Pergamon

PII: S0031-9422(97)00476-7

Phytochemistry, Vol. 48, No. 6, pp. 1003–1011, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

NAHOCOLS AND ISONAHOCOLS, ENDOTHELIN ANTAGONISTS FROM THE BROWN ALGA, *SARGASSUM AUTUMNALE*

NAOKO TSUCHIYA, AIYA SATO*, HIDEYUKI HARUYAMA†, TOHRU WATANABE‡ and YASUTERU IIJIMA‡

Biomedical Research Laboratories, †Analytical and Metabolic Research Laboratories, and ‡Pharmacology and Molecular Biology Research Laboratories, Sankyo Co. Ltd. 2-58 1-chome, Shinagawa-ku, Tokyo 140-0005, Japan

(*Received in revised form* 21 *April* 1997)

Key Word Index—*Sargassum autumnale*; Sargassaceae; brown alga; nahocols; isonahocols; aryl prenyl ether; prenyl hydroquinone; endothelin antagonists.

Abstract—Novel endothelin antagonists, nahocols A, A_1, B, C, D_1 and D_2, and isonahocols D_1 and D_2, were isolated from the brown alga, *Sargassum autumnale*. Their structures were determined through detailed analysis of NMR spectra and chemical reactions. Nahocols have an aryl prenyl ether structure, which are speculated to be biogenetic precursors of the ubiquitous prenyl hydroquinones or prenyl benzoquinones in the plant and animal kingdoms. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Endothelin (ET-1) [1] is an endothelium-derived 21-amino acid peptide. It has a very potent vasoconstriction activity in isolated blood vessels and constitutes an ET family with its two isoforms, ET-2 and ET-3, and four highly homologous cardiotoxic peptides, sarafotoxin S6a–d. ET has specific binding-sites on many cell membranes, such as smooth muscle and the endothelial and epithelial cells of many organs. The binding-sites have been grouped into two subtypes, ET_A and ET_B based on ligand affinity. The obvious increase of ET-1 blood levels has been clinically observed in various diseases, including acute renal insufficiency, acute myocardial infarction, hypertension and arteriosclerosis. ET receptor antagonists may therefore be effective for the prevention and treatment of these diseases. A variety of natural and synthetic antagonists [2] has been found, some of these are in clinical trials.

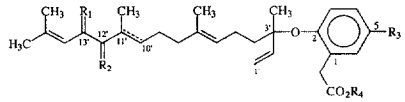
In the course of a programme for investigating new ET antagonists in marine organisms, we screened extracts of marine organisms for inhibition of ET-1 binding to its receptors (ET_A and ET_B) and found that the brown alga, *Sargassum autumnale*, produced endothelin antagonists [3]. We herein report on the isolation, characterization and antagonistic activities of the novel antagonists (**1a–f**, **2a–b**). *Sargassum autumnale* is a relatively rare brown alga, found in shallow and protected waters of limited coasts washed by the Tsushima Current. Of great biological interest is that *S. autumnale* matures in summer, while most other brown algae belonging to this taxon, mature in early spring through early summer in Japan.

RESULTS AND DISCUSSION

Sargassum autumnale was air-dried, powdered and extracted with methanol at room temperature. The methanol extract was partitioned between hexane and 90% aqueous methanol. The antagonistic activity remained in the 90% aqueous methanol fraction, containing a complex mixture of closely related compounds. The 90% aqueous methanol extract was repeatedly separated by a combination of normal and reversed-phase chromatography guided by endothelin binding assay [4].

Nahocol A (**1a**), a colourless oil, analysed for $C_{29}H_{42}O_6$ by HREI mass spectrometry ($[M]^+$ m/z 486.3001, Δ +1.9 mmu). The IR indicated the presence of hydroxyl (3450 cm^{-1}), an ester (1730 cm^{-1}) and carbonyl (1705 cm^{-1}). The ^1H NMR (Table 1) was consistent with the presence of a secondary, a tertiary and three olefinic methyls, a proton on each carbon bearing an oxygen atom, five olefinic protons, a methoxyl, an isolated methylene and a trisubstituted aromatic ring. In the ^{13}C NMR (Table 1), signals assignable to 15 sp^3 and 14 sp^2 carbons were observed. The sp^3 carbons contained six methyls, six methylenes, two tertiary and a quaternary one. The sp^2 carbons comprised two trisubstituted double bonds, a vinyl double bond and the trisubstituted aromatic ring, in addition to the ketone and ester carbonyls. **1a** was converted to a diacetate (**3a**) and a methyl ether (**3b**),

* Author to whom correspondence should be addressed.



Nahocol A (**1a**) :R_1=H, OH;R_2=O;R_3=OH;R_4=CH_3;C_{10}-C_{11}= single bond
Nahocol A_1(**1b**) :R_1=H, OH;R_2=O;R_3=OH;R_4=CH_3;C_{10}-C_{11}= single bond
Nahocol B (**1c**) :R_1=H, H;R_2=O;R_3=OH;R_4=CH_3;C_{10}-C_{11}= single bond
Nahocol C (**1d**) :R_1=R_2=H, OH;R_3=OH;R_4=CH_3;C_{10}-C_{11}= single bond
Nahocol D_1(**1e**) :R_1=R_2=H, OH;R_3=OH;R_4=CH_3;C_{10}-C_{11}= *E*-double bond
Nahocol D_2 (**1f**) :R_1=R_2=H, OH;R_3=OH;R_4=CH_3;C_{10}-C_{11}= *E*-double bond
3a :R_1=H, OAc;R_2=O;R_3=OAc;R_4=CH_3;C_{10}-C_{11}= single bond
3b :R_1=H, OH;R_2=O;R_3=OCH_3;R_4=CH_3;C_{10}-C_{11}= single bond
4a :R_1=O;R_2=H, OH;R_3=OH;R_4=H;C_{10}-C_{11}= single bond
4b :R_1=O;R_2=H, OH;R_3=OH;R_4=H;C_{10}-C_{11}= single bond

Fig. 1. Structures of nahocols and their derivatives.

respectively, compatible with the presence of a secondary and a phenolic hydroxyl. Treatment of **3a** with dilute NaOH transposed the carbonyl from C-12′ to C-13′ to yield a diastereomeric mixture of **4a** and **4b**.

HMQC and relayed COSY experiments indicated the presence of 10 substructures (**A**–**J**) (Fig. 2). Three protons at δ 2.68 (H-11′), 4.86 (H-13′) and 1.05 (H-19′) showed cross-peaks with C-12 at δ 214.6, suggesting the connectivities of **A**, **B** and **C**. The proton (H-11′) also showed a cross-peak with C-9′ (δ 25.3), consistent with the connectivity of **C** and **D**. The connectivity of **D** and **E** was supported by the long-range couplings of H-6′ (δ 5.05) and H-18′ (δ 1.55) to C-8′ (δ 39.4). The proton (H-18′) coupled to C-2′ (δ 143.6) and C-4′ (δ 42.0), and three vinyl protons [δ 5.17, 5.18 (H-1′), 6.04 (H-2′)] also coupled to C-3′ (δ 81.7). These H-C correlations were compatible with the connectivity of **E**–**G**. Substructures (**H**–**J**) constituted a 2,5-dihydroxyphenylacetic acid (homogenistic acid) moiety, any of whose hydroxyls made an ether linkage with **F**.

