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Establishment of absolute stereostructure of falcarindiol, algicidal principle against *Heterocapsa circularisquama* from Notopterygii Rhizoma

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ABSTRACT

Falcarindiol (**1**) was isolated as an algicidal principle against the harmful red tide dinoflagellate, *Heterocapsa circularisquama*, from Notopterygii Rhizoma through bioassay-guided separation. In order to determine the ambiguous absolute structure of this active principle, all three stereoisomers as well as falcarindiol (**1**) were synthesized. As a result of intensive analysis of their physicochemical properties, the configuration of **1** was revealed to be 3*R*,8*S*. On the other hand, (3*S*,8*S*)- and (3*S*,8*R*)-isomers were found to exhibit more potent algicidal activity than (3*R*,8*S*)-falcarindiol (**1**) isolated from Notopterygii Rhizoma. In addition, the diyne moiety of **1** was established as the crucial structural requirement for algicidal potency.

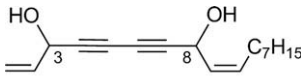
In recent years, harmful algal blooms (HABs) have been reported to occur world-wide affecting public health and fisheries industries. The HABs frequently caused large-scale red tides and mass mortalities of cultured fishes and shellfish, resulting in significant damage to ecosystem. *Heterocapsa circularisquama*,[1] recorded as a new variety of dinoflagellate in 1995, has been widely recognized to be one of the most troublesome algae causing the noxious red tide. In particular, this species showed potent toxicity against bivalves, such as oysters, pearl oysters, and Japanese littlenecks leading to enormous damage for aquaculture industries.[2] However, there have been few useful and environmentally benign methods to control HAB of this causative alga so far. Hence, we have been engaged in exploration of algicidal principles against *H. circularisquama* from medicinal plants. In this Letter, we deal with the establishment of the absolute structure of falcarindiol (**1**), isolated from Notopterygii Rhizoma as an active principle, by the synthesis of all stereoisomers. Furthermore, we wish to describe the structural requirement of **1** for algicidal potency.

H. circularisquama is a unicellular alga with about 20 μm of size and whirls briskly in the sphere of life, while dead individuals settle without moving. So, algicidal activity was evaluated by direct count for the number of whirling and settling *H. circularisquama* under microscope observation after co-incubation with each sample for 48 h in artificial sea water.[3]

As a result of screening about 400 extracts from medicinal plants by using this assay, the MeOH extract of Notopterygii Rhizoma (rhizoma of *Notopterygium incisum*) was found to exhibit potent algicidal activity. Bioassay-guided separation of the extract through successive DIAION HP-20, SiO_2, and ODS column chromatography, and ODS HPLC disclosed an active principle (0.066% from the crude drug) with MIC of 0.5 μg/mL. The gross structure of the algicidal principle was identified to be that of falcarindiol (**1**) by comparison of the spectroscopic features such as the NMR and FAB-MS data with those described in the literature (Fig. 1).[4]

Although falcarindiol (**1**) and its two stereoisomers were isolated from a variety of natural resources, the optical rotation of falcarindiol (**1**) from Notopterygii Rhizoma in the present study was not in accordance with any reported data. In addition, there seems no consistency between the reported optical rotations and the absolute stereochemistry (See the Supplementary data). In this context, the synthesis of falcarindiol (**1**) and all the stereoisomers of **1** was conducted to establish the conclusive index resolving the confusion in stereochemistry of falcarindiols as well as to elucidate the participation of the two chiral centers of **1** in algicidal activity.

Retrosynthetic analysis of **1** exemplified by the 3*R*,8*S* diastereomer is depicted in Figure 2. This route enables to synthesize the four possible stereoisomers of **1** by changing the chirality of the asymmetric catalysis, namely, the conjugated diyne of **1** would be constructed by Cadiot–Chodkiewicz reaction[5] from two alkynes **i** and **ii**. The chiral centers bearing the hydroxyl groups of **i** and **ii**



falcarindiol (**1**)

Figure 1. Gross structure of falcarindiol (**1**).

