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Vasodilatation effect of farnesylacetones, active constituents of *Sargassum siliquastrum*, on the basilar and carotid arteries of rabbits

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ABSTRACT

Two farnesylacetones, **311** and **312**, major active constituents of *Sargassum siliquastrum* collected from the coast of the East Sea in Korea, showed a moderate vasodilatation effect on the basilar arteries of rabbits. Therefore, treatment with farnesylacetones **311** and **312** may selectively accelerate cerebral blood flow through dilatation of the basilar artery.

Natural products obtained from terrestrial plants and microorganisms play an important role in the development of clinical medicines.[1] Recently, marine resources have received the attention of researchers in drug discovery because of their considerable biodiversity in the widespread oceans which cover over 70% of the earth.[1] The marine natural resources are the treasury of a large group of structurally unique secondary metabolites useful to medicine which have yielded a large number of drug candidates.[1] Various marine natural products have been found to be useful tools for physiological and pharmacological studies[2] and many researchers performed clinical trials or preclinical evaluation for cancer,[3–5] pain,[6,7] inflammation,[8,9] and alzheimer[10] from them.

Because vascular-related diseases such as hypertension, stroke, subarachnoid hemorrhage and Alzheimer's dementia are a threat to the public health, the development of modulators that control vascular tone and then alleviate the symptoms is an urgent endeaver.[11,12] The vascular tone is essentially dependent on the cytoplasmic Ca^{2+} concentration of vascular smooth muscle cells (vSMCs) which is regulated by Ca^{2+} entry from the extracellular space through L-type Ca^{2+} channels and inositol 1,4,5-triphosphate (IP_3)-mediated Ca^{2+} release from the sarcoplasmic reticulum (SR) or removal of Ca^{2+} from the cytoplasm to the extracellular space or SR by Ca^{2+} removal mechanisms.[13,14] Among the various targets for regulation of the vascular tone, the voltage-dependent L-type Ca^{2+} channels are effective targets to modulate cytoplasmic Ca^{2+} concentration in physiological myogenic tone regulation and pathophysiological conditions including atherosclerosis and hypertention.[15–17]

Vascular tone modulation of a large cerebral artery, such as the basilar artery, plays a pivotal role in maintaining cerebral blood flow and pressure in physiological and pathophysiological conditions.[18–20] Recently, it was reported that diabetic patients have increased susceptibility to hypoxic and ischemic injury in cerebral blood vessels.[21] Diabetes increases the risk of stroke and stroke mortality, and decreases recovery after stroke due to the increased plasma endothelin-1 (ET-1) levels and basal ET-1 constrictor tone.[22,23] As reported previously, delayed cerebral ischemia may cause death and disability in patients with subarachnoid hemorrhage (SAH) because the extracellular Ca^{2+} influx through L-type Ca^{2+} channels into vascular smooth muscle cells plays a fundamental role in the development and chronic effects of vasospasm after SAH.[24–26]

Therefore, because vasodilatation of the basilar artery is potentially useful in mitigating vasospasm following SAH and cerebral ischemia induced by cerebral thrombosis, and as it decreases the

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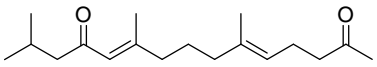
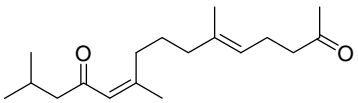


(5*E*,10*E*)-6,10,14-trimethylpentadeca-5,10-diene-2,12-dione(**311**)



(5*E*,10*Z*)-6,10,14-trimethylpentadeca-5,10-diene-2,12-dione(**312**)

Figure 1. 10*E*-Farnesylacetone (**311**) and 10*Z*-farnesylacetone (**312**) purified from *Sargassum siliquastrum* by an activity-guided separation.

risk of stroke in patients of diabetes mellitus, the discovery of vasodilatation molecules with low toxicity profiles for clinical use is essential. These new molecules will be promising drug candidates for the treatment of cerebrovascular diseases.