In the NOESY experiment, NOEs were observed between an aromatic proton (H-3) and H-1′, 4′ and 17′ (Fig. 2), thus indicating that C-3′ was connected to C-2 through an ether linkage, because two aromatic protons must show NOE if the alternative ether linkage at C-5 is correct. Nahocol-A, consequently, could be depicted as methyl 5-hydroxy-2-(*O*)-[13′-hydroxy-

Table 1. ^{1}H and ^{13}C NMR data of compounds **1a** and **1b** ($CDCl_3$)

No.	**1a** ^{13}C δ	**1a** ^{1}H δ [*J* (Hz)]	**1b** ^{1}H δ [*J* (Hz)]
1	127.2 (C)		
2	148.9 (C)		
3	119.7 (CH)	6.91 (*d*, 8.8)	6.93 (*d*, 9.0)
4	114.1 (CH)	6.57 (*dd*, 3.4, 8.8)	6.59 (*dd*, 2.9, 9.0)
5	149.9 (C)		
6	117.7 (CH)	6.68 (*d*, 3.4)	6.69 (*d*, 2.9)
1′	114.2 (CH_2)	5.17 (*dd*, 0.9, 11.2)	5.18 (*dd*, 1.0, 11.1)
		5.18 (*dd*, 0.9, 17.6)	5.19 (*dd*, 1.0, 18.4)
2′	143.6 (CH)	6.04 (*dd*, 11.2, 17.6)	6.05 (*dd*, 11.1, 18.4)
3′	81.7 (C)		
4′	42.0 (CH_2)	1.72 (*m*)	1.72 (*m*)
5′	22.3 (CH_2)	2.07 (*m*)	2.09 (*m*)
		2.07 (*m*)	2.09 (*m*)
6′	126.4 (CH)	5.09 (*t*, 7.0)	5.09 (*t*, 6.8)
7′	134.6 (C)		
8′	39.4 (CH_2)	1.94 (*t*, 6.8)	1.92 (*t*, 6.8)
		1.94 (*t*, 6.8)	1.92 (*t*, 6.8)
9′	25.3 (CH_2)	1.32 (*m*)	1.2–1.4 (*m*)
		1.32 (*m*)	1.2–1.4 (*m*)
10′	33.6 (CH_2)	1.32 (*m*)	1.2–1.4 (*m*)
		1.58 (*m*)	1.5–1.7 (*m*)
11′	41.3 (CH)	2.68 (*sixt*, 6.8)	2.68 (*sixt*, 6.8)
12′	214.6 (C)		
13′	74.3 (CH)	4.86 (*dd*, 3.9*, 9.8)	4.93 (*br d*, 9.7)
14′	121.1 (CH)	4.98 (*ddd*, 1.0, 1.5, 9.8)	4.96 (*br d*, 9.7)
15′	139.9 (C)		
16′	25.9 (CH_3)	1.80 (*d*, 1.0)	1.81 (*br s*)
17′	22.5 (CH_3)	1.36 (*s*)	1.38 (*s*)
18′	15.7 (CH_3)	1.55 (*s*)	1.56 (*s*)
19′	16.1 (CH_3)	1.05 (*d*, 6.8)	1.10 (*d*, 6.8)
20′	18.6 (CH_3)	1.86 (*d*, 1.5)	1.87 (*br s*)
1″	36.4 (CH_2)	3.55 (*d*, 16.2)	3.55 (*d*, 16.1)
		3.61 (*d*, 16.2)	3.61 (*d*, 16.1)
2″	172.3 (C)		
OMe	51.8 (CH_3)	3.68 (*s*)	3.69 (*s*)

* Disappeared with D_2O.

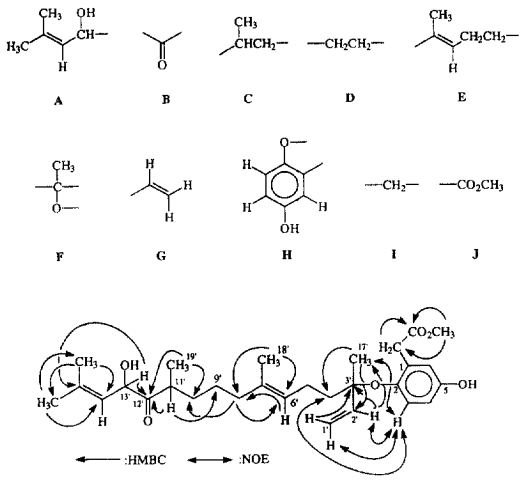


Fig. 2. Substructures of compound **1a** and their HMBC and NOE correlations.

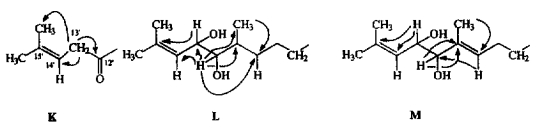


Fig. 3. Substructures of compounds **1c**–**f** correlated by HMBC.

12′-oxo-3′,7′,11′,15′-tetramethyl-1′,6′(*E*),14′-hexadecatrien-3′-yl]phenylacetate (**1a**). The relative and absolute configurations at C-3′,-11′ and -13′ remain unclear.

Nahocol-A_1 (**1b**), a colourless oil, analysed for $C_{29}H_{42}O_6$, isomeric to **1a**, by HREI mass spectrometry ($[M]^+$ *m/z* 486.2991, Δ +0.9 mmu). The IR indicated the presence of hydroxyl (3600 and 3350 cm^{-1}), ester (1730 cm^{-1}) and carbonyl (1710 cm^{-1}). The ^1H NMR of **1b** also closely resembled **1a** (Table 1), indicating that they shared the same planar structure. They were, however, stereoisomeric to each other at C-3′, -11′ or -13′.

Nahocol-B (**1c**), a colourless oil, analysed for $C_{29}H_{42}O_5$ by HREI mass spectrometry ($[M]^+$ *m/z* 470.3019, Δ −1.3 mmu). Although the spectral features were closely related to those of **1a**, the ^1H and ^{13}C NMR suggested a substructure **K**, in which the hydroxyl at C-13′ of **1a** was replaced by a hydrogen atom. The diagnostic cross-peak between H-13′ (δ 3.14) and C-12′ (δ 213.8), together with other cross-peaks (Fig. 3), were observed in the HMBC experiment. Therefore, nahocol-**B** could be assigned as methyl 5-hydroxy-2-(*O*)-[12′-oxo-3′,7′,11′,15′-tetramethyl-1′,6′(*E*),14′-hexadecatrien-3′-yl]phenylacetate (**1c**).

Nahocol-C (**1d**), a colourless oil, analysed for $C_{29}H_{44}O_6$ by HREI mass spectrometry ($[M-H_2O]^+$ *m/z* 470.3017, Δ −1.5 mmu) and had no carbonyl. The DQFCOSY spectrum indicated the presence of a substructure **L** (Fig. 3), in addition to **E**–**J**. The HMBC could connect them to methyl 2-(*O*)-[12′,13′-dihydroxy-3′,7′,11′,15′-tetramethyl-1′,6′(*E*),14′-hexadecatrien-3′-yl]-5-hydroxyphenylacetate (**1d**). On treatment with acetone dimethyl acetal in the presence of *p*-TsOH, **1d** gave a *cis*-acetonide (**5a**) (Fig. 4), whose stereochemistry was assigned by NOE [5] between H-12′ (δ 3.80) and H-13′ (δ 4.78). The vicinal hydroxyls were therefore *erythro* to each other.

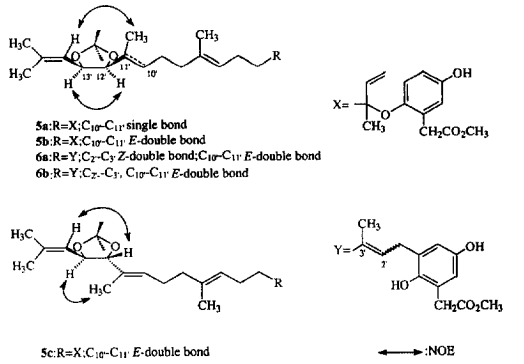


Fig. 4. Structures of acetonides.

Nahocol-D_1 (**1e**), and -D_2 (**1f**), had the same molecular formula $C_{29}H_{42}O_6$ determined by HREI mass spectrometry (**1e**: $[M-H_2O]^+$ *m/z* 468.2854, Δ −2.2 mmu, **1f**: $[M-H_2O]^+$ *m/z* 468.2866, Δ −1.0 mmu), and were diastereomeric to each other at C-12′ or C-13′. Although they resembled **1d** in spectral features, they were different from **1d** in the number of olefinic methyls and double bonds: four methyls and four double bonds for **1e** and **1f** and three methyls and three double bonds for **1d**. The chemical shifts and splitting patterns [**1e**: δ 3.87 (*d*, *J* = 6.8 Hz), **1f**: δ 3.81 (*d*, *J* = 7.8 Hz)] of each H-12′, which were related to C-11′, -12′ and -19′ by HMBC (Fig. 3), indicated that the double bond was located between C-10′ and C-11′, and **1e** and **1f** could be assigned as diastreoisomers of methyl 2-(*O*)-[12′,13′-dihydroxy-3′,7′,11′,15′-tetramethyl-1′,6′(*E*),10′(*E*),14′-hexadecatetraen-3′-yl]-5-hydroxy phenylacetate. **1e** and **1f** also gave *cis*- (**5c**) and *trans*- (**5b**) acetonides (Fig. 4), respectively, whose stereochemistry were determined by the respective NOEs between H-12′ (δ 4.53) and H-13′ (δ 4.94), H-12′ (δ 3.97) and H-14′ (δ 5.17) and H-13′ (δ 4.45) and H-19′ (δ 1.66). Therefore, **1e** was *erythro* while **1f** was *threo*.

