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MACROCARQUINOID D, NEW MEROTERPENOID FROM BROWN ALGA, *SARGASSUM MACROCARPUM*

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Abstract – A new meroterpenoid, macrocarquinoid D (**1**) and 11 known meroterpenoids were isolated from brown algae, *Sargassum macrocarpum*. The structure of **1** was elucidated with NMR spectral data using 2D techniques. The DPPH radical scavenging and AGEs inhibitory activity of isolated compounds were examined.

Brown algae, as *Sargassum* species are known, are a rich source of bioactive compounds such as flavonoids, terpenoids, polysaccharides, and polyphenols.[1] One brown alga, *Sargassum macrocarpum* (Sargassaceae), reportedly has bioactivity, with neuroprotective effects,[2] anticancer effects,[3] and anti-inflammatory activity.[4] Our earlier studies revealed that macrocarquinoids A–C, contained in *S. macrocarpum*, possess advanced glycation end products (AGEs) inhibitory activities.[5] During our continued component analyses of *S. macrocarpum*, we isolated a new compound, macrocarquinoid D (**1**), along with 11 known compounds. This report describes the isolation, structure elucidation of **1**, and the DPPH radical scavenging activity and AGEs inhibitory activity of isolated compounds.

Whole dried *S. macrocarpum* was extracted using EtOH. The EtOH extracts were partitioned between hexane and 90% MeOH; the 90% MeOH layer was partitioned between EtOAc and H_2O. The EtOAc soluble portion was subjected to silica gel chromatography, followed by reversed-phase column chromatography and then reversed-phase HPLC (MeOH-H_2O) to afford a new compound, macrocarquinoid D (**1**) and 11 known compounds: sargachromenol (**2**),[6,7] 7-methylsargachromenol (**3**),[8] sargaquinoic acid

(**4**),[6,7] sargaquinal (**5**),[7] sargahydroquinoic acid (**6**),[6] sargahydroquinal (**7**),[9] 3-methylsargaquinoic acid (**8**),[8] sargaquinone (**9**),[10] fallahydroquinone (**10**),[11] and macrocarquinoid B (**11**), and C (**12**).[5] These structures of known compounds were elucidated from comparison of ^{1}H and ^{13}C NMR data.

