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3,4-Dihydroxystyrene Dimers, Inducers of Larval Metamorphosis in Ascidians, from a Marine Sponge *Jaspis* sp.

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Abstract: Four new 3,4-dihydroxystyrene dimers (**3**, **4**, **5**, and **9**) have been isolated from a marine sponge *Jaspis* sp. along with the known narains (**1** and **2**), of which **3-5** induced metamorphosis of ascidian *Halocynthia roretzi* larvae. Structures of these metabolites were elucidated on the basis of spectral data. Biogenesis of these compounds is also discussed.

INTRODUCTION

Larvae of marine benthic organisms, hydroids, barnacles, bryozoans, and tunicates, swim for a while before automatically embarking on a search for a place to settle and grow. Although settlement and metamorphosis of larvae are believed to be initiated by chemical cues,[1] signal transduction mechanisms of the larvae are unknown.

As part of our research on the mechanism of larval settlement and metamorphosis, we have isolated several compounds which promoted larval metamorphosis in two ascidians, *Halocynthia roretzi* and *Ciona savignyi*: urochordamines A and B[2a, 2b] from two ascidians *C. savignyi* and *Botrylloides* sp., and phlorotannins and sulfoquinovosyl diacylglycerols[2c] from the brown alga *Sargassum thunbergii*. Subsequently, we found strong activity in the MeOH extract of a marine sponge, *Jaspis* sp. collected off the Izu Peninsula, and isolated from this sponge two active 3,4-dihydroxystyrene derivatives, (*E*)- (**1**) and (*Z*)-narains (**2**).[2d, 3] Further examination of the MeOH extract led to the isolation of three more 3,4-dihydroxystyrene dimers **3**, **4**, and **5**. We also isolated another dimer **9** from the acetone extract of the sponge. This paper describes isolation, structural elucidation, and biogenesis of these compounds.

RESULTS AND DISCUSSION

The water soluble portion of the MeOH extract was partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer was subjected to ODS with aqueous MeOH; the active 30 % MeOH-H_2O eluate was further

purified by gel filtration or reversed phase HPLC to afford four new compounds (3-6) along with the previously isolated (*E*)- and (*Z*)-narains (1 and 2). The less active Et_2O layer was purified by silica gel column chromatography to afford compound 7.

Structures of compounds 3-5 and 7.

Compound 3 exhibited a prominent peak at *m/z* 483 in the negative FAB mass spectrum, which gave a formula of $C_{16}H_{12}O_{12}S_2Na$ (*m/z* 482.9674, Δ +0.6 mmu) by HRFABMS. The presence of two sulfate groups was substantiated by an IR band at 1260 cm^{-1} and a FABMS ion peak at *m/z* 381 $(483\text{-}SO_3Na\text{+}H)^-$.