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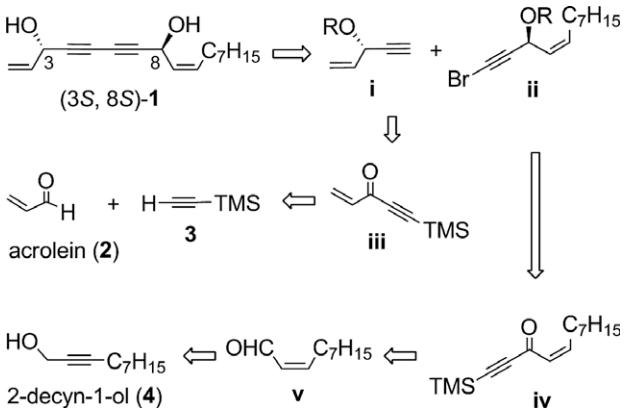


Figure 2. Retrosynthetic analysis of falcarindiol (**1**).

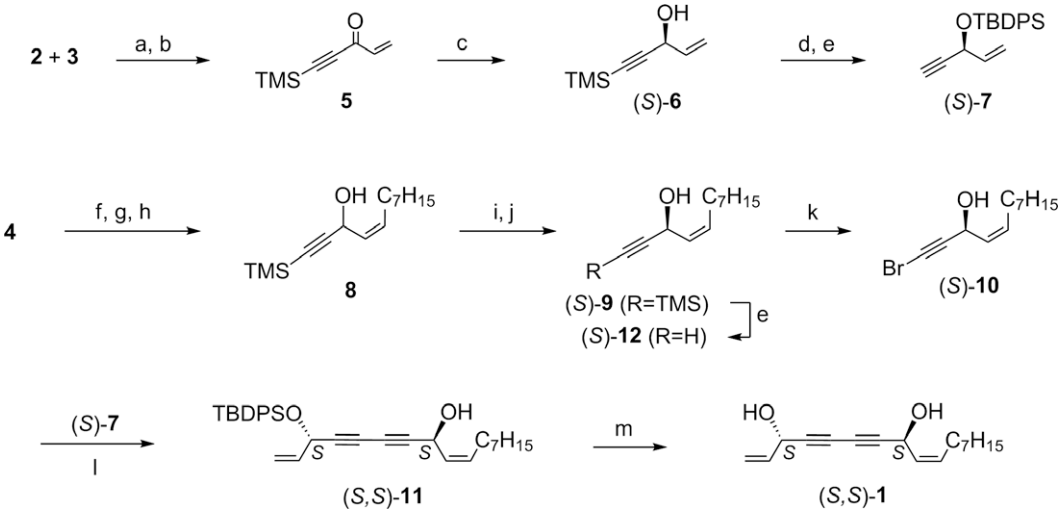
were provided by asymmetric reduction of corresponding ketones **iii** and **iv**, respectively. The conjugated ketone **iii** was yielded by condensation of acrolein **2** and protected acetylene **3**, while the ketone **iv** was also prepared from **3** and aldehyde **v** in a similar manner. *Z*-Olefin **v** was prepared from 2-decyn-1-ol (**4**) by hydrogenation with Lindlar catalysis.

The synthesis of falcarindiol (**1**) is summarized in Scheme 1. Coupling of acrolein (**2**) with trimethylsilylacetylene (**3**) in the presence of *n*-BuLi followed by MnO_2 oxidation afforded conjugated ketone **5**. The resulting ketone **5** was subjected to asymmetric reduction by the complex of (*S*)-2-methyl-CBS-oxazaborolidine and BH_3 [(*S*)-CBS catalyst][6,7] to furnish chiral alcohol (*S*)-**6**. Protection of the hydroxyl group in (*S*)-**6** as *t*-butyldiphenylsilyl (TBDPS) ether and succeeding removal of the trimethylsilyl (TMS) function provided the left-side segment, alkyne (*S*)-**7**, in 50% yield from **3**. Hydrogenation to 2-decyn-1-ol (**4**) by Lindlar catalyst under a hydrogen atmosphere gave *Z*-olefin**,** which was submitted to MnO_2 oxidation and subsequent condensation with **3** to afford alcohol **8**. After conversion of alcohol **8** to the corresponding ketone, asymmetric reduction of the ketone by (*S*)-CBS catalyst provided chiral secondary alcohol (*S*)-**9**. The predictable configurations of both (*S*)-**6** and (*S*)-**9** were confirmed to be *S* by application of modified Mosher's method[8] (See the Supplementary data), while the optical purities higher than 99% ee of (*S*)-**7**, (*R*)-**7**, (*S*)-**9**, and (*R*)-**9** were determined by chiral HPLC analysis. Treatment of (*S*)-**9** with *N*-bromosuccinic imide (NBS) in the presence of silver nitrate prompted deprotection of the TMS residue and bromination to give brominated alkyne (*S*)-**10**.[9] Cadiot–Chodkiewicz reaction between (*S*)-**7** and (*S*)-**10** with the aid of cuprous chloride, *n*-butylamine, and hydoxylamine hydrochloride afforded diyne (3*S*,8*S*)-**11**. Finally, deprotection of the TBDPS group of **11** by tetra-*n*-butylammonium fluoride furnished our desired (3*S*,8*S*)-**1**[10] in 23% overall yield for 8 steps from **4**. In addition, (*R*)-**7** and (*R*)-**10** were prepared by using (*R*)-CBS catalyst and the remaining three stereoisomers of (3*S*,8*S*)-**1**[10] were synthesized from the corresponding segments (**7** and **10**) in the same manner.

