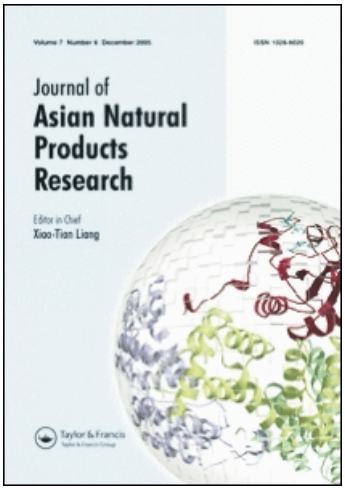

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CRASSICAULISINE, A NEW SULPHONOGLYCOLIPID FROM THE RED ALGA *CHONDRIA CRASSICAULIS* HARV.

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A new sulphonoglycolipid, crassicaulisine, has been isolated from the red alga *Chondria crassicaulis* Harv.. Four known compounds were also found from the title plant. The structure of the new compound was elucidated on the basis of chemical reactions and spectroscopic analysis.

Keywords: *Chondria crassicaulis* Harv.; Rhodomelaceae; Glycolipids; Sulphonoglycolipids; Crassicaulisine

INTRODUCTION

Red algae of the genus *Chondria* (Rhodomelaceae, Rhodophyta) are known for the production of cyclic polysulfides [1], terpenoids [2], aminoacids [3], and indole derivatives [4–5], which exhibit antiviral, antifungal, cytotoxic and anthelmintic activities.

As part of our research project on the study of marine organisms from the Chinese coasts, we made a collection of a red alga *Chondria crassicaulis* Harv. off the Nanji Islands, Zhejiang Province, China. On separation of the CH_2Cl_2-soluble fraction of an ethanol extract of this alga, we isolated a new sulphonoglycolipid, crassicaulisine (**1**), together with four known compounds. This paper deals with the isolation and structural elucidation of the new sulphonoglycolipid from the red alga.

RESULTS AND DISCUSSION

The alga was exhaustively extracted with EtOH and the extract was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble portion was subjected to column chromatography on silica gel eluting with $CHCl_3$/MeOH system. This procedure resulted in the isolation of a new

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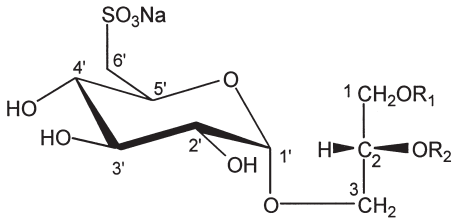
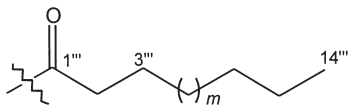
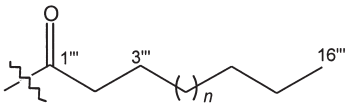
sulphonoglycolipid, named crassicaulisine (**1**), together with four known compounds. The known compounds were identified as *Z*-liagosterol, cholesta-5, 25-diene-3β, 24ζ-diol, 3β-hydroxy-cholesta-5,25-dien-24-one and laurinterol, respectively, by analysis of their spectral data and by comparison with the data reported in literatures.



1 R_1 = 

$m = 8$

R_2 = 

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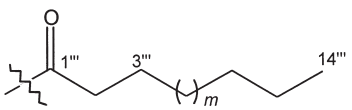
3 $R_1 = R_2$ = 

$m = 8$

Crassicaulisine (**1**), a white amorphous powder, $[\alpha]_D + 43.0$ (*c* 0.13, MeOH), showed the quasimolecular ion $(M + H)^+$ at *m/z* 789 and $(M + Na)^+$ at *m/z* 811 in the FABMS and the molecular formula $C_{39}H_{73}O_{12}SNa$ was established by HRFABMS [*m/z* 789.4717 $(M + H)^+$]. The NMR spectral data of **1** shows a characteristic signal pattern due to a glyceroglycolipid [6–7]: a triplet (6H) at δ 0.96 (two terminal methyls), a broad signal centered at δ 1.37 (44H, methylene in the fatty acid chains), and a doublet (1H) at δ 5.38 (anomeric proton). From the $^1H-^1H$ COSY data, a spin system was easily assigned to an unsymmetrical and fully substituted glycerol moiety. The methylene protons at C-1 and C-3 of glycerol were distinctly different and characteristic chemical shifts (Table I) reflecting the acyl versus glycosidic substitution at these two positions. Moreover, the $^1H-^1H$ COSY spectrum revealed that the coupling constants and splitting patterns of the protons in the sugar moiety of **1** were very similar to those of α-glucopyranoside, but the chemical shifts were in some ways different. Namely, observation of the C-6′ methylene at δ 4.28 and 3.75 indicated the attachment of a sulphonyl group on the C-6′ carbon, so the sugar moiety was sulphoquinovose. The ^{13}C NMR spectrum of **1** (Table I), in which the C-6′ carbon signal appeared at δ 54.99, also substantiated this conclusion. Treatment of **1** with 10% NaOMe in MeOH afforded 6-sulphoquinovopyranosyl glycerol and a mixture of two fatty acid methyl esters. The former, $[\alpha]_D + 43.5$ (*c* 0.60, MeOH), was identified, by analysis of its ^1H, ^{13}C and ^{13}C DEPT NMR spectra and by comparison with NMR data reported in the literature [6], as 3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol (**2**), which was previously obtained by NaOMe treatment of the sulphonoglycolipid **3** isolated from the leaves of the tropical tree *Byrsonima crassifolia* [6]. The composition of the fatty acid methyl esters was shown to be methyl myristate and methyl palmitate, respectively, by GC-MS analysis. In order to