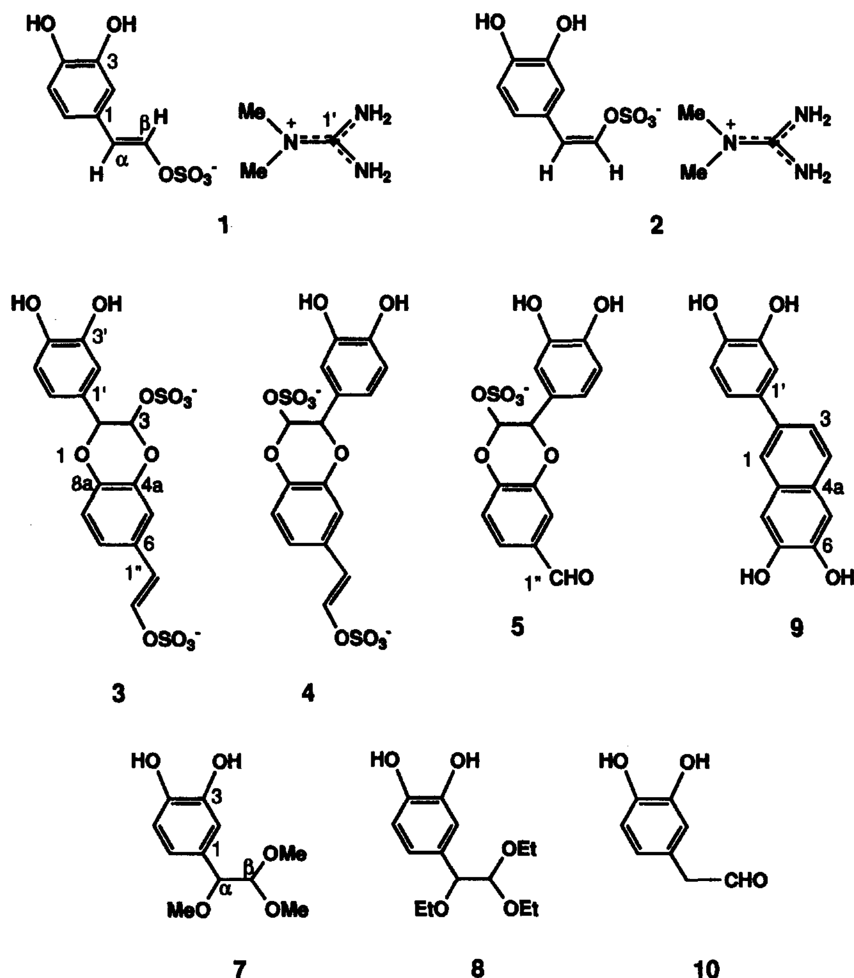

Fig. 1 Structures of narains and related compounds

Table 1. ^1H and ^{13}C NMR Data for 3-5 in DMSO-d_6

	3		4		5	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
2	5.10 (s)	75.4 (d)	5.79 (br.s)	92.5 (d)	5.92 (s)	92.6 (d)
3	5.79 (s)	92.4 (d)	5.12 (br.s)	75.3 (d)	5.21 (s)	75.4 (d)
4a		140.26 (s)		142.3 (s)		142.7 (s)
5	6.71 (d, 2.0)	114.2 (d)	6.82 (s)	113.0 (d)	7.45 (s)	117.2 (d)
6		128.9 (s)		129.9 (s)		131.2 (s)
7	6.82 (dd, 8.0, 2.0)	118.9 (d)	6.75 (d, 8.4)	118.1 (d)	7.46 (d, 8.4)	123.7 (d)
8	6.81 (d, 8.0)	116.6 (d)	6.71 (d, 8.4)	117.4 (d)	7.06 (d, 8.4)	118.1 (d)
8a		140.7 (s)		138.7 (s)		146.0 (s)
1'		127.7 (s)		127.7 (s)		126.9 (s)
2'	6.67 (d, 2.0)	113.6 (d)	6.68 (s)	113.5 (d)	6.69 (s)	113.6 (d)
3'		145.3 (s)		145.17 (s)[c]		145.5 (s)
4'		145.2 (s)		145.21 (s)[c]		145.3 (s)
5'	6.66 (d, 8.0)	115.5 (d)	6.66 (d, 8.4)	115.5 (d)	6.68 (d, 8.4)	115.6 (d)
6'	6.54 (dd, 8.0, 2.0)	117.5 (d)	6.53 (d, 8.4)	117.2 (d)	6.56 (d, 8.4)	117.4 (d)
1"	5.89 (d, 13)	110.1 (d)	5.90 (d, 13)	110.1 (d)	9.84 (s)	191.4 (d)
2"	7.08 (d, 13)	140.29 (d)	7.10 (d, 13)	140.5 (d)		
3'-OH	8.90 (br.s)[a]		8.88 (br.s)[b]		9.00 (br.s)	
4'-OH	9.00 (br.s)[a]		9.02 (br.s)[b]		8.96 (br.s)	

Multiplicity of signals and coupling constants (Hz) are shown in parentheses. [a-c] May be interchangeable.