In the course of searching for vasoactive molecules from marine organisms, we previously reported, for the first time, that the sargahydroquinoic acid extracted from the Brown algae, *Sargassum micracanthum*, showed a selective vasodilatation effect on the basilar artery of more than 10-fold over carotid the artery of white rabbits.[27] Brown algae of the *Sargassum* are known to contain plastoquinones[28,29] and cromanols,[30,31] which have been reported to show antioxidant,[32] antiviral,[33] and anticancer activity.[34]

Based on a similar approach, we discovered that an organic extract of *Sargassum siliquastrum* showed vasodilatation effects on the basilar and carotid artery of white rabbits (data not shown).[35] In addition, it was reported that chromenes extracted from *Sargassum siliquastrum* exhibit the cytotoxicity, antioxidant activity, and inducement of the larval settlement of the hydrozoan.[36,37]

The brown alga *S. siliquastrum* was collected from the coast of the East Sea in Korea and kept in a deep freezer at −70 °C. The specimens (wet wt 140 g) were chopped and then immersed in an 80% methanol (MeOH)-water solution with mechanical stirring for 1 day at room temperature. After evaporating the MeOH, the samples were extracted with ethyl acetate (2 L × 2), and then evaporated to give a crude extract (19 g). The extract (1 g) was separated by C-18 reverse phase HPLC (Phenomenex ODS-aqua (250 × 10 mm) 80% MeOH, 3 mL/min) and grouped into seven parts by retention time. Among the active fractions **3, 4**, and **6**, fraction **3** exhibited a strong vasodilatation effect. With successive HPLC separation using different eluents and flow rates (85% MeOH, 1 mL/min), we can divide fraction 3 into two parts, **31** and **32**. Finally we have obtained two pure active farnesolacetones, **311** (5.7 mg) and **312** (9.6 mg) from the more active fraction **31** by using HPLC (YMC ODS-aqua prep 75% MeOH, 3 mL/min).

^{1}H NMR spectrums of **311** and **312** showed that the structure of the two molecules is almost the same except for the C-10 methyl configuration. Namely, the chemical shift of the C-10 methyl peak of **311** is downshifted to 2.12 ppm due to neighboring group effect of the C-12 carbonyl group, whereas the one of **312** is shown at 1.86 ppm. Therefore, **311** has an *E* geometry and **312** is the *Z* isomer as shown in Figure 1. Because the structure[38,39] and some biological activities[40,41] of two farnesylacetones, **311** and **312**, were already reported, we have confirmed their final structures by comparison with our spectral data (Fig. 1).[42]

Although compound **312** and related compound exhibited an anticholinesterase effects,[40,41] because there is no report that **311** and **312** show a relaxation effect in the cerebral and systemic artery in our knowledge, we tested the vasodilatation effects on the basilar and carotid artery of white rabbits to discover drug candidates against vascular diseases.[43]

The application of a high K^+-containing physiological salt solution on the basilar and common carotid artery generated anisometric contraction by intracellular Ca^{2+} increase through L-type Ca^{2+} channels and reached a steady-state within 20 min. As shown in Figure 2, a single application of **311** in log scale concentration induced vasodilatation of the basilar and common carotid arteries causing them to contract with a high K^+-containing salt solution (50 mM K^+). Serial application of **311** induced vasodilatation of the basilar and common carotid arteries in a concentration-dependent manner. The curve fittings of the concentration–response relationship using the Hill equation confirm that the concentration of the half maximal dilatation (EC_{50}) for basilar and carotid artery