In comparison with the algicidal falcarindiol (**1**) from Notopterygii Rhizoma, the synthesized (3*R*,8*S*)-**1** showed the superimposable ^{1}H, ^{13}C NMR, IR, and FAB-MS spectra. In addition, the optical rotation of the synthesized **1**, +232.0° (*c* 0.33, $CHCl_3$), is the closest to that of the isolated **1**, +232.8° (*c* 0.33, $CHCl_3$), among the four synthesized stereoisomers as shown in Table 1. Furthermore, the two diastereomers exhibited the fairly different absolute values from the isolated and synthesized falcarindiols (**1**). Thus, the absolute configuration of **1** from Notopterygii Rhizoma was unambiguously concluded to be 3*R*,8*S*.

Finally, algicidal activity of the four synthesized stereoisomers against *H. circularisquama* was evaluated. As depicted in Table 2, the (3*S*,8*S*)- and (3*S*,8*R*)-isomers exhibited more potent activity than the naturally occurring (3*R*,8*S*)-**1**. Therefore, 3*S*-configuration was revealed to enhance algicidal activity with regard to the four stereoisomers. Moreover to elucidate the participation of the diyne portion of **1** in bioactivity, both enantiomers [(*S*)-**12** and (*R*)-**12**] of (*E*)-dodeca-4-ene-1-yne-3-ol, the right half structure of **1**, were prepared from **9**. Interestingly, both the compounds exhibited little algicidal activity at the concentration of 4 μM. Consequently, the conjugated diyne moiety of falcarindiol (**1**) was clarified to be essential for potent algicidal activity against *H. circularisquama*.

In conclusion, we disclosed falcarindiol (**1**) as the algicidal principle against *H. circularisquama* from Notopterygii Rhizoma through bioassay-guided separation. By intensive comparison with the optical rotations among the four synthesized stereoisomers, **1** was securely elucidated to possess 3*R*,8*S*-configuration. In addition, the conjugated diyne portion was established as the crucial



Scheme 1. Synthesis of falcarindiol and stereoisomers. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C to rt, 95%; (b) MnO_2, CH_2Cl_2, rt, 82%; (c) (*S*)-2-methyl-CBS-oxazaborolidine, BH_3·THF, THF, −40 °C; (d) TBDPSCl, imidazole, CH_2Cl_2, rt; (e) KOH, MeOH, THF, rt, *S*: 64%, *R*: 65% for **7** from **5**, *S*: 94%, *R*: quant. for **12** from **9**; (f) H_2, Pd–$CaCO_3$, MeOH, rt, 98%; (g) MnO_2, CH_2Cl_2, rt, 87%; (h) **3**, *n*-BuLi, THF, −78 °C to rt, 82%; (i) MnO_2, CH_2Cl_2, rt, 75%; (j) (*S*)-2-methyl-CBS-oxazaborolidine, BH_3·THF, THF, −40 °C, *S*: 85%, *R*: 82%; (k) NBS, $AgNO_3$, acetone, rt, *S*: 87%, *R*: 86%; (l) (*S*)-**7**, CuCl, *n*-$BuNH_2$, NH_2OH·HCl, MeOH, 0 °C; (m) *n*-Bu_4NF, THF, rt, 3*S*,8*S*: 60%, 3*S*,8*R*: 61%, 3*R*,8*S*: 64%, 3*R*,8*R*: 62% from **10**.