Isonahocol D_1 (**2a**) and isonahocol D_2 (**2b**) could not be separated by HPLC on ODS and silica gel with a combination of solvents. They could be only successful purified by normal phase HPLC using nitrated silica gel, NO_2 with 10% *i*-propanol in hexane.

Isonahocol D_1 (**2a**), a colourless oil, had a molecular formula $C_{29}H_{42}O_6$ (HREI mass spectrum $[M-H_2O]^+$

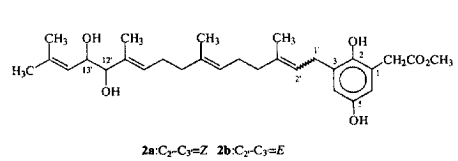
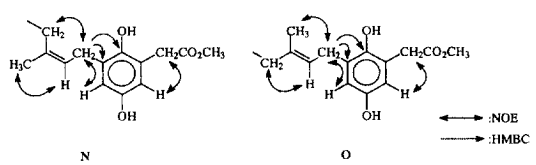


Fig. 5. Structures of compounds **2a** and **2b**.

Table 2. ^1H and ^{13}C NMR data of compounds **1c** and **1d** ($CDCl_3$)

No.	**1c** ^{13}C δ	**1c** ^1H δ [J (Hz)]	**1d** ^{13}C δ	**1d** ^1H δ [J (Hz)]
1	126.9 (C)		127.1 (C)	
2	147.6 (C)		148.0 (C)	
3	119.7 (CH)	6.91 (*d*, 9.0)	119.7 (CH)	6.91 (*d*, 9.0)
4	114.1 (CH)	6.58 (*dd*, 3.0, 9.0)	114.1 (CH)	6.57 (*dd*, 3.0, 9.0)
5	150.3 (C)		149.9 (C)	
6	117.8 (CH)	6.68 (*d*, 3.0)	117.7 (CH)	6.68 (*d*, 3.0)
1′	114.2 (CH_2)	5.17 (*dd*, 1.0, 11.2)	114.2 (CH_2)	5.18 (*dd*, 1.0, 11.2)
		5.18 (*dd*, 1.0, 17.3)		5.19 (*dd*, 1.0, 17.4)
2′	143.6 (CH)	6.04 (*dd*, 11.2, 17.3)	143.7 (CH)	6.05 (*dd*, 11.2, 17.4)
3′	81.6 (C)		81.7 (C)	
4′	42.1 (CH_2)	1.75 (*m*)	41.9 (CH_2)	1.75 (*m*)
		1.75 (*m*)		1.75 (*m*)
5′	22.3 (CH_2)	2.10 (*m*)	22.3 (CH_2)	2.07 (*m*)
		2.10 (*m*)		2.07 (*m*)
6′	124.3 (CH)	5.10 (*t*, 7.0)	124.0 (CH)	5.09 (*t*, 6.8)
7′	134.9 (C)		135.3 (C)	
8′	39.6 (CH_2)	1.94 (*t*, 7.0)	39.8 (CH_2)	1.92 (*t*, 6.8)
		1.94 (*t*, 7.0)		1.92 (*t*, 6.8)
9′	25.5 (CH_2)	1.36 (*m*)	25.1 (CH_2)	1.31 (*m*)
		1.36 (*m*)		1.46 (*m*)
10′	32.6 (CH_2)	1.31 (*m*)	32.9 (CH_2)	1.19 (*m*)
		1.64 (*m*)		1.35 (*m*)
11′	44.7 (CH)	2.68 (*m*)	34.2 (CH)	1.65 (*m*)
12′	213.8 (C)		77.1 (CH)	3.45 (*t*, 5.4)
13′	41.0 (C)	3.14 (*d*, 6.8)	69.5 (CH)	4.39 (*dd*, 5.4, 9.3)
14′	116.0 (CH)	5.29 (*t*, 6.8)	123.3 (CH)	5.34 (*d*, 9.3)
15′	135.5 (C)		138.5 (C)	
16′	25.7 (CH_3)	1.75 (*br s*)	26.0 (CH_3)	1.77 (*br s*)
17′	22.5 (CH_3)	1.36 (*s*)	22.6 (CH_3)	1.37 (*s*)
18′	15.7 (CH_3)	1.55 (*s*)	15.8 (CH_3)	1.56 (*s*)
19′	16.4 (CH_3)	1.06 (*d*, 6.8)	14.3 (CH_3)	0.96 (*d*, 6.8)
20′	18.5 (CH_3)	1.80 (*br s*)	18.5 (CH_3)	1.73 (*br s*)
1″	36.3 (CH_2)	3.55 (*d*, 16.0)	36.3 (CH_2)	3.55 (*d*, 16.1)
		3.61 (*d*, 16.0)		3.61 (*d*, 16.1)
2″	172.7 (C)		172.4 (C)	
OMe	51.8 (CH_3)	3.68 (*s*)	51.9 (CH_3)	3.68 (*s*)



N O

Fig. 6. Substructures of compounds **2a** and **2b** correlated by HMBC and NOE.

m/z 468.2884, Δ −0.8 mmu) and its ^1H–^1H COSY and ^1H–^{13}C COSY spectra indicated the presence of a substructure **N**, in addition to **E** and **M**. The cross-peaks of H-1′ with C-1, -2 and -6 in the HMBC were consistent with **N** (Fig. 6). The *Z*-configuration of the C-2–C-3, double bond was determined by NOEs. **2a** was therefore depicted as methyl 2,5-dihydroxy-3-[12′,13′-dihydroxy-3′,7′,11′,15′-tetramethyl-2′(*Z*),6′(*E*),10′(*E*),14′-hexadecatetraen-1′-yl]phenylacetate.

Isonahocol D_2 (**2b**) had the same molecular formula $C_{29}H_{42}O_6$ (HREI mass spectrometry [M-H_2O]$^+$ *m/z* 468.2870, Δ −0.5 mmu) as **2a**. The NOEs in a substructure **O** (Fig. 6), in addition to other spectral properties, showed that **2b** differed from **2a** in the stereochemistry of the C-2–C-3, double bond, and could be depicted as methyl 2,5-dihydroxy-3-[12′,13′-dihydroxy-3′,7′,11′,15′-tetramethyl-2′(*E*),6′(*E*),10′(*E*),14-hexadecatetraen-1′-yl]phenylacetate. The vicinal hydroxyls of **2a** and **2b** were both *erythro* based on the formation of the corresponding *cis*-acetonides (**6a**, **b**) (Fig. 4).

Many *Sargassums* [5–11] and their relatives [11–13] frequently produce prenyl hydroquinones or prenyl benzoquinones, while *S. autumnale* contains nahocols, with an aryl prenyl ether structure, and isonahocols with a prenyl hydroquinone one. It may be speculated that the latter are biosynthesized through Claisen rearrangement of the former. Some isonahocol-type metabolites of **1a**–**f** were found in the extract, but they have not yet been purified. Attempts to rearrange **1e** to **2a** chemically did not succeed because of their

Table 3. ^1H and ^{13}C NMR data of compounds **1e** and **1f** ($CDCl_3$)

