
NH		8.54 d (9)	H-1, H-2, H-3, H-4	

* Coupling constants (J in Hz) are given in parentheses.

In order to determine the structure of sphingosine unit of **1**, which would lead to a gross structure, compound **1** was hydrolyzed with 1 mol/L HCl in 82% MeOH and followed by silica gel chromatography affording compound **2**,[6] which was identified by its EI mass spectrum and ^{1}H NMR data as follows:



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So, the structure of the sphingosine unit was determined, and as a result, compound **1** was assigned as 1-*O*-β-*D*-glucopyranosyl-2-[(4′*Z*)-2′-hydroxytetracosene amido]-4-tetradecyl-1, 3, 4-butantriol.

Acknowledgments

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