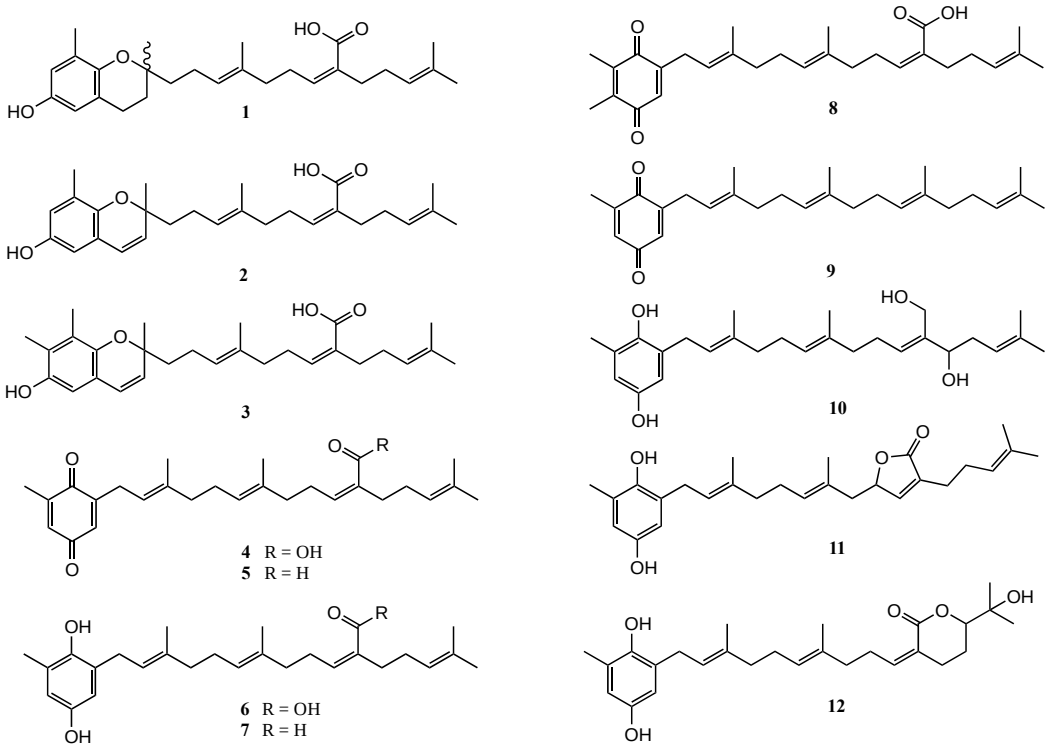

Figure 1. Compounds isolated from *Sargassum macrocarpum*

The molecular formula of macrocarquinoid D (**1**), $C_{27}H_{38}O_4$, was established using HRESIMS [*m/z* 427.28468 $(M+H)^+$, -0.15 mmu]. The IR spectrum suggested the presence of hydroxy groups (3386 cm^{-1}) and unsaturated carbonyl group (1687 cm^{-1}). The gross structure of **1** was deduced from detailed analyses of ^{1}H and ^{13}C NMR data (Table 1) aided by 2D NMR experiments (^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY). The ^{13}C NMR data indicated that the presence of 27 carbons, 13 of which were sp^2 carbons (three bearing an oxygen atom), and 14 of which were sp^3 carbons (one bearing an oxygen atom). The ^{1}H-^{1}H COSY spectrum indicated the connectivities of C-3 to C-4, C-2' to C-3', C-5' to C-7', and C-10' to C-11' (Figure 2). The HMBC correlations of H-5 (δ_H 6.43) to C-6 (δ_C 148.5) and C-8a (δ_C 145.9), H-7 (δ_H 6.48) to C-6, H_3-18' (δ_H 2.19) to C-7 (δ_C 115.4), C-8, (δ_C 125.9) and C-8a, H-4 (δ_H 2.60) to C-8a, H-3 (δ_H 1.73) to C-4a (δ_C 129.7), H_3-17' (δ_H 1.22) to C-2 (δ_C 73.9), and C-3 (δ_C 41.6) suggest that **1** possesses a chromane moiety attached methyl groups at C-2 and C-8 (Figure 2). The presence of carboxy group attached C-8' was revealed the HMBC correlations of H-7' (δ_H 5.92) to C-15' (δ_C 172.2) and H_2-9' (δ_H 2.26) to C-8' (δ_C 132.4) and C-15' (Figure 2). The structures of the other regions were inferred from detailed analyses of HMBC correlations. The *E* configurations of olefins were confirmed by NOESY correlations of H-3' (δ_H

5.15) to H_2-5' (δ_H 2.07) and H-7' to H_2-9'. Consequently, the planar structure of **1** was established (Figure 2). The absolute stereochemistry of meroterpenoids, which possess a chromane ring, was elucidated from CD measurements,[12] but no remarkable cotton effect was observed in CD measurements of **1**. This result and the small optical rotation value indicated **1** as an enantiomeric mixture.

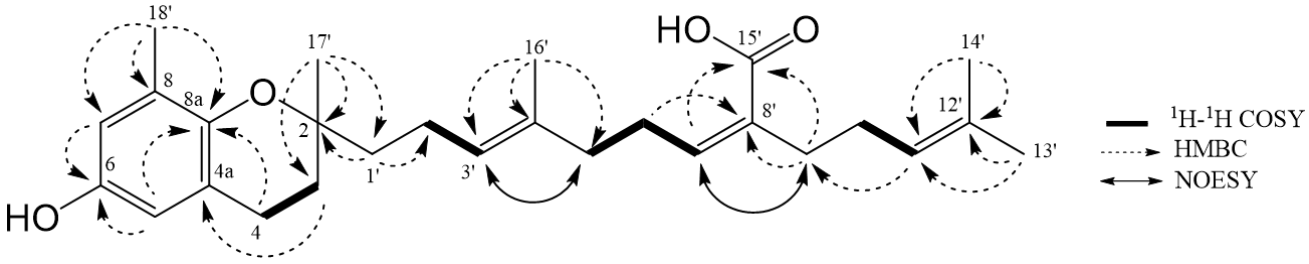

Figure 2. 2D NMR correlations of macrocarquinoid D (**1**)

Table 1. ^{1}H and ^{13}C NMR data for macrocarquinoid D (**1**) in $CDCl_3$

Position	δ_C	δ_H (*J* in Hz)
1		
2	73.9	
3	41.6	1.73 (m)
4	24.6	2.60 (m)
4a	129.7	
5	114.0	6.43 (s)
6	148.5	
7	115.4	6.48 (s)
8	125.9	
8a	145.9	
1'	41.4	1.53 (m)
2'	22.9	2.10 (m)
3'	124.6	5.15 (t, 6.6)
4'	135.4	
5'	39.1	2.07 (m)
6'	28.4	2.58 (m)
7'	143.6	5.92 (t, 7.1)
8'	132.4	
9'	34.7	2.26 (t, 7.3)
10'	27.8	2.12 (m)
11'	123.5	5.09 (t, 6.2)
12'	132.2	
13'	25.7	1.67 (s)
14'	17.7	1.58 (s)
15'	172.2	
16'	16.1	1.61 (s)

