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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.

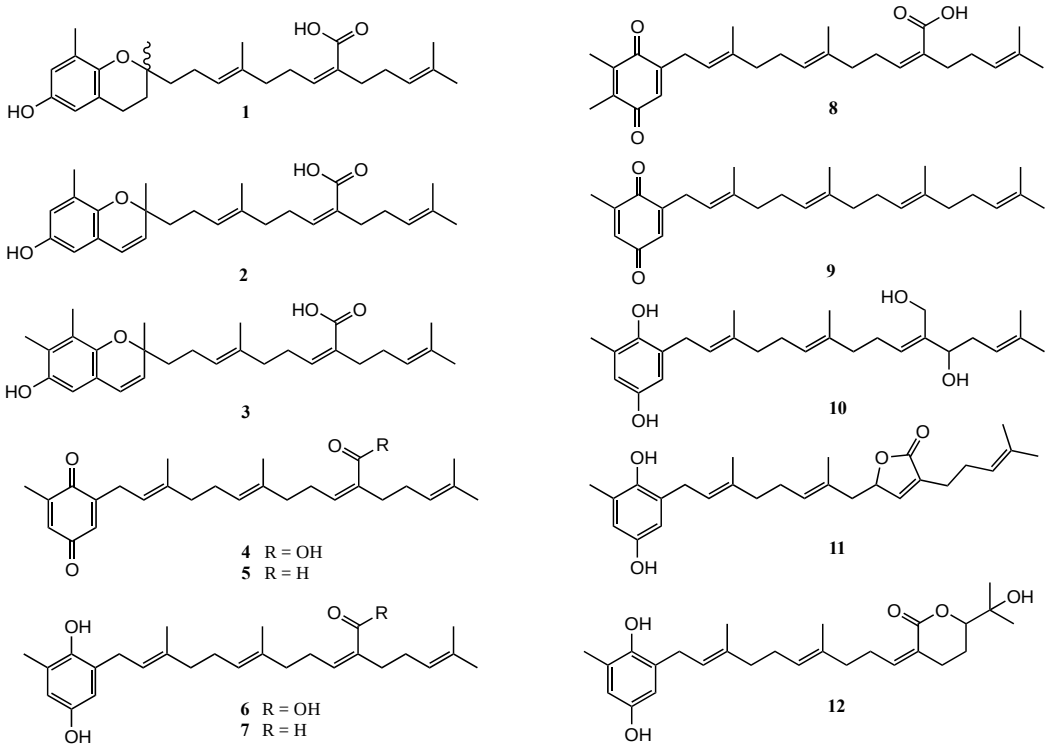


Figure 1. Compounds isolated from *Sargassum macrocarpum*

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 ($\delta_H$ 6.43) to C-6 ($\delta_C$ 148.5) and C-8a ($\delta_C$ 145.9), H-7 ($\delta_H$ 6.48) to C-6, $H_3$-18' ($\delta_H$ 2.19) to C-7 ($\delta_C$ 115.4), C-8, ($\delta_C$ 125.9) and C-8a, H-4 ($\delta_H$ 2.60) to C-8a, H-3 ($\delta_H$ 1.73) to C-4a ($\delta_C$ 129.7), $H_3$-17' ($\delta_H$ 1.22) to C-2 ($\delta_C$ 73.9), and C-3 ($\delta_C$ 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' ($\delta_H$ 5.92) to C-15' ($\delta_C$ 172.2) and $H_2$-9' ($\delta_H$ 2.26) to C-8' ($\delta_C$ 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' ($\delta_H$

5.15) to $H_2$-5' ($\delta_H$ 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.

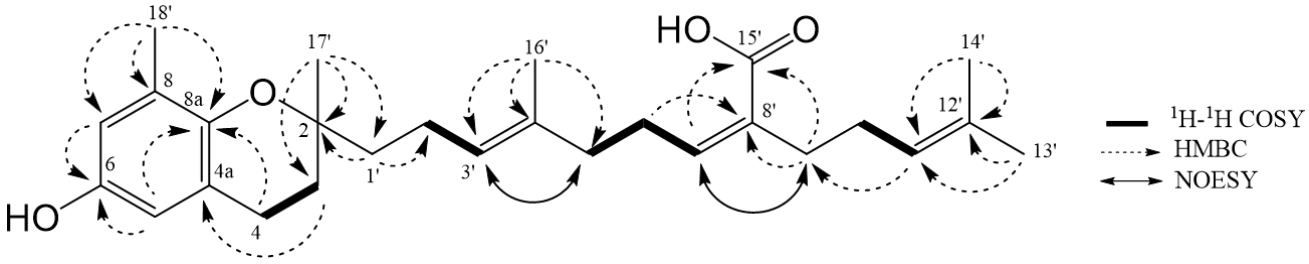


Figure 2. 2D NMR correlations of macrocarquinoid D (**1**)

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

| Position | $\delta_C$ | $\delta_H$ (*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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