	1e		**1f**	
No.	^{13}C δ	^1H δ [J (Hz)]	^{13}C δ	^1H δ [J (Hz)]
1	127.2 (C)		127.1 (C)	
2	148.0 (C)		148.0 (C)	
3	119.7 (CH)	6.91 (*d*, 9.0)	119.7 (CH)	6.91 (*d*, 9.0)
4	114.1 (CH)	6.57 (*dd*, 3.0, 9.0)	114.1 (CH)	6.58 (*dd*, 3.0, 9.0)
5	149.9 (C)		149.9 (C)	
6	117.7 (CH)	6.67 (*d*, 3.0)	117.7 (CH)	6.68 (*d*, 3.0)
1′	114.2 (CH_2)	5.16 (*dd*, 1.0, 11.2)	114.2 (CH_2)	5.16 (*dd*, 1.0, 11.1)
		5.17 (*dd*, 1.0, 17.3)		5.18 (*dd*, 1.0, 17.4)
2′	143.6 (CH)	6.04 (*dd*, 11.2, 17.3)	143.6 (CH)	6.05 (*dd*, 11.1, 17.4)
3′	81.7 (C)		81.7 (C)	
4′	41.9 (CH_2)	1.69 (*m*)	41.9 (CH_2)	1.70 (*m*)
		1.69 (*m*)		1.70 (*m*)
5′	22.3 (CH_2)	2.09 (*m*)	22.4 (CH_2)	2.07 (*m*)
		2.09 (*m*)		2.07 (*m*)
6′	124.6 (CH)	5.11 (*t*, 7.0)	124.4 (CH)	5.11 (*t*, 7.0)
7′	134.8 (C)		134.8 (C)	
8′	39.1 (CH_2)	2.00 (*t*, 7.0)	39.1 (CH_2)	1.99 (*t*, 7.6)
		2.00 (*t*, 7.0)		1.99 (*t*, 7.6)
9′	26.0 (CH_2)	2.15 (*m*)	26.0 (CH_2)	2.11 (*m*)
		2.15 (*m*)		2.11 (*m*)
10′	129.4 (C)	5.47 (*t*, 6.8)	128.7 (CH)	5.40 (*dd*, 5.9, 7.3)
11′	133.7 (C)		133.7 (C)	
12′	80.2 (CH)	3.87 (*d*, 6.8)	81.0 (CH)	3.81 (*d*, 7.8)
13′	69.3 (CH)	4.31 (*dd*, 6.8, 8.8)	70.2 (CH)	4.29 (*dd*, 7.8, 8.3)
14′	123.5 (CH)	5.19 (*t*, 8.8)	123.6 (CH)	5.13 (*d*, 8.3)
15′	139.0 (C)		138.5 (C)	
16′	26.0 (CH_3)	1.78 (*br s*)	25.9 (CH_3)	1.71 (*br s*)
17′	22.5 (CH_3)	1.36 (*s*)	22.6 (CH_3)	1.37 (*s*)
18′	15.8 (CH_3)	1.58 (*s*)	15.8 (CH_3)	1.57 (*s*)
19′	11.9 (CH_3)	1.66 (*s*)	12.4 (CH_3)	1.60 (*s*)
20′	18.6 (CH_3)	1.73 (*br s*)	18.5 (CH_3)	1.68 (*br s*)
1″	36.3 (CH_2)	3.55 (*d*, 16.0)	36.3 (CH_2)	3.55 (*d*, 16.0)
		3.61 (*d*, 16.0)		3.61 (*d*, 16.0)
2″	172.7 (C)		172.4 (C)	
OMe	51.8 (CH_3)	3.68 (*s*)	51.9 (CH_3)	3.68 (*s*)

instability under the reaction conditions. In addition, we are uncertain if nahocols and isonahocols are naturally occurring or obtained by esterification of the corresponding carboxylic acids during methanolic extraction.

Endothelin antagonistic activities of the nahocols and isonahocols are listed in Table 5. Although they are not always potent and selective, they appeared to be somewhat ET_B-selective.

EXPERIMENTAL

General. ^1H (400 MHz) and ^{13}C NMR (100 MHz) were recorded in $CDCl_3$ with TMS as an int. standard. IR were recorded in $CHCl_3$. UV and optical rotations were recorded in EtOH. Silica gel (230–400 mesh) and packed Lobar columns [Si-60 (normal phase) and RP-18 (reversed-phase)] were used for low pressure CC or TLC. Packed columns (20ϕ × 250 mm) of silica gel, NO_2 and ODS were used for prep. HPLC.

Plant material. Sargassum autumnale was collected in shallow water (1 m deep) at the end of Omosu Bay, Okino Shima, Shimane Pref., Japan, in June 1991. It was authenticated by Prof. M. Kajimura of Oki Marine Biological Experimental Station, Shimane University, where a voucher specimen is kept.

Extraction and isolation. The alga (3.5 kg), after washing, was air-dried, powdered and extracted ×3 with MeOH (20 l) at room temp. The combined extracts were concd to dryness under red. pres. and then partitioned between hexane and 90% aq. MeOH. Concn of the active 90% aq. MeOH layer under red. pres., left a brown oil (140 g). Sepn was monitored by inhibition of each fr. against ET-1 binding to a porcine lung ET_B receptor [4]. The 90% aq. MeOH extract (120 g) was subjected to silica gel CC (1.2 kg). Elution with a 10% gradient mixt. of EtOAc in hexane gave Fr-1 (20–30% EtOAc, 32.87 g, 59% inhibition at 10 $\mu g\ m^{-1}$), Fr-2 (20–30% EtOAc, 9.94 g, 100% inhibition at 10 $\mu g\ m^{-1}$), Fr-3 (30–40% EtOAc, 15.01

Table 4. ^1H and ^{13}C NMR data of compounds **2a** and **2b** ($CDCl_3$)

No.	**2a** ^{13}C δ	**2a** ^1H δ [J (Hz)]	**2b** ^{13}C δ	**2b** ^1H δ [J (Hz)]
1	121.7 (C)		127.1 (C)	
2	146.6 (C)		146.7 (C)	
3	131.0 (C)		148.0 (C)	
4	116.0 (CH)	6.56 (*d*, 3.0)	116.0 (CH)	6.56 (*d*, 3.0)
5	149.3 (C)		149.3 (C)	
6	115.1 (CH)	6.46 (*d*, 3.0)	115.1 (CH)	6.46 (*d*, 3.0)
1′	28.9 (CH_2)	3.32 (*d*, 6.8)	29.1 (CH_2)	3.33 (*d*, 6.8)
		3.32 (*d*, 6.8)		3.33 (*d*, 6.8)
2′	122.6 (CH)	5.34 (*t*, 6.8)	129.1 (CH)	5.29 (*t*, 6.8)
3′	137.4 (C)		137.2 (C)	
4′	31.8 (CH_2)	2.17 (*m*)	39.5 (CH_2)	2.15 (*m*)
		2.17 (*m*)		2.15 (*m*)
5′	26.2 (CH_2)	2.13 (*m*)	26.1 (CH_2)	2.12 (*m*)
		2.13 (*m*)		2.12 (*m*)
6′	124.6 (CH)	5.12 (*t*, 6.3)	124.4 (CH)	5.10 (*t*, 6.8)
7′	134.8 (C)		134.8 (C)	
8′	39.0 (CH_2)	2.05 (*m*)	39.1 (CH_2)	2.01 (*m*)
		2.05 (*m*)		2.01 (*m*)
9′	25.7 (CH_2)	2.20 (*m*)	26.2 (CH_2)	2.17 (*m*)
		2.20 (*m*)		2.17 (*m*)
10′	129.9 (CH)	5.46 (*t*, 6.8)	130.0 (CH)	5.46 (*t*, 6.8)
11′	133.5 (C)		133.5 (C)	
12′	80.4 (CH)	3.86 (*d*, 6.8)	80.4 (CH)	3.85 (*d*, 6.8)
13′	69.3 (CH)	4.31 (*dd*, 6.8, 8.8)	69.3 (CH)	4.30 (*dd*, 6.8)
14′	123.4 (CH)	5.19 (*t*, 8.8)	123.5 (CH)	5.20 (*d*, 8.8)
15′	139.5 (C)		139.4 (C)	
16′	26.1 (CH_3)	1.77 (*s*)	26.1 (CH_3)	1.78 (*s*)
17′	23.4 (CH_3)	1.74 (*s*)	23.4 (CH_3)	1.77 (*s*)
18′	15.8 (CH_3)	1.60 (*s*)	15.9 (CH_3)	1.59 (*s*)
19′	11.8 (CH_3)	1.66 (*s*)	11.8 (CH_3)	1.65 (*s*)
20′	18.6 (CH_3)	1.73 (*s*)	18.6 (CH_3)	1.74 (*s*)
1″	37.4 (CH_2)	3.59 (*s*)	37.3 (CH_2)	3.60 (*s*)
		3.59 (*s*)		3.60 (*s*)
2″	174.0 (C)		174.0 (C)	
OMe	52.6 (CH_3)	3.73 (*s*)	52.6 (CH_3)	3.73 (*s*)

Table 5. Endothelin antagonistic activities of nahocols, isonahocols, and their derivatives

Compounds	Antagonistic activities (μM) ET_A (Bovine arota)	ET_B (Porcine lung)
1a	76.1	21.6
1b	84.3	27.7
1c	89.3	23.4
1d	82.3	27.1
1e	100.8	29.4
1f	78.1	27.7
2a	112.7	18.5
2b	100.0	19.1
3a	>3500	>350
4a	152.5	103.8
4b	152.5	101.6

g, not tested), Fr-4 (40–50% EtOAc, 30.90 g, 67% inhibition at 10 μg ml^{-1}), Fr-5 (50–60% EtOAc, 11.30 g), Fr-6 (60–90% EtOAc, 26.55 g, 100% inhibition at 10 μg ml^{-1}), Fr-7 (EtOAc, 7.51 g, 86% inhibition at 10 μg ml^{-1}) and Fr-8 (10–30% MeOH in EtOAc, 12.14 g, 35% inhibition at 10 μg ml^{-1}).