17'	26.5	1.22 (s)
18'	16.5	2.19 (s)

The DPPH radical scavenging activity was found for isolated compounds (Figure 1). Comparing IC_{50} of isolated 12 compounds (Table 2), compounds which possess hydroquinone (**6**, **7**, **10**, **11**, **12**), chromene (**2**, **3**), or chromane (**1**) were found to have higher radical scavenging activity than that of quinone (**4**, **5**, **8**, **9**), which was the same trend as that reported earlier.[13] Results also clarified that the chromane type compound **1** was slightly weaker than hydroquinone or chromene-type compounds. Furthermore, because the previously isolated related compounds possessed AGEs-inhibitory activity, the compounds isolated this time were evaluated (Table 2). Since the DPPH radical scavenging and the AGEs-inhibitory activity tend to be different, it is necessary to investigate in detail in the future.

Table 2. The IC_{50} of DPPH radical scavenging and AGEs-inhibitory activity of isolated 12 compounds

Compound	IC_{50} (mM)	
	DPPH radical scavenging activity	AGEs-inhibitory activity
Macrocarquinoid D (**1**)	0.35	0.45
Sargachromenol (**2**)	0.25	1.4
7-Methylsargachromenol (**3**)	0.41	0.87
Sargaquinoic acid (**4**)	0.84	1.6[a]
Sargaquinal (**5**)	1.2	2.2 [a]
Sargahydroquinoic acid (**6**)	0.29	2.5[a]
Sargahydroquinal (**7**)	0.34	1.6[a]
3-Methylsargaquinoic acid (**8**)	> 2	1.1
Sargaquinone (**9**)	> 2	2.6
Fallahydroquinone (**10**)	0.31	0.60
Macrocarquinoid B (**11**)	0.30	2.6[a]
Macrocarquinoid C (**12**)	0.31	1.0[a]
Trolox[b]	0.26	—
Aminoguanidine[b]	—	3.3

[a]: previously reported data[5], [b]: positive control

EXPERIMENTAL

General experimental procedures

Optical rotations were determined using a digital polarimeter (P-1020; Jasco Corp.). The UV and IR spectra were obtained respectively on a UV/VIS spectrometer (GeneQuant 1300; GE Healthcare Life Science) and

a Fourier transform infrared spectrometer (FT/IR-410; Jasco Corp.). Also, ESI/MS was measured using a mass spectrometer (JMS-T100LP AccuTOF LC-plus 4G; JEOL). CD spectra were measured using a circular dichroism spectrometer (J-715; Jasco Corp.). The ^{1}H and ^{13}C NMR spectra were recorded using an NMR spectrometer (JNM-ECX400; JEOL). Tetramethylsilane (TMS) was used as an internal standard. The UV absorption and fluorescence intensity were measured using a multimode microplate reader (Varioskan LUX; Thermo Fisher Scientific Inc.).

Material

The brown alga *Sargassum macrocarpum* was collected in February 2020 at the coast of the Tsukumo-wan in Ishikawa Prefecture in Japan. After collection, the alga sample was washed with water, air-dried, and stored at -30 ℃ until use.

