Glycerol Derivatives and Sterols from *Sargassum parvivesiculosum*

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Five glycerol derivatives (1—5) and three sterols (6—8) were isolated from the EtOH extraction of the brown alga of *Sargassum parvivesiculosum*. On the basis of spectroscopic methods, their structures were elucidated as 1,3-di-*O*-[2′,2′-di-(*p*-phenylene) isopropylidene] glycerol (1), (2*S*)-1-*O*-heptatriacontanoyl glycerol (2), (2*S*)-1,2-di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl) glycerol (3), (2*S*)-1-*O*-palmitoyl glycerol (4), (2*S*)-1,3-di-(*O*-palmitoyl)-2-*O*-octadecanoyl glycerol (5), 24-ethylcholest-5,23*Z*-dien-3β,28ζ-diol (6), 24-vinylcholest-5-en-24ζ-hydroperoxy (7), 24-ethylcholest-4,24(28)-dien-3β-ol (8), respectively. Among them, 1 and 2 were new.

Key words *Sargassum parvivesiculosum*; glycerol derivative; sterol

Sargassum parvivesiculosum is a common brown alga, widely distributed in southeast China. It is used for treating tracheitis and thyroiditis in folk medicine, and also has the activity of inhibiting tumor.[1)] The same genus of *S. angustifolium* (TURN.) C. AG. and *S. fusiforme* (HARV.) SETCH, both known as a "marine alga", have been collected in the Chinese pharmacopeia. The EtOH extraction of *S. carpophyllum* J. AG. can clearly induce the morphological abnormality of *Pyricularia oryzae*.[2)] Glycerides and sterols are rich in this genus.[2—5)] Some of them have activity against *Pyricularia oryzae*.[5)] During the course of searching the chemical constituents of *S. parvivesiculosum*, five glycerol derivatives and three sterols were obtained. On the basis of spectroscopic methods, their structures were elucidated as 1,3-di-*O*-[2′,2′-di-(*p*-phenylene) isopropylidene] glycerol (**1**), (2*S*)-1-*O*-heptatriacontanoyl glycerol (**2**), (2*S*)-1,2-di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl) glycerol (**3**),[6)] (2*S*)-1-*O*-palmitoyl glycerol (**4**),[5)] (2*S*)-1,3-di-(*O*-palmitoyl)-2-*O*-octadecanoyl glycerol (**5**),[5)] 24-ethylcholest-5,23*Z*-dien-3β,28ζ-diol (**6**),[7)] 24-vinylcholest-5-en-24ζ-hydroperoxy (**7**),[2)] 24-ethylcholest-4,24(28)-dien-3β-ol (**8**),[4)] respectively. Among them, **1** and **2** were new. Furthermore, according to the literature, **3** exhibited activity against *Pyricularia oryzae*, cytotoxicity against P388 and HL-60 tumor cells, and weak cytotoxicity against MCF-7 and A549 human tumor cells[8)]; and **6** showed cytotoxic activity against HL-60 tumor cells (IC_{50}=7.8 μg/ml).[7)]

Results and Discussion

Compound **1** was obtained as white powder, showing the molecular formula of $C_{18}H_{20}O_3$ as determined by EI-MS and NMR spectra. Its IR spectrum showed absorption bands for –OH (3433 cm^{-1}) and aromatic ring (1562, 1510 cm^{-1}), while the UV spectrum exhibited maximum absorption at 206, 228, 255, 276 and 280 nm (aromatic ring). With the assistance of 2D-NMR studies, including ^1H–^1H COSY, HMQC and HMBC experiments, all of the assignments of ^{13}C- and ^1H-NMR data of **1** were determined. The signals at δ_C 157.4 (s, C-1a, 1b), 143.6 (s, C-4a, 4b), 127.9 (d, C-3a, 5a, 3b, 5b), 114.8 (d, C-2a, 6a, 2b, 6b), and corresponding δ_H 7.09 (4H, d, J=8.0 Hz, H-3a, 5a, 3b, 5b), 6.90 (4H, d, J=8.0 Hz, H-2a, 6a, 2b, 6b) in the ^{13}C- and ^1H-NMR spectra of **1**, indicated the existence of the same two *p*-substituted phenylenes, which was further supported by the HMBC spectrum showing correlations of H-3a, 5a/3b, 5b, H-2a, 6a/2b, 6b with C-1a/1b, C-4a/4b, and ^1H–^1H COSY spectrum showing correlations of H-3a/5a/3b/5b with H-2a/6a/2b/6b, respectively (Fig. 2).