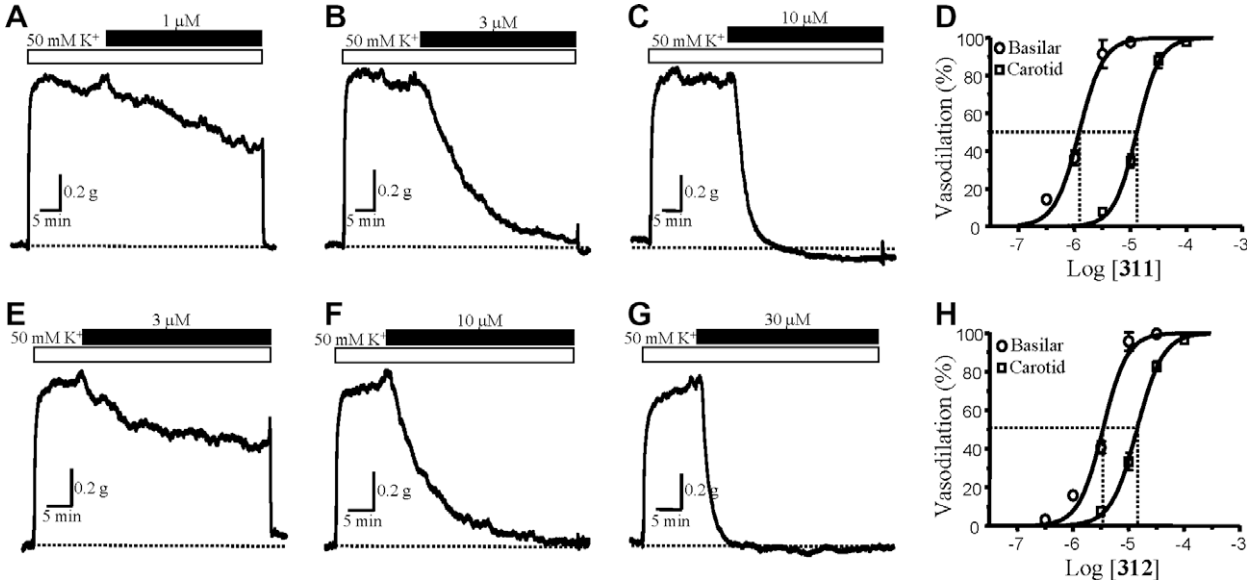


Figure 2. Vasodilatative effects of **311** and **312** from *Sargassum siliquastrum* on depolarization-induced vasoconstriction of rabbit basilar and carotid artery. Representative traces were acquired by single application of **311** in log scale concentration (10^{-6}, $10^{-5.5}$, 10^{-5} M) (A–C) and **312** in log scale concentration ($10^{-5.5}$, 10^{-5}, $10^{-4.5}$ M) (E–G) for 40 min after contraction induced by high K^+ (50 mM) reached a stationary phase (20 min) on the basilar artery (A–C). Vasodilatation effects of **311** and **312** were also investigated in log scale concentration (traces not shown). The vasodilatation efficacy was plotted as a function of log scale concentration for **311** (D) and **312** (H) and the curves were fitted using Hill equation, $E = (1 + EC_{50}/[\mathbf{311}\text{ or }\mathbf{312}]^n)^{-1}$. Data are represented as mean ± SD ($n = 4$).

Table 1
Vasodilatation potency of farnesylacetones **311** and **312** on depolarization-induced constriction of rabbit basilar and common carotid artery.

Compounds	Vasodilatation efficacy (EC_{50})[a]	
	Basilar artery (μM) [b]	Carotid artery (μM)[b]
311	1.22 (±0.25)	13.7 (±2.4)
312	3.72 (±0.65)	14.5 (±1.8)

[a] Concentration–response curves were fitted by a function of non-linear regression assuming a Hill equation ($E = [1 + EC_{50}/[\mathbf{311}\text{ or }\mathbf{312}]^n]^{-1}$).
[b] Values are means of three experiments, standard deviation is given in parentheses.

were 1.22 ± 0.28 and 13.7 ± 2.4 μM ($n = 4$), respectively. These results indicated that **311** dilated the cerebral and systemic artery of rabbits.

In the same way, we found the EC_{50} of **312** for the basilar and carotid arteries to be 3.72 ± 0.65 and 14.5 ± 1.8 μM ($n = 4$), respectively. (Table 1) When we compared the EC_{50} of the dilation effect of each compound against a basilar and carotid artery of rabbits, two farnesylacteones, **311** and **312**, selectively relaxed the basilar artery over the carotid artery. Specifically, **311** showed a selectivity of more than 10-fold against the basilar over the carotid artery. Thus, treatment of farnesylacetones extracted from *Sargassum siliquastrum* may accelerate cerebral blood flow through vasodilatation of the basilar artery without influencing the systemic blood pressure. Although this is preliminary result for vasodilatation of the basilar artery from ex vivo screening, we have discovered a promising lead structure of a basilar dilatation agent for human cerebral vascular diseases.