Fr-2 was subjected to silica gel CC (300 g), eluted with 25% EtOAc in hexane to yield Fr-2-1 (3.850 g), Fr-2-2 (1.342 g), Fr-2-3 (2.584 g) and Fr-2-5 (2.643 g). The combined frs Fr-2-2 and Fr-2-3 were again chromatographed on silica gel (180 g). Elution with $CHCl_3$, followed by a combination of CC [Si-60 (25% Me_2CO in hexane) and RP-18 (85% aq. MeOH)], gave **1c** (193.6 mg).

Fr-4 (20 g) was subjected to silica gel CC (600 g), eluted stepwise with a mixt. of EtOAc and hexane to give Fr-4-1 (30% EtOAc, 334 mg, 25% inhibition at 5 μg ml^{-1}), Fr-4-2 (30–40% EtOAc, 13.494 g, 38–45% inhibition at 5 μg ml^{-1}), Fr-4-3 (50% EtOAc, 1.327 g, 55.2% inhibition at 5 μg ml^{-1}), Fr-4-4 (50–100% EtOAc, 1.547 g, 33–43% inhibition at 5 μg ml^{-1}) and Fr-4-5 (50% MeOH in EtOAc, 2.175 g, 20.1% inhibition at 5 μg ml^{-1}). Fr-4-2 (12.13 g) gave a major fr. (7.217 g) after elution with 0.5% MeOH in $CHCl_3$

on silica gel CC (1.2 kg). Prep. HPLC (ODS, 60% aq. MeOH) gave a yellow oil (3.130 g) containing **1a** and **1b**. This was subjected to RP-18 chromatography and elution with 70–80% aq. MeOH gave **1a** (916 mg) and a **1b**-rich fr. (86 mg). The latter was purified by HPLC (silica gel 4% *i*-PrOH in hexane) to give **1b** (23.9 mg).

Fr-6 (1.82 g) was first subjected to CC [RP-18, 75% aq. MeOH and Si-60 (15% *i*-PrOH in hexane)], the major fr. from which was finally purified to **1e** (151.6 mg) by RP-18 chromatography (50% aq. ACN). The remainder (24.25 g) was subjected to silica gel CC (400 g × 2) and eluted with 2.5% MeOH in $CHCl_3$ to give Fr-6-1 (3.81 g), Fr-6-2 (1.32 g), Fr-6-3 (19.36 g), Fr-6-4 (0.21 g), Fr-6-5 (1.02 g) and Fr-6-6 (1.15 g). Fr-6-5 (1.02 g) was subsequently subjected to Si-60 (2% MeOH in $CHCl_3$) and RP-18 chromatography (55% aq. ACN) to give **1f** (175 mg). Fr-6-3 (19.36 g) was separated by HPLC (ODS, 50% aq. ACN) into 10 frs [Fr-6-3-1 (297.7 mg), -2 (2.307 g), -3 (1.006 g), -4 (1.122 g), -5 (2.682 g), -6 (**1e**, 512.8 mg), -7 (1.013 g), -8 (1.443 g), -9 (713 mg) and -10 (172 mg)]. Fr-6-9 (713 mg) was further purified to **1d** (80.5 mg), **1e** (46.2 mg) and **1f** (43.4 mg) by CC [RP-18 (60% aq. ACN), Si-60 ($CHCl_3$)] and HPLC (ODS, 70% aq. ACN)]. Fr-6-2 (1.32 g) was subjected to RP-18 chromatography (50% aq. ACN) and HPLC (NO_2, 10% *i*-PrOH in hexane) to give **2a** (36.0 mg) and **2b** (40.0 mg).

Nahocol A (**1a**). Colourless oil. $[\alpha]_D^{25}$ −168.7° (*c* 0.99). $C_{29}H_{42}O_6$, HREIMS (*m/z*) 486.3001 ($[M]^+$, Δ +1.9 mmu). IR (ν_{max} cm^{-1}) 3450, 1730, 1610, 1590, 1495, 1460, 1410, 1380, 1360, 1220, 1200, 1165, 1100, 920. UV [λ_{max} nm (log ε)] 205 (4.3), 225 (3.8), 293 (3.3). ^1H and ^{13}C NMR (Table 1).

Nahocol A_1 (**1b**). Colourless oil. $[\alpha]_D^{25}$ +10.5° (*c* 0.78). $C_{29}H_{42}O_6$, HREIMS (*m/z*) 486.2291 ($[M]^+$, Δ +0.9 mmu). IR (ν_{max} cm^{-1}) 3600, 3350, 1730, 1710, 1510, 1460, 1440, 1380, 1350, 1310, 1220, 1190, 1100, 1000, 860. UV [λ_{max} nm (log ε)] 207 (4.5), 293 (3.3). ^1H NMR (Table 1).

Nahocol B (**1c**). Colourless oil. $[\alpha]_D^{25}$ −9.7° (*c* 0.96). $C_{29}H_{42}O_5$, HREIMS (*m/z*) 470.3019 ($[M]^+$, Δ −1.3 mmu). IR (ν_{max} cm^{-1}) 3600, 2925, 1730, 1600, 1435, 1408, 1370, 1340, 1280, 1165, 1090, 990. UV [λ_{max} nm (log ε)] 204 (4.4), 228 (3.9), 293 (3.4). ^1H and ^{13}C NMR (Table 1).

Nahocol C (**1d**). Colourless oil. $[\alpha]_D^{25}$ +6.3° (*c* 0.71). $C_{29}H_{44}O_6$, HREIMS (*m/z*) 470.3017 ($[M-H_2O]^+$, Δ −1.5 mmu). IR (ν_{max} cm^{-1}) 3600, 3350, 1730, 1705, 1600, 1495, 1435, 1410, 1370, 1340, 1165. UV [λ_{max} nm (log ε)] 206 (4.2), 229 (3.8), 293 (3.3). ^1H and ^{13}C NMR (Table 2).

Nahocol D_1 (**1e**). Colourless oil. $[\alpha]_D^{25}$ +2.6° (*c* 0.76). $C_{29}H_{42}O_6$, HREIMS (*m/z*) 468.2854 ($[M-H_2O]^+$, Δ −1.5 mmu). IR (ν_{max} cm^{-1}) 3600, 3350, 1730, 1610, 1590, 1495, 1440, 1170, 1100, 1010. UV [λ_{max} nm (log ε)] 206 (4.5), 228 (3.9), 293 (3.5). ^1H and ^{13}C NMR (Table 3).

Nahocol D_2 (**1f**). Colourless oil. $[\alpha]_D^{25}$ +4.5° (*c* 0.95). $C_{29}H_{42}O_6$, HREIMS (*m/z*) 468.2866 ($[M-H_2O]^+$, Δ −1.0 mmu). IR (ν_{max} cm^{-1}) 3600, 3350, 1730, 1610, 1590, 1495, 1440, 1170, 1100, 1010. UV [λ_{max} nm (log ε)] 206 (4.4), 229 (3.9), 293 (3.4). ^1H and ^{13}C NMR (Table 3).

Isonahocol D_1 (**2a**). Colourless oil. $[\alpha]_D^{25}$ +8.4° (*c* 0.83). $C_{29}H_{42}O_6$, HREIMS (*m/z*) 468.2884 ($[M-H_2O]^+$, Δ +0.8 mmu). IR (ν_{max} cm^{-1}) 3600, 3350, 1710, 1600, 1460, 1435, 1375, 1220, 1140, 1010. UV [λ_{max} nm (log ε)] 210 (4.4), 293 (3.5). ^1H and ^{13}C NMR (Table 4).