Extraction and purification

The dried *S. macrocarpum* (410 g) was extracted using EtOH (1500 mL × 3) to obtain 43.5 g. The part of EtOH extract (3.9 g) was partitioned between hexane (300 mL × 3) and 90% MeOH (300 mL). The 90% MeOH layer was partitioned between EtOAc (300 mL × 3) and H_2O (300 mL). The EtOAc soluble portions (1.2 g) were subjected to silica gel column chromatography (hexane/acetone, 10:1 → 0:1). The fraction eluted with hexane/acetone (10:1), 6.8 mg, was sequentially subjected to silica gel column chromatography (hexane/EtOAc, 19:1 → 0:1) to give sargaquinone (**9**, 2.8 mg) from hexane/EtOAc (19:1), and sargaquinal (**5**, 2.8 mg) from hexane/EtOAc (9:1). The fraction eluted with hexane/acetone (5:1), 30.3 mg was purified using ODS HPLC (InertSustaine AQ-C18, GL Science Inc 4.6 × 250 mm, MeOH/H_2O 85:15, 1 mL/min, UV 254 nm) to afford sargaquinoic acid (**4**, t_R 17.0 min, 6.3 mg) and 3-methylsargaquinoic acid (**8**, t_R 22.5 min, 2.1 mg). The fraction eluted with hexane/acetone (3:1) was subjected to C18 (Wakogel 100C18) column chromatography (MeOH/H_2O, 60:40 → 100:0), and MeOH/H_2O (70:30), 671 mg was purified continuously using ODS HPLC (InertSustaine AQ-C18, GL Science Inc 10 × 250 mm, MeOH/H_2O 75:25 → 95:5, 1 mL/min, UV 230 nm) to afford macrocarquinoid C (**12**, t_R 13.5 min, 3.4 mg), fallahydroquinone (**10**, t_R 16.0 min, 2.0 mg), macrocarquinoid B (**11**, t_R 17.5 min, 9.5 mg), sargahydroquinoic acid (**6**, t_R 19.0 min, 565 mg), sargahydroquinal (**7**, t_R 23.0 min, 2.0 mg), and sargachromenol (**2**, t_R 26.0 min, 9.5 mg). The fraction eluted with MeOH/H_2O (80:20) was purified using ODS HPLC (InertSustaine AQ-C18, GL Science Inc 4.6 × 250 mm, MeOH/H_2O 90:10, 1 mL/min, UV 254 nm) to afford 7-methylsargachromenol (**3**, t_R 7.5 min, 0.7 mg).

The remaining extract of 39.6 g was partitioned using the same procedure. The EtOAc layer (39.6 g) was subjected to silica gel column chromatography (hexane/acetone, 10:1 → 0.1). The fraction eluted with hexane/acetone (3:2), 1716 mg, was then applied to C18 column chromatography (MeOH/H_2O, 60:40 →

100:0), and MeOH/H_2O (75:25), 1036 mg, was purified continuously using ODS HPLC (InertSustaine AQ-C18, GL Science Inc 10 × 250 nm, MeOH/H_2O 70:30 → 95:5, 1 mL/min, UV 290 nm) to afford a new compound, macrocarquinoid D (**1**, t_R 14.5 min, 3.8 mg) together with macrocarquinoid C (**12**, t_R 11.0 min, 9.1 mg), fallahydroquinone (**10**, t_R 14.0 min, 5.5 mg), macrocarquinoid B (**11**, t_R 16.0 min, 21.5 mg), sargahydroquinoic acid (**6**, t_R 19.5 min, 903 mg), and sargaquinoic acid (**4**, t_R 29.0 min, 56.8 mg).

Macrocarquinoid D (**1**): light yellow amorphous solid; $[\alpha]_D^{22}$ +2.9° (*c* 0.5, $CHCl_3$); IR (KBr) cm^{-1}: 3386, 2923, and 1687; UV(MeOH) nm (log ε) 291 (3.46) and 207 (4.32); ^1H and ^{13}C NMR ($CDCl_3$) see Table 1; HRESI-MS *m/z* 427.28468 (Calcd for $C_{27}H_{39}O_4$: 427.28483).

Evaluation of antioxidant activity

Antioxidant activity was quantified using a DPPH radical antioxidant assay kit. The measurement of each compound was conducted using Trolox as an index.

Evaluation of inhibitory activity of production of AGEs using glyceraldehyde

The antiglycation activity assay was evaluated using a GA and BSA model.[5] Briefly, the sample solution (dissolved in DMSO, 10 μL), GA solution (50 mM, 20 μL), BSA solution (50 mg/mL, 40 μL), and PBS buffer (130 μL) were added to wells in 96-well black plates. Measurements were taken with fluorescence intensity at excitation of 370 nm and emission of 440 nm using a 96-well black plate. After incubation at 37 °C for 24 h, the fluorescence intensities of the respective wells were measured under identical conditions. Aminoguanidine sulfate dissolved in DMSO was used as a positive control; instead of the sample solution, DMSO solution was used as a blank control. The inhibition rate (%) of fluorescent AGE formation was calculated using the following formula.

Inhibition rate (%) = [1– (fluorescence intensity of sample for 24 h incubation – fluorescence intensity of sample at 0 h) / (fluorescence intensity of blank control for 24 h incubation – fluorescence intensity of blank control at 0 h)]

The IC_{50} values were calculated from the logarithmic function given by the inhibition rate of fluorescent AGE formation against the sample concentration.

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