In conclusion, the activity-guided purification for an organic extract of *Sargassum siliquastrum* gave vasodilatative constituents, farnesylacetones **311** and **312**, which effectively dilated the basilar and carotid arteries of rabbits with a moderate selectivity for the basilar artery. These molecules will be a novel lead compounds as well as drug candidates against the treatment of cerebral vascular diseases.

Acknowledgment

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42. *Spectral data for **311**:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.03 (1H, s, H-11), 5.08 (1H, t, J = 7.1 Hz, H-5), 2.47 (2H, t, J = 7.3Hz, H-13), 2.28 (3H, m, H-7 and H-14), 2.14 (3H, s, H-1), 2.12 (3H, s, 10-CH_3), 1.61 (3H, s, 6-CH_3), 1.55 (2H, t, H-8), 0.92 (6H, d, J = 6.6 Hz, 14-CH_3 2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.7, 201.3, 158.2, 135.8, 123.6, 123.2, 53.5, 43.7, 40.7, 39.1, 29.9, 25.7, 25.2, 22.7, 22.4, 19.3, 15.8 ppm; HRTOFMS m/z 301.2774 $[M+Na]^+$, calcd for $C_{18}H_{30}O_2Na$ 301.2143; for **312**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.03 (1H, s, H-11), 5.08 (1H, t, J = 7.1 Hz, H-5), 2.53 (2H, m, H-13), 2.46 (2H, m, H-7), 2.27 (3H, m, H-7 and H-14), 2.12 (3H, s, H-1), 2.01(2H, t, J = 7.4 Hz, H-9), 1.86 (3H, s, 10-CH_3), 1.62 (3H, s, 6-CH_3), 1.52 (2H, m, H-8), 0.92 (6H, d, J = 6.6 Hz, 14-CH_3 2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.9, 200.6, 158.9, 136.3, 124.2, 122.7, 53.5, 43.8, 39.7, 33.4, 29.9, 26.5, 25.5, 25.1, 22.7, 22.6, 22.5, 15.9 ppm; HRTOFMS m/z 301.2775 $[M+Na]^+$, calcd for $C_{18}H_{30}O_2Na$ 301.2143.
43. *Experimental procedures:* After anesthetizing twelve male white rabbits weighing 2–2.5 Kg by inhalation of enflurane, the basilar and common carotid arteries were isolated quickly under sterile conditions and placed in a physiological salt solution (PSS) that contained (in mM): 137 NaCl, 5.4 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 23.8 $NaHCO_3$, and 5.5 glucose. Residual blood was rinsed from the lumen and adherent connective tissue, fat, and adventitia were carefully removed. The basilar and carotid arteries were cut into rings (3 mm) in a dissecting chamber filled with PSS saturated with a 95% O_2 and 5% CO_2 mixture. Basilar and carotid rings were mounted using a pair of stainless steel hooks under a resting tension of 0.6 g and 1.5 g, respectively, in organ baths containing 15 mL of PSS, which was maintained at 37 °C and bubbled with a 95% O_2 and 5% CO_2 mixture. One of the hooks was connected to a force displacement transducer (MLT050; AD Instruments, Colorado Springs, CO, USA) and the tension was recorded with Powerlab/400 on a chart program (AD Instruments). After equilibration for 30 min, each ring specimen was repeatedly exposed to the high K^+ solution (50 mM K^+), prepared by replacing NaCl with an equimolar concentration of KCl, until the responses became stable. Functional endothelial cells were confirmed by the ability of acetylcholine (10 μM) to induce relaxation. Concentration-response relationships were obtained by a single application of **311** or **312** in a log scale concentration after precontraction induced by high K^+ reached a steady-state on the basilar artery and common carotid artery.