Isonahocol D_2 (**2b**). Colourless oil. $[\alpha]_D^{25}$ +8.6° (*c* 2.89). $C_{29}H_{42}O_6$, HREIMS (*m/z*) 468.2870 ($[M-H_2O]^+$, Δ −0.5 mmu). IR (ν_{max} cm^{-1}) 3600, 3350, 1710, 1600, 1460, 1435, 1375, 1340, 1220, 1140, 1100, 1010. UV [λ_{max} nm (log ε)] 205 (4.6), 294 (3.5). ^1H and ^{13}C NMR (Table 4).

Nahocol A_1 diacetate (**3a**). To a soln of **1a** (294.6 mg) in dry pyridine (0.5 ml), was added Ac_2O (0.5 ml). The mixt. was kept at room temp. overnight. The residue, after usual work-up, was purified by Si-60 chromatography, eluted with 30% EtOAc in hexane to give **3a** (275 mg) as a colourless oil. $[\alpha]_D^{25}$ −123.8° (*c* 0.77). $C_{33}H_{46}O_8$, HREIMS (*m/z*) 570.3184 ($[M]^+$, Δ −0.9 mmu). IR (ν_{max} cm^{-1}) 1730, 1610, 1490, 1435, 1370, 1340, 1220, 1195, 1100, 1015, 965, 920. UV [λ_{max} nm (log ε)] 202 (4.4), 225 (3.8), 278 (2.9). ^1H NMR: δ 1.02 (3H, *d*, *J* = 6.7 Hz), 1.24–1.5 (2H, *m*), 1.44 (3H, *s*), 1.5–1.8 (2H, *m*), 1.82 (3H, *d*, *J* = 1.2 Hz), 1.86 (3H, *d*, *J* = 1.2 Hz), 1.96 (2H, *t*, *J* = 7.5 Hz), 2.0–2.1 (2H, *m*), 2.13 (3H, *s*), 2.26 (3H, *s*), 2.65 (1H, *sixtet*, *J* = 6.8 Hz), 3.58 (1H, *d*, *J* = 16.1 Hz), 3.62 (1H, *d*, *J* = 16.1 Hz), 3.68 (3H, *s*), 5.09 (1H, *t*, *J* = 7.1 Hz), 5.14 (1H, *d*, *J* = 11.1 Hz), 5.19 (1H, *d*, *J* = 17.6 Hz), 5.21 (1H, *d*, *J* = 10.0 Hz), 5.80 (1H, *d*, *J* = 10.0 Hz), 6.06 (1H, *dd*, *J* = 11.1, 17.6 Hz), 6.83 (1H, *dd*, *J* = 2.9, 8.9 Hz), 6.92 (1H, *d*, *J* = 2.9 Hz), 7.03 (1H, *d*, *J* = 8.9 Hz). ^{13}C NMR: δ 15.7 (CH_3), 16.2 (CH_3), 18.9 (CH_3), 20.8 (CH_3), 21.1 (CH_3), 22.2 (CH_2), 22.6 (CH_3), 25.3 (CH_2), 26.0 (CH_3), 33.1 (CH_2), 36.5 (CH_2), 39.6 (CH_2), 42.1 (CH), 42.4 (CH), 51.8 (CH_3), 75.9 (CH), 82.0 (CH), 114.5 (CH_2), 116.9 (CH), 117.8 (CH), 120.2 (CH), 123.8 (CH), 124.2 (CH), 126.6 (C), 135.1 (C), 142.6 (C), 143.4 (CH), 144.0 (C), 152.1 (C), 169.7 (C), 170.1 (C), 171.8 (C), 208.5 (C).

Nahocol A_1 methyl ether (**3b**). To a soln of **1a** (130.1 mg) and MeI (0.17 ml) in dry DMF (1 ml), was added Ag_2O (75 mg) and the mixt. stirred at room temp. overnight. After the reaction was complete, H_2O and EtOAc were added to the reaction mixt. The EtOAc layer was washed with H_2O and satd. NaCl soln, successively, and then dried over Na_2SO_4. Concn of the EtOAc layer under red. pres. gave a yellow oil (107.4 mg), which was purified by Si-60 chromatography (25% EtOAc in hexane) to give **3b** (44.8 mg) as a colourless oil. $[\alpha]_D^{25}$ −14.1° (*c* 0.49). $C_{30}H_{44}O_6$, EIMS (*m/z*) 500, 482, 468, 196, 164, 83. IR (ν_{max} cm^{-1}) 3450, 1730, 1705, 1600, 1495, 1430, 1370, 1340, 1300, 1270, 1210, 1160, 1100, 1000, 920. UV [λ_{max} nm (log ε)] 207 (4.3), 227 (4.1), 290 (3.5). ^1H NMR: δ 1.05 (3H, *d*, *J* = 6.7 Hz), 1.2–1.4 (2H, *m*), 1.42 (3H, *s*), 1.56 (3H, *s*), 1.7–1.75 (2H, *m*), 1.80 (3H, *d*, *J* = 1.1 Hz), 1.86

(3H, *d*, J = 1.2 Hz), 1.94 (2H, *t*, J = 7.0 Hz), 2.08 (2H, *m*), 2.68 (1H, *sixtet*, J = 6.5 Hz), 3.58 (1H, *d*, J = 15.9 Hz), 3.63 (1H, *d*, J = 15.9 Hz), 3.68 (3H, *s*), 3.75 (3H, *s*), 3.79 (1H, *d*, J = 4.6 Hz, D_2O-exchangeable), 4.86 (1H, *dd*, J = 4.6, 9.8 Hz), 4.98 (1H, *br d*, J = 9.8 Hz), 5.10 (1H, *t*, J = 7.0 Hz), 5.17 (1H, *d*, J = 17.6 Hz), 5.19 (1H, *d*, J = 11.1 Hz), 6.05 (1H, *dd*, J = 11.1, 17.6 Hz), 6.65 (1H, *dd*, J = 3.1, 9.9 Hz), 6.75 (1H, *d*, J = 3.1 Hz), 6.98 (1H, *d*, J = 9.9 Hz). ^{13}C NMR: δ 15.7 (CH_3), 16.2 (CH_3), 18.6 (CH_3), 22.3 (CH_2), 22.5 (CH_3), 25.3 (CH_2), 25.9 (CH_3), 33.6 (CH_2), 36.6 (CH_2), 39.7 (CH_2), 41.3 (CH), 42.2 (CH_2), 51.8 (CH_3), 55.5 (CH_3), 74.3 (CH), 81.7 (CH), 112.4 (CH), 114.2 (CH_2), 116.4 (CH), 119.4 (CH), 121.3 (CH), 124.6 (CH), 127.1 (C), 134.6 (C), 139.8 (C), 143.7 (CH), 148.2 (C), 153.8 (C), 172.1 (C), 214.6 (C).