The ^{13}C- and ^1H-NMR spectra of **1** also showed typical signals for a fully substituted glycerol moiety [δ_C 69.9 (t, C-1, 3), 68.7 (d, C-2), δ_H 4.48 (4H, m, H-1, 3), 4.64 (1H, m, H-2)],[9,10)] which was identified by the correlations of H-1, 3 with C-2, and H-2 with C-1, 3 in the HMBC spectrum (Fig. 2). The HMBC correlations of H-1/3 with C-1a/1b, respectively, suggested that the same two *p*-substituted phenylenes were symmetrically attached to C-1 and C-3 by ether bond, respectively. Furthermore, in the HMBC spectrum, correlations of H-3a, 5a, 3b, 5b, H-1′, 3′ (δ_H 1.58, 6H, s) with C-2′ (δ_C 41.5, s), and H-1′, 3′ with C-4a, 4b, respectively, suggested the same two *p*-substituted phenylenes also simultaneously attached to an isopropylidene. Based on the above evidence, the structure of **1** was elucidated to be 1,3-di-*O*-[2′,2′-di-(*p*-phenylene) isopropylidene] glycerol (Fig. 1).

Compound **2** was obtained as white powder. It showed in its negative-ion FAB-MS spectrum a quasimolecular ion peak at *m/z* 483 $[M-1]^-$ in accordance with the formula of $C_{30}H_{60}O_4$ as determined by HR-FAB-MS, and confirmed

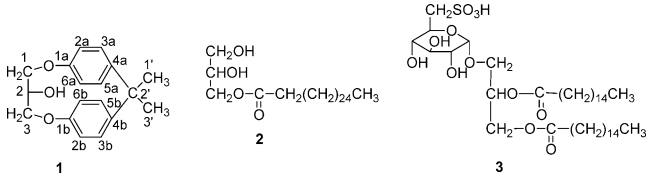

Fig. 1. Structures of Compounds **1**—**3**

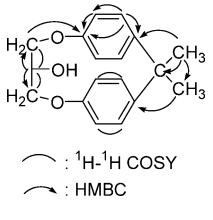

Fig. 2. Key ^1H–^1H COSY and HMBC Correlations of Compound **1**

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from the ^{13}C- and DEPT NMR spectra. Its IR spectrum revealed absorption bands for –OH at 3419 cm^{-1} and C=O at 1733 cm^{-1}. The ^{13}C- (DEPT) and ^{1}H-NMR spectra of **2** showed signals for a fatty acid moiety [δ_C 14.0 (q), 22.6 (t), 24.8 (t), 29.0—29.6 (t), 31.8 (t), 34.1 (t), 174.3 (s), δ_H 0.85 (3H, t, J=6.4 Hz), 1.22 (br s, $n\times CH_2$), 1.60 (2H, m), 2.33 (2H, t, J=7.5 Hz)], two oxymethylene [δ_C 63.2, 65.1, δ_H 4.17 (2H, m), 3.67 (1H, dd, J=11.6, 3.8 Hz), 3.55 (1H, dd, J=11.6, 5.8 Hz)], and one oxymethine [δ_C 70.2, δ_H 3.92 (1H, m)]. These data suggested that **2** was a fatty acid-1-glyceride[9,10]; this was supported by the HMBC spectrum. In the HMBC spectrum, correlations of H-1 (δ_H 4.17, 2H, m), H-3a (δ_H 3.55, 1H, dd, J=11.6, 5.8 Hz) with C-2 (δ_C 70.2, d), and H-1, H-2 (δ_H 3.92, 1H, m) with C-3 (δ_C 63.2, t) indicated the presence of an unsymmetrical substitution glycerol moiety, furthermore, correlations of H-1, H-2′ (δ_H 2.33, 2H, t, J=7.5 Hz), H-3′ (δ_H 1.60, 2H, m) with C-1′ (δ_C 174.3, s) indicated the acyl of fatty acid moiety attached to C-1. According to the FAB-MS spectrum showing the molecular weight, the fatty acid moiety should be heptacosanoic acid. Based on the literature which reported that the analogue right optical rotation glyceride had an absolute configuration of 2*S*,[11] the structure of **2** was elucidated as (2*S*)-1-*O*-heptatriacontanoyl glycerol {$[\alpha]_D^{26.2}$ +7.5° (c=0.20, pyridine)} (Fig. 1).