Table 1
Optical rotations of falcarindiol and stereoisomers

		$[\alpha]_D^{24}$ (c 0.33)
Synthetic product	3*R*,8*S*	+232.0°
	3*R*,8*R*	−187.3°
	3*S*,8*S*	+188.1°
	3*S*,8*R*	−233.2°
Natural product	3*R*,8*S*	+232.8°

Table 2
Algicidal activity of falcarindiol and stereoisomers

		Algicidal activity		
		4 μM	2 μM	1 μM
Synthetic compound	3*R*,8*S*	+	±	−
	3*R*,8*R*	±	±	−
	3*S*,8*S*	+	+	±
	3*S*,8*R*	+	+	±
Natural product	3*R*,8*S*	+	±	−

Algicidal activity; +: 100%, 100%> ± ≧50%, − <50%.

structural requirement for algicidal potency of **1** on the basis of structure activity relationship of the synthesized congeners of **1**. On the other hand, it should be noted that the (3*S*,8*S*)- and (3*S*,8*R*)-stereoisomers showed twofold potent algicidal activity in comparison with falcarindiol (**1**). So far, synthetic hydroxy fatty acid amides[11] and polyunsaturated fatty acids[12] were only found out as low-molecule algicides against *H. circularisquama*. Among the former, the amide containing L-aspartic acid showed MIC of 0.3 μg/mL, while the latter displayed growth inhibition ranging from 30% to 69% at the concentration of 25 μg/mL. Taking these findings into account, falcarindiol (**1**) as well as the (3*S*,8*S*)- and (3*S*,8*R*)-isomers should be regarded as significantly potent algicidal principles against *H. circularisquama*.

Supplementary data

Supplementary data (the previously reported absolute configurations and optical rotations of falcarindiol, and distributions of $\Delta\delta$ values in the modified Mosher's method for (*S*)-**6** and (*S*)-**9** are available) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.047.

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10. *(3S,8S)*-**1**: colorless oil. IR (KBr): 3250, 2155, 1646 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.94 (1H, ddd, J = 16.8, 10.2, 5.3 Hz, 2-H), 5.60 (1H, dt, J = 10.4, 7.3 Hz, 10-H), 5.51 (1H, dd, J = 10.4, 8.0 Hz, 9-H), 5.47 (1H, d, J = 16.8 Hz, 1-Ha), 5.26 (1H, d, J = 10.2 Hz, 1-Hb), 5.20 (1H, br d, J = 8.0 Hz, 8-H), 4.94 (1H, br d, J = 5.3 Hz, 3-H), 2.11 (2H, dt, J = 7.3, 7.1 Hz, 11-H), 1.98 (2H, br s, 3, 8-OH), 1.38 (2H, quint, J = 7.1 Hz, 12-H), 1.27 (8H, m, 13, 14, 15, 16-H), 0.88 (3H, t, J = 7.1 Hz, 17-H). FAB-MS m/z: 261 $[M+H]^+$. FAB-HRMS m/z: calcd for $C_{17}H_{25}O_2$: 261.1854, found: 261.1855.
(3R,8S)-**1**: colorless oil. IR (KBr): 3252, 2155, 1645 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.95 (1H, ddd, J = 16.5, 10.5, 5.5 Hz, 2-H), 5.60 (1H, dt, J = 11.0, 7.5 Hz, 10-H), 5.52 (1H, dd, J = 11.0, 7.5 Hz, 9-H), 5.47 (1H, d, J = 16.5 Hz, 1-Ha), 5.26 (1H, d, J = 10.5 Hz, 1-Hb), 5.20 (1H, dd, J = 7.5, 5.1 Hz, 8-H), 4.95 (1H, dd, J = 6.5, 5.5 Hz, 3-H), 2.12 (2H, dt, J = 7.5, 7.0 Hz, 11-H), 2.02 (1H, d, J = 6.5 Hz, 3-OH), 1.92 (1H, d, J = 5.1 Hz, 8-OH), 1.39 (2H, quint, J = 7.0 Hz, 12-H), 1.28 (8H, m, 13, 14, 15, 16-H), 0.89 (3H, t, J = 7.0 Hz, 17-H). FAB-MS m/z: 261 $[M+H]^+$. FAB-HRMS m/z: calcd for $C_{17}H_{25}O_2$: 261.1854, found: 261.1850.
(3S,8R)-**1**: colorless oil. FAB-MS m/z: 261 $[M+H]^+$. FAB-HRMS m/z: calcd for $C_{17}H_{25}O_2$: 261.1854, found: 261.1854. The IR and ^1H NMR spectra were superimposable on those of (3*R*,8*S*)-**1**.
(3R,8R)-**1**: colorless oil. FAB-MS m/z: 261 $[M+H]^+$. FAB-HRMS m/z: calcd for $C_{17}H_{25}O_2$: 261.1854, found: 261.1853. The IR and ^1H NMR spectra were superimposable on those of (3*S*,8*S*)-**1**.
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