TABLE I ^{1}H NMR and ^{13}C NMR data for compound **1** (in pyridine-d_5) and ^{13}C NMR data for compound **2** (in $D_2O + CD_3OD$)*

No.	**1**		**2**
	δ ^{1}H (mult. *J* in Hz)	δ ^{13}C (mult.)	δ ^{13}C (mult.)
1a	4.84 (dd, 3.1, 11.9)	63.67 (t)	64.29(t)
1b	4.64 (dd, 7.0, 11.9)		
2	5.77 (m)	71.06 (d)	72.54(d)
3a	4.72 (dd, 5.2, 10.7)	66.68 (t)	70.73(t)
3b	4.01 (dd, 6.1, 10.7)		
1′	5.38 (d, 3.7)	100.5 (d)	100.0(d)
2′	4.18 (dd, 3.7, 9.6)	73.68 (d)	73.37(d)
3′	4.60 (dd, 9.3, 9.6)	75.17 (d)	74.52(d)
4′	3.92 (dd, 9.3, 9.3)	75.29 (d)	74.92(d)
5′	5.08 (ddd, 2.3, 8.1, 9.3)	69.86 (d)	69.84(d)
6′a	4.28 (dd, 2.3, 14.3)	54.99 (t)	54.00(t)
6′b	3.75 (dd, 8.1, 14.3)		
1″, 1‴	–	173.55 (s), 173.48 (s)	
2″, 2‴	2.47 (t, 7.4)	34.63 (t), 34.41 (t)	
3″, 3‴	1. 73 (m)	25.40 (t)	
4″–11″, 4‴–13‴	1. 37 (m)	29.56 (t)-30.15 (t)	
12″, 14‴	1. 37 (m)	32.26 (t)	
13″, 15‴	1. 37 (m)	23.07 (t)	
14″, 16‴	0.96 (t, 6.8)	14.40 (q)	

* Bruker DRX-400; δ values are reported in ppm referenced to TMS as an internal standard. Assignments were deduced from analysis of one-dimensional and two-dimensional spectra and comparison with known sulphonoglycolipid [6].

determine the sequence of fatty acid residues in crassicaulisine, we subjected glycolipid **1** to enzymatic hydrolysis [6]. On regio-selective enzymic hydrolysis using Lipase type XIII in dioxane–H_2O (1:1) at 37°C for 3 h, **1** furnished mostly myristic acid, which was identified after CH_2N_2 treatment by GC-MS analysis. Thus, it was concluded that a myristyl residue was attached 1-OH of the glycerol moiety in **1**. As a consequence, the palmitoyl should be connected with 2-OH. Finally, the absolute stereochemistry of C-2 in the glycerol portion of compound **1** was tentatively assigned as *R*, the same as that of model compound **3** by either comparing the specific rotation of **2** with that reported previously [8] or comparing the optical rotation of **1** with that of **3** $\{[\alpha]_D + 43.0$ (*c* 1, MeOH)$\}$ [6] according to Hyraeb's rule [9]. On the basis of these findings, the structure of the new glycolipid **1** was determined as (2*R*)-1-*O*-myristoyl-2-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol. In fact, the novelty of our compound **1** comparing with **3** resides only in the fatty acid residues in glycerol portion.

Numberous diacylglyceroglycolipid have been isolated from algae, higher plants, as well as animals. However, in most cases glyceroglycolipids were not reported as single components, neither have the sequence of their acyl moieties been determined. In this report, the sequence of acyl moieties of **1** was determined by regio-selective enzymic hydrolysis. Several glycolipids are reported to exhibit hemolytic activity and other biological activities [10]. Compound **1** was tested for cytotoxicity against HL-60 and MCF-7 cell lines, but it showed no significant bioactivity. Other bioassays are currently on-going.