Conversion of **1b** *to* **4a** *and* **4b**. To a soln of **1b** (150 mg) in MeOH (2 ml), was added 5% NaOH soln. The mixt. was kept at room temp. for 2 hr. The residue obtained by usual work-up, was subjected to RP-18 chromatography (60% aq. ACN) to give **4a** (23.9 mg) and **4b** (less mobile, 18.1 mg). **4a**. Colourless oil. $[\alpha]_D^{25}$ −18.9° (*c* 0.7). $C_{28}H_{40}O_6$, EIMS (*m/z*) 472, 454, 353, 271, 243, 150, 122, 83. IR (ν_{max} cm^{-1}) 3350, 1710, 1675, 1615, 1495, 1440, 1400, 1375, 1280, 1210, 1090, 1030, 1000, 920. UV [λ_{max} nm (log ε)] 206 (4.2), 234 (4.2), 293 (3.3). ^{1}H NMR: δ 1.09 (3H, *d*, J = 7.0 Hz), 1.0–1.1 (2H, *m*), 1.2–1.26 (2H, *m*), 1.45 (3H, *s*), 1.52 (3H, *s*), 1.7–2.1 (7H, *m*), 1.96 (3H, *s*), 2.21 (3H, *s*), 3.58 (1H, *d*, J = 16.1 Hz), 3.62 (1H, *d*, J = 16.1 Hz), 4.08 (1H, *d*, J = 2.5 Hz), 5.06 (1H, *t*, J = 6.8 Hz) 5.18 (1H, *d*, J = 17.6 Hz), 5.21 (1H, *d*, J = 11.0 Hz), 6.60 (1H, *dd*, J = 11.0, 17.6 Hz), 6.11 (1H, *br s*), 6.60 (1H, *dd*, J = 2.9, 8.9 Hz), 6.69 (1H, *d*, J = 2.9 Hz), 6.95 (1H, *d*, J = 8.9 Hz). ^{13}C NMR: δ 15.6 (CH_3), 17.2 (CH_3), 21.4 (CH_3), 22.2 (CH_3), 22.4 (CH_2), 25.3 (CH_2), 28.1 (CH_3), 29.1 (CH_2), 36.7 (CH), 36.8 (CH_2), 39.5 (CH_2), 42.1 (CH_2), 81.1 (CH), 82.6 (C), 114.4 (CH), 114.6 (CH_2), 117.8 (CH), 119.7 (CH), 120.0 (CH), 124.1 (CH), 126.7 (C), 135.0 (C), 143.3 (CH), 149.7 (C), 150.2 (C), 159.5 (C), 176.2 (C), 200.1 (C). **4b**. Colourless oil. $[\alpha]_D^{25}$ +22.8° (*c* 0.47). $C_{28}H_{40}O_6$, EIMS (*m/z*) 472, 454, 353, 271, 243, 150, 122. IR (ν_{max} cm^{-1}) 3325, 1710, 1675, 1615, 1495, 1440, 1400, 1380, 1280, 1210, 1090, 1030, 1000, 920. UV [λ_{max} nm (log ε)] 204 (4.5), 233 (4.3), 293 (3.5). ^{1}H NMR: δ 0.65 (3H, *d*, J = 6.8 Hz), 1.2–2.1 (11H, *m*), 1.42 (3H, *s*), 1.58 (3H, *s*), 1.96 (3H, *d*, J = 0.9 Hz), 2.22 (3H, *d*, J = 0.6 Hz), 3.59 (2H, *s*), 4.21 (1H, *d*, J = 2.3 Hz), 5.12 (1H, *t*, J = 6.9 Hz), 6.21 (1H, *d*, J = 17.6 Hz), 5.22 (1H, *d*, J = 11.1 Hz), 6.06 (1H, *d*, J = 11.1, 17.6 Hz), 6.08 (1H, *br s*), 6.60 (1H, *dd*, J = 2.9, 8.8 Hz), 6.68 (1H, *d*, J = 2.9 Hz), 6.95 (1H, *d*, J = 8.8 Hz). ^{13}C NMR: δ 12.7 (CH_3), 15.7 (CH_3), 21.4 (CH_3), 22.3 (CH_3), 22.4 (CH_2), 25.5 (CH_2), 28.1 (CH_3), 33.4 (CH_2), 36.1 (CH), 36.7 (CH_2), 39.6 (CH_2), 42.2 (CH_2), 78.7 (CH), 82.3 (C), 114.4 (CH), 114.5 (CH_2), 117.9 (CH), 119.2 (CH), 120.0 (CH), 124.3 (CH), 126.7 (C), 135.0 (C), 143.3 (CH), 147.7 (C), 150.2 (C), 159.8 (C), 175.5 (C), 200.8 (C).

Nahocol C acetonide (**5a**). A mixt. of **1d** (8.3 mg) and *p*-TsOH (1 mg) in Me_2CO dimethyl acetal (1 ml) was kept at room temp. for 30 min. After addition of EtOAc and satd $NaHCO_3$ soln, the reaction mixt. was stirred at room temp. for 30 min. The EtOAc extract, after usual work-up, was purified by HPLC (ODS, 90% aq. MeOH) to give **5a** (7.3 mg) as a colourless oil. $[\alpha]_D^{25}$ +25.1° (*c* 0.26). $C_{32}H_{48}O_6$, FABMS (*m/z*) 551 [(M + Na)$^+$]. IR (ν_{max} cm^{-1}) 3602, 2988, 2936, 1736, 1678, 1602, 1497, 1438, 1413, 1380, 1345, 1413, 1168, 1100, 1040, 1003, 882. UV [λ_{max} nm (log ε)] 204 (4.5), 233 (4.3), 293 (3.5). ^{1}H NMR: δ 0.95 (1H, *m*), 1.00 (3H, *d*, J = 6.7 Hz), 1.13 (1H, *m*), 1.26 (1H, *m*), 1.35 (3H, *s*), 1.39 (3H, *s*), 1.4–1.6 (2H, *m*), 1.47 (3H, *s*), 1.55 (3H, *s*), 1.7–1.8 (2H, *m*), 1.87 (2H, *m*), 2.06 (2H, *m*), 3.5 (1H, *d*, J = 15.9 Hz), 3.62 (1H, *d*, J = 15.9 Hz), 3.68 (3H, *s*), 3.80 (1H, *dd*, J = 5.8, 9.0 Hz), 4.55 (1H, *s*), 4.78 (1H, *dd*, J = 5.8, 10.2 Hz), 5.08 (1H, *t*, J = 7.0 Hz), 5.17 (1H, *d*, J = 17.6 Hz), 5.19 (1H, *d*, J = 11.0 Hz), 5.27 (1H, *dd*, J = 1.1, 9.0 Hz), 6.05 (1H, *dd*, J = 11.0, 17.6 Hz), 6.59 (1H, *dd*, J = 3.0, 8.9 Hz), 6.69 (1H, *d*, J = 3.0 Hz), 6.93 (1H, *d*, J = 8.9 Hz). ^{13}C NMR: δ 15.8 (CH_3), 16.7 (CH_3), 17.9 (CH_3), 22.35 (CH_2), 22.44 (CH_3), 25.0 (CH_2), 25.7 (CH_3), 26.1 (CH_3), 28.3 (CH_3), 32.8 (CH_2), 33.0 (CH), 36.3 (CH_2), 40.0 (CH_2), 42.2 (CH_2), 51.8 (CH_3), 74.8 (CH), 81.7 (C), 82.6 (CH), 107.4 (C), 114.1 (CH), 114.3 (CH_2), 117.7 (CH), 120.0 (CH), 121.6 (CH), 124.0 (CH), 127.2 (C), 135.4 (C), 136.3 (C), 143.7 (CH), 148.2 (C), 149.7 (C), 172.2 (C).

Nahocol D_1 acetonide (**5b**). According to the method of the prepn for **5a**, **5b** (8.7 mg) was obtained as a colourless oil from **1e** (18 mg). $[\alpha]_D^{25}$ +11.1° (*c* 0.4). $C_{32}H_{46}O_6$, FABMS (*m/z*) 549 [(M + Na)$^+$]. IR (ν_{max} cm^{-1}) 3600, 2988, 2936, 1736, 1677, 1497, 1438, 1413, 1382, 1344, 1288, 1166, 1263, 887. UV [λ_{max} nm (log ε)] 204 (4.5), 233 (4.3), 293 (3.5). ^{1}H NMR: δ 1.37 (3H, *s*), 1.40 (3H, *s*), 1.53 (3H, *s*), 1.59 (3H, *s*), 1.67 (3H, *d*, J = 1.1 Hz), 1.71 (3H, *s*), 1.7–1.8 (2H, *m*), 1.95–2.15 (2H, *m*), 3.56 (1H, *d*, J = 16.0 Hz), 3.62 (1H, *d*, J = 16.0 Hz), 3.68 (3H, *s*), 4.53 (1H, *d*, J = 7.2 Hz), 4.55 (1H, *s*), 4.94 (1H, *dd*, J = 7.2, 8.3 Hz), 5.12 (1H, *d*, J = 8.3 Hz), 5.13 (1H, *t*, J = 7.0 Hz), 5.17 (1H, *d*, J = 17.5 Hz), 5.18 (1H, *d*, J = 11.0 Hz), 5.44 (1H, *t*, J = 7.0 Hz), 6.05 (1H, *dd*, J = 11.0, 17.5 Hz), 6.58 (1H, *dd*, J = 3.0, 8.8 Hz), 6.69 (1H, *d*, J = 3.0 Hz), 6.93 (1H, *d*, J = 8.8 Hz). ^{13}C NMR: δ 13.8 (CH_3), 15.9 (CH_3), 18.3 (CH_3), 22.4 (CH_2), 22.5 (CH_3), 25.0 (CH_3), 25.9 (CH_3), 26.2 (CH_2), 27.2 (CH_3), 36.3 (CH_2), 39.3 (CH_2), 42.1 (CH_2), 51.9 (CH_3), 75.1 (CH), 81.7 (C), 82.9 (CH), 107.8 (C), 114.1 (CH), 114.2 (CH_2), 117.7 (CH), 120.0 (CH), 121.4 (CH), 124.1 (CH), 127.0 (CH), 127.2 (C), 131.6 (C) 135.2 (C), 136.9 (C), 143.7 (CH), 148.2 (C), 149.7 (C), 172.3 (C).