3 showed a quasimolecular ion peak at m/z 793 $[M-1]^-$ in its negative-ion ESI-MS spectrum. Together with ^{1}H- and ^{13}C-NMR spectral data, a molecular formula $C_{41}H_{78}O_{12}S$ was established. Its IR spectrum indicated the presence of a sulphonyl group (1121, 1028, 792 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra closely resembled those of (2*S*)-1,2-di-*O*-miristoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl) glycerol and other analogous compounds.[6] Treatment of **3** with sodium methoxide–methanol gave 6-sulpho-quinovopyranosyl glycerol (**3a**) (identified by ^{1}H-, ^{13}C-NMR spectra and by comparison with literature data[12]) and methyl palmitate (analysed by GC mass spectrometry). Based on these findings, **3** was determined to be (2*S*)-1,2-di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl) glycerol which had even been isolated from the brown alga of *Ishige okamurai*. The element of sulphur was rich in the brown alga, but this was the first report of **3** obtained from the genus of *Sargassum*.

Experimental

General Experimental Procedures All the mps were obtained on an XRC-1 micromelting apparatus and were uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H-, ^{13}C-NMR and 2D NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with TMS as internal standard. EI-MS and FAB-MS spectral data were obtained on a VG Autospec-3000 spectrometer, and 70 eV for EI-MS. ESI-MS spectral data were obtained on a LCQ^{DECA} XP HPLC/MS^n spectrometer. Silica gel (200—300 mesh) for column chromatography and GF_{254} for TLC were obtained from Qindao Haiyang Chemical Co., Ltd, Qindao, China.

Plant Material The brown alga of *Sargassum parvivesiculosum* was collected from Sanya, Hainan province, China, in May 2002. It was identified by Prof. S. Zhang, South China Sea Institute of Oceanology, Academia Sinica. The voucher specimen (No. 2002-5) was deposited in the herbarium of the Department of Taxonomy, South China Sea Institute of Oceanology, Academia Sinica, Guangzhou, China.

Extraction and Isolation The air-dried and powdered bodies (18.0 kg) of *S. parvivesiculosum* were extracted with EtOH three times under reflux, and the solvent was evaporated *in vacuo*. The residue was partitioned in H_2O and extracted with EtOAc and *n*-BuOH three times, respectively. The EtOAc and *n*-BuOH extracts were concentrated *in vacuo* to afford 130 g and 53 g of residue, respectively. The EtOAc portion was subjected to column chromatography (CC) on silica gel, using petroleum ether–EtOAc gradients (from 1 : 0 to EtOAc) as eluents. Combining the fractions with TLC (GF_{254}) monitoring, eight fractions were obtained. Fraction **1** (10 g) was then subjected to CC on silica gel, eluted with petroleum ether–EtOAc gradient to give **5** (18 mg). Fraction **2** (23 g) was subjected to CC on silica gel, eluted with $CHCl_3$–EtOAc (10 : 1 to 10 : 3) to give **1** (8 mg). Fraction **3** (7 g) was subjected to CC on silica gel, eluted with $CHCl_3$–EtOAc gradients (from 5 : 1 to 4 : 1) to give **2** (10 mg), **4** (9 mg), 6 (11 mg). Fraction 5 (23 g) was subjected to CC on silica gel, eluted with $CHCl_3$–Me_2CO (from 10 : 1 to 10 : 3) to give **7** (8 mg) and **8** (9 mg). The *n*-BuOH portion was subjected to column chromatography (CC) on silica gel, eluted with $CHCl_3$–MeOH gradients (from 1 : 0 to MeOH) to give **3** (26 mg).