EXPERIMENTAL SECTION

General Experimental Procedures

The IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a 400 MHz Bruker DRX-400 (400 MHz for ^{1}H and

100 MHz for ^{13}C) spectrometer. Chemical shifts are reported in ppm relative to an internal TMS standard. ^{1}H- and ^{13}C-NMR assignments were supported by $^{1}H-^{1}H$ COSY, HMQC and HMBC experiments. HRFABMS spectrum was recorded on a Finnigan MAT 95 mass spectrometer. GC/MS: MD800 GC/MS, injector temperature 280°, column DB-1701 (30 m × 0.25 mm), column temperature 100(3)–250/10. Optical rotations were determined on a Perkin–Elmer 241MC Polarimeter. Commercial Merck Si gel plates were used for TLC. The chromatograms were sprayed with 0.1% $Ce(SO_4)_2$ in 2N H_2SO_4 and heated at 80° for 5 min to detect the spots.

Collection of the Biological Material

The examined sample was collected from Nanji Island, Zhejiang, China in May 2000 and identified by Associate Professor Lin-Di Gu of Shanghai Normal University. A voucher sample is available for inspection at the Herbarium of Institute of Materia Medica, SIBS-CAS.

Extraction and Isolation

The air-dried alga (4.6 kg) was extracted with 95% EtOH (15 l) at room temperature. The EtOH extract was concentrated *in vacuo* and the resulting residue partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 extract (70 g) was chromatographed on a silica gel column using eluents of increasing polarity from light petroleum ether to EtOAc, MeOH. The fractions eluted with 20% MeOH/EtOAc was further purified by second silica gel column with $CHCl_3$/MeOH (9:1) as eluents affording **1** (538 mg).

Crassicaulisine (1)

A white amorphous powder, $[\alpha]_D + 43.0$ (*c* 0.13, MeOH). IR ν_{max} (KBr) cm^{-1}: 3435, 1741, 1170, 1035, 721. FAB-MS, *m/z*: 789$(M + 1)^+$, 811$(M + Na)^+$; HRFAB-MS *m/z*: 789.4717 $(M + H)^+$ (calcd. 789.4799). ^{1}H NMR (pyridine-d_5, 400 MHz): see Table I; ^{13}C NMR (pyridine-d_5, 100 MHz): see Table I.

Alkaline Hydrolysis of 1

A solution of **1** (30 mg) in 10% dry NaOMe–MeOH (4 ml) was stirred at 40°C for 2 h. The reaction mixture was neutralized with 2 N HCl–MeOH and partitioned between MeOH and *n*-hexane. The MeOH layer was concentrated under reduced pressure and purified by C-18 reverse-phase CC [eluents: MeOH–H_2O(1:4) → MeOH–H_2O(1:1)] and Sephadex LH-20 CC eluting with MeOH to afford (**2**), $[\alpha]_D + 43.5$ (*c* 0.60, MeOH). ^{13}C–NMR data of **2**: see Table I. The *n*-hexane layer was concentrated under reduced pressure to yield fatty acid methyl esters, which were analyzed by GC/MS.

Enzymic Hydrolysis of 1

A solution of **1** (4 mg) and Lipase type XIII (from *Pseudomonas* sp. Lot83H0885, Sigma. 3.0 mg, 70 units) in 1 ml dioxane–H_2O (1:1) was incubated at 37°C for 3 h. The reaction was quenched by adding 5% HOAc (0.5 ml), then EtOH was added to the reaction mixture. Solvent was removed under reduced pressure and the resulting residue dissolved in THF. The THF solution was treated with CH_2N_2 gas and the reaction mixture was extracted with *n*-hexane. The *n*-hexane layer was concentrated under reduced pressure and analyzed by GC/MS.

Isolation and Identification of Fatty Acids of 1

GC–MS analysis were carried out on a Finnigan instrument consisting of an MD 800 apparatus equipped with a DB-1701 capillary column (30 m × 0.25 mm, 0.25 μm film thickness; cross-linked 100% methyl–silicone), and of an electron impact source operating at 70 eV and 250°C. The column temperature was programmed to increase from 100 to 250°C at a rate of 10°C/min. The injector temperature was 280°C and the detector temperature was 250°C. The sample was injected into GC in MeOH. Methyl esters of fatty acids were recognized by comparison of EIMS spectra with that of standards. The retention times of methyl myristate and methyl palmitate were 13.20 and 15.44 min, respectively.

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