Nahocol D_2 acetonide (**5c**). According to the method of the prepn for **5a**, **5c** (11.1 mg) was obtained as a colourless oil from **1f** (18 mg). $[\alpha]_D^{25}$ +2.2° (*c* 0.4). $C_{32}H_{46}O_6$, FABMS (*m/z*) 549 [(M + Na)$^+$]. IR (ν_{max} cm^{-1}) 3600, 2988, 2936, 1736, 1681, 1497, 1438, 1413, 1381, 1344, 1288, 1165, 1101, 1039, 888. ^{1}H NMR: δ

1.39 (3H, *s*), 1.44 (3H, *s*), 1.45 (3H, *s*), 1.58 (3H, *s*), 1.64 (3H, *d*, J = 1.2 Hz), 1.66 (3H, *s*), 1.75 (3H, *d*, J = 1.1 Hz), 1.55–1.8 (2H, *m*), 1.95–2.2 (2H, *m*), 3.57 (1H, *d*, J = 15.8 Hz), 3.61 (1H, *d*, J = 15.8 Hz), 3.68 (3H, *s*), 3.97 (1H, *d*, J = 8.5 Hz), 4.45 (1H, *t*, J = 8.5 Hz), 4.60 (1H, *s*), 5.12 (1H, *t*, J = 6.4 Hz), 5.16 (1H, *d*, J = 7.9 Hz), 5.18 (1H, *d*, J = 17.6 Hz), 5.19 (1H, *d*, J = 11.0 Hz), 5.49 (1H, *t*, J = 6.9 Hz), 6.05 (1H, *dd*, J = 11.0, 17.6 Hz), 6.58 (1H, *dd*, J = 3.0, 8.8 Hz), 6.68 (1H, *d*, J = 3.0 Hz), 6.93 (1H, *d*, J = 8.8 Hz). ^{13}C NMR: δ 11.7 (CH_3), 15.9 (CH_3), 18.5 (CH_3), 22.3 (CH_2), 22.5 (CH_3), 26.0 (CH_3), 26.5 (CH_2), 27.0 (CH_3), 27.4 (CH_3), 36.3 (CH_2), 39.1 (CH_2), 42.1 (CH_2), 51.9 (CH_3), 76.0 (CH), 81.7 (C), 86.4 (CH), 108.1 (C), 114.1 (CH), 114.3 (CH_2), 117.7 (CH), 119.6 (CH), 121.6 (CH), 124.1 (CH), 127.2 (C), 129.4 (CH), 130.2 (C) 135.0 (C), 139.3 (C), 143.7 (CH), 148.1 (C), 150.0 (C), 172.3 (C).

Isonahocol D_1 acetonide (**6a**). According to the method of the prepn for **5a**, **6a** (1.8 mg) was obtained as a colourless oil from **2a** (7.4 mg). $[\alpha]_D^{25}$ −29.4° (*c* 0.09). $C_{32}H_{46}O_6$, FABMS (*m/z*) 549 $[(M+Na)^+]$. IR (ν_{max} cm^{-1}) 3604, 3364, 2988, 2934, 1733, 1660, 1606, 1481, 1460, 1440, 1382, 1164, 1023, 886. ^{1}H NMR: δ 1.40 (3H, *s*), 1.52 (3H, *s*), 1.54 (3H, *s*), 1.60 (3H, *s*), 1.67 (3H, *s*), 1.71 (3H, *s*), 1.76 (3H, *s*), 2.00 (2H, *t*, J = 7.3 Hz), 2.05–2.25 (6H, *m*), 3.34 (2H, *d*, J = 7.3 Hz), 3.60 (2H, *s*), 3.74 (3H, *s*), 4.55 (1H, *d*, J = 7.1 Hz), 4.84 (1H, *s*), 4.95 (1H, *dd*, J = 7.1, 8.8 Hz), 5.12 (1H, *d*, J = 8.8 Hz), 5.15 (1H, *t*, J = 6.6 Hz), 5.34 (1H, *t*, J = 6.5 Hz), 5.45 (1H, *t*, J = 6.9 Hz), 6.47 (1H, *d*, J = 3.0 Hz), 6.56 (1H, *d*, J = 3.0 Hz), 6.74 (1H, *s*). ^{13}C NMR: δ 13.9 (CH_3), 15.9 (CH_3), 18.2 (CH_3), 23.5 (CH_3), 24.9 (CH_3), 25.8 (*t*), 25.9 (CH_3), 26.4 (CH_3), 27.2 (CH_3), 29.0 (CH_2), 32.0 (CH_2), 37.5 (CH_2), 39.2 (CH_2), 52.6 (CH_3), 75.0 (CH), 82.2 (CH), 107.8 (C), 115.0 (CH), 115.9 (CH), 121.2 (CH), 121.6 (C), 122.5 (CH), 124.2 (CH), 127.0 (CH), 130.9 (C) 131.4 (C), 135.1 (C), 137.1 (C), 137.5 (C), 146.8 (C), 149.2 (C), 174.0 (C).

Isonahocol D_2 acetonide (**6b**). According to the method of the prepn for **5a**, **6b** (5.4 mg) was obtained as a colourless oil from **2b** (11.8 mg). $[\alpha]_D^{25}$ −16.7° (*c* 0.3). $C_{38}H_{40}O_6$, FABMS (*m/z*) 549 $[(M+Na)^+]$. IR (ν_{max} cm^{-1}) 3604, 3364, 2988, 2935, 1733, 1709, 1659, 1607, 1481, 1459, 1440, 1382, 1163, 1023, 886. ^{1}H NMR: δ 1.40 (3H, *s*), 1.52 (3H, *s*), 1.53 (3H, *s*), 1.59 (3H, *s*), 1.67 (3H, *d*, J = 1.0 Hz), 1.71 (3H, *s*), 1.76 (3H, *s*), 2.00 (2H, *t*, J = 7.3 Hz), 1.99 (2H, *m*), 2.05–2.20 (6H, *m*), 3.34 (2H, *d*, J = 7.2 Hz), 3.61 (2H, *s*), 3.74 (3H, *s*), 4.52 (1H, *d*, J = 7.0 Hz), 4.58 (1H, *s*), 4.94 (1H, *dd*, J = 7.0, 8.8 Hz), 5.1–5.15 (2H, *m*), 5.30 (1H, *t*, J = 6.2 Hz), 5.43 (1H, *t*, J = 6.2 Hz), 6.48 (1H, *d*, J = 3.0 Hz), 6.56 (1H, *d*, J = 3.0 Hz), 6.64 (1H, *s*). ^{13}C NMR: δ 13.8 (CH_3), 16.0 (CH_3), 18.3 (CH_3), 23.5 (CH_3), 25.0 (CH_3), 25.9 (CH_3), 26.1 (CH_2), 26.4 (CH_2), 27.2 (CH_3), 29.1 (CH_2), 32.0 (CH_2), 37.4 (CH_2), 39.3 (CH_2), 52.6 (CH_3), 75.1 (CH), 83.0 (CH), 107.8 (C), 115.1 (CH), 115.9 (CH), 121.3 (CH), 121.7 (C), 121.8 (CH), 124.2 (CH), 127.2 (C), 130.7 (C), 131.4 (C) 135.0 (C), 137.0 (C), 137.5 (C), 146.9 (C), 149.1 (C), 173.9 (C).

Acknowledgements—The authors are indebted to Prof. M. Kajimura of Oki Marine Biological Experimental Station, Shimane University, and Prof. Y. Yokohama, Director of Shimoda Marine Research Center, University of Tsukuba, for the collection and identification of plant material.

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