Alkaline Hydrolysis and GC Analysis Compound **3** (5.0 mg) in dry MeOH (1 ml) was separately treated with 5% NaOMe–MeOH (0.5 ml) at room temperature for 10 min. The reaction mixture was neutralized with Dowex 50 W×8 and the resin removed by filtration. The filtrate was extracted with hexane and the hexane layer was concentrated to yield fatty acid methyl esters. This was analysed by GC-MS: Hewlett-Packard 5890 GC equipped with a mass-selective detector MSD 5970 MS, a split injector and a fused-silica column HP-5 (25 m×0.2 mm; i.d. 0.33 mm film); column temp. 230 °C, carrier N_2, flow rate 30 ml/min. t_R (min) of methyl palmitate was 12.5, which was identical to the authentic standard methyl palmitate. Removal of the solvent from the MeOH layer under reduced pressure gave a residue which was purified by silica gel CC ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1) to furnish **3a**.

1,3-Di-*O*-[2′,2′-di-(*p*-phenylene) Isopropylidene] Glycerol (**1**): White powder. $C_{18}H_{20}O_3$. ^{1}H-NMR (500 MHz, pyridine-d_5) δ_H: 7.09 (4H, d, J=8.0 Hz, H-3a, 5a, 3b, 5b), 6.90 (4H, d, J=8.0 Hz, H-2a, 6a, 2b, 6b), 1.58 (6H, s, H-1′, 3′), 4.48 (4H, m, H-1 and H-3), 4.64 (1H, m, H-2). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 157.4 (s, C-1a, 1b), 143.6 (s, C-4a, 4b), 127.9 (d, C-3a, 5a, 3b, 5b), 114.8 (d, C-2a, 5a, 2b, 5b), 30.4 (q, C-1′, 3′), 41.5 (s, C-2′), 69.9 (t, C-1, 3), 68.7 (d, C-2). IR (KBr) cm^{-1}: 3435, 3000, 2923, 2850, 1730, 1630, 1510, 1104, 826, 802. UV λ_{max}(MeOH) nm: 209, 229, 256, 277, 284. EI-MS m/z: 284 $[M]^+$ (10), 269 $[M-15]$ (30), 256 (13), 228 (8), 213 (25), 191 (8), 168 (5), 139 (20), 119 (16), 99 (20), 85 (18), 71 (30), 60 (100), 43 (85). HR-EI-MS m/z: 284.1408 $[M-H]^-$ (Calcd for $C_{18}H_{20}O_3$: 284.1412).

(2*S*)-1-*O*-Heptatriacontanoyl Glycerol (**2**): White powder. mp 64—65 °C. $C_{30}H_{60}O_4$. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H: 0.85 (3H, t, J=6.4 Hz, Me-27′), 1.22 (46H, br s, H-4′ to H-26′), 1.60 (2H, m, H-3′), 2.33 (2H, t, J=7.5 Hz, H-2′), 3.55 (1H, dd, J=11.6, 5.8 Hz, H-3a), 3.67 (1H, dd, J=11.6, 3.8 Hz, H-3b), 3.92 (1H, m, H-2), 4.17 (2H, m, H-1). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 65.1 (C-1), 70.2 (d, C-2), 63.3 (t, C-3), 174.4 (s, C-1′), 34.1 (t, C-2′), 24.8 (t, C-3′), 29.0—29.6 (t, C-4′ to C-24′), 31.8 (t, C-26′), 22.6 (t, C-25′), 14.0 (q, C-27′). IR (KBr) cm^{-1}: 3419, 2920, 2850, 1733, 1469, 1389, 1180, 992. Negative FAB-MS m/z: 483 $[M-1]^-$ (70), 376 (13), 255 (100). HR FAB-MS m/z: 483.4443 $[M-H]^-$ (Calcd for $C_{30}H_{59}O_4$: 483.4413). $[\alpha]_D^{26.2}$ +7.5° (c=0.20, pyridine).

(2*S*)-1,2-Di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl) Glycerol (**3**): White powder. ^{1}H-NMR (500 MHz, $CDCl_3$+CD_3OD) δ_H: 4.40 (1H, dd, J=11.6, 3.0 Hz, H-1a), 4.11 (1H, dd, J=11.6, 6.8 Hz, H-1b), 5.26 (1H, m, H-2), 3.56 (1H, dd, J=11.0, 6.0 Hz, H-3a), 3.86 (1H, dd, J=11.0, 5.0 Hz, H-3b), 4.81 (1H, d, J=3.6 Hz, H-1′), 3.56 (1H, overlapped, H-2′), 3.73 (1H, dd, J=9.0, 9.5 Hz, H-3′), 3.57 (1H, overlapped, H-4′), 3.98 (1H, ddd, J=2, 9.2, 9.5 Hz, H- 5′), 3.34 (1H, dd, J=2, 14.3 Hz, H-6′a), 3.24 (1H, dd, J=9.2, 14.3 Hz, H-6′b), 0.85 (6H, t, J=6.4 Hz, Me-16″), 1.22 (48H, br s, H-4″ to H-15″), 1.55 (4H, m, H-3″), 2.29 (4H, m, H-2″). ^{13}C-NMR (125 MHz, $CDCl_3$+CD_3OD) δ_C: 63.0 (t, C-1), 70.1 (d, C-2), 67.3 (t, C-3), 98.9 (d, C-1′), 71.4 (d, C-2′), 72.7 (d, C-3′), 73.4 (d, C-4′), 67.8 (d, C-5′), 53.6 (t, C-6′), 174.2 (s, C-1″), 34.4 (t, C-2″), 25.0 (t, C-3″), 29.2—29.8 (t, C-4″—13″), 31.9 (t, C-14″), 22.7 (t, C-15″), 14.1 (q, C-16″). IR (KBr) cm^{-1}: 3350, 2922, 1734, 1121, 1028, 1104, 792. Negative ESI-MS m/z: 793 $[M-1]^-$. $[\alpha]_D^{25}$ +42.5° (c=1, MeOH).

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References

1) Zhang J. B., "Marine Medicament and Effection," Traditional Chinese Medical Science Ancient Books Press, Beijing, 1998, pp. 186—187.
2) Tang H. F., Yi Y. H., Yao X. S., *Chin. Pharm. J.*, **37**, 262—265 (2002).
3) Xu S. H., Ding L. S., Wang M. K., *Chin. J. Org. Chem.*, **22**, 138—140 (2002).
4) Liu H. B., Cui Z., Li Y. S., *Chin. Pharm. J.*, **33**, 464—466 (1998).
5) Tang H. F., Yi Y. H., Yao X. S., *Chin. Marine Medic.*, **5**, 5—9 (2002).
6) Luca R., Nunziatina D. T., Ingeborg B., *Phytochemistry*, **45**, 647—650 (1997).
7) Tang H. F., Yi Y. H., Yao X. S., Lin H. W., *J. Asian Nat. Prod. Res.*, **4**, 95—101 (2002).
8) Tang H. F., Yi Y. H., Yao X. S., "Marine Natural Productions and Natural Biochemistry Medicaments Collected Papers," Beihai, China, 2001, pp. 20—30.
9) Yang H., Jiang B., Hou A. J., Lin Z. W., Sun H. D. J., *Asian Nat. Prod. Res.*, **2**, 177—185 (2000).
10) Kong L. Y., Wen Z. D., Shi J. J., *Acta Botanica Sinica*, **38**, 161—166 (1996).
11) Dharma R. K., Trevor G. R., Donald M. S., *Biochemistry*, **24**, 519—525 (1985).
12) Jung J. H., Lee H., Kang S. S., *Phytochemistry*, **42**, 447—452 (1996).