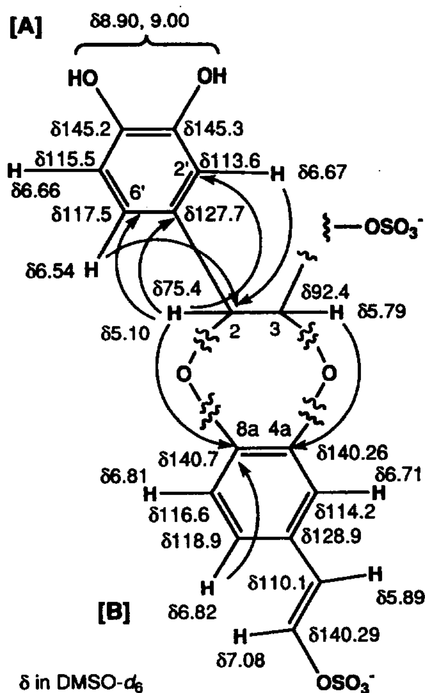

Fig. 2 Partial HMBC correlations of 3

Interpretation of the NMR spectra (Table 1) including HMQC and HMBC data (Fig. 2) led to partial structure A, consisting of a 3,4-dihydroxyphenyl group and two oxymethine carbons [δ 75.4 (d)/δ 5.10 (s) and δ 92.4 (d)/δ 5.79 (s)], and partial structure B, a 3,4-disubstituted phenyl group linked to an (*E*)-ethenyl sulfate similar to (*E*)-narain (1). These structural features were also supported by UV absorption [215.5 (ε 17200), 263.5 (7700), 281.5 (5300, sh.), and 302 (2500, sh.) nm]. HMBC cross peaks; δ 140.26 (C4a)/δ 5.79 (H-3) and δ 140.7 (C8a)/δ 5.10 (H-2) inferred connectivities of C2-O-C8a and C3-O-C4a, thereby constructing a 2,3-dihydro-1,4-dioxanaphthalene framework. Chemical shift (δ 92.4) of C3 allowed to place another sulfate group at C3. Dihedral angle (~90°) between C2 and C3 obtained from coupling constant, $J_{H\text{-}2,\,H\text{-}3}$=0 Hz and NOESY cross peaks; H-2/H-3, H-2/H-2', H-2/H-6', H-3/H-2', and H-3/H-6' were consistent with both *erythro* and *threo* configurations, indicating that relative stereochemistry of 3 was ambiguous. Moreover, specific rotation of 3, $[\alpha]_D^{20}$ +0.13° (*c* 0.35, 90 % MeOH-H_2O) and the absence of a CD maximum indicated that 3 was a racemate. Thus, structure of 3 was as shown in Fig. 1.

Compound **4** had a same molecular formula as **3**. The ^{1}H NMR spectrum was almost superimposable on that of **3**, whereas the assignments were partially different (Table 1). HMBC correlations of H-2/C8a, H-3/C4a, and H-7/C8a indicated that a sulfate group and 3,4-dihydroxyphenyl group were linked to C2 and C3, respectively; the position of the substituents was reversed from that in **3** (Fig. 1). The specific rotation of **4** was also 0°.

The negative FAB mass spectrum of **5** showed a peak at *m/z* 367, matching a formula of $C_{15}H_{11}O_9S$ (*m/z* 367.0132, Δ +0.8 mmu) by HRFABMS; a CHO_3SNa unit smaller than **3** or **4**. The ^{1}H and ^{13}C NMR spectra revealed signals characteristic of an aldehyde group at δ 9.84 (s, H-1") and δ 191.4 (d, C1"), respectively. HMBC cross peaks (H-5/C1"; H-7/C1"; H-1"/C5, C6, and C7) pointed to an aldehyde group at C6 of 2,3-dihydro-1,4-dioxanaphthalene instead of an (*E*)-ethenyl sulfate group in **4** (Fig. 1). Again, no optical rotation was observed.

Compound **7** had a molecular formula of $C_{11}H_{16}O_5$ as determined by HRFAB mass spectrum [*m/z* 227.0913, Δ -0.6 mmu, $(M-H)^-$ for $C_{11}H_{15}O_5$]. NMR data suggested the presence of three methoxy groups [δ 3.06/δ 55.8; δ 3.09/δ 54.6; δ 3.30/δ 53.6] and two methine carbons [δ 3.92 (1 H, d, *J*=6.5 Hz, H-α)/δ 82.8 (d, Cα); δ 4.26 (1 H, d, *J*=6.5 Hz, H-β)/δ 105.8 (d, Cβ)] as well as a 3,4-dihydroxyphenyl group. HMBC correlations allowed to connect $CH(OMe)-CH(OMe)_2$ unit [δ 3.06/δ 82.8; δ 3.09/δ 105.8; δ 3.30/δ 105.8; δ 3.92/δ 55.8 and 105.8; δ 4.26/δ 53.6 and 54.6] to the 3,4-dihydroxyphenyl group [δ 3.92 (H-α)/δ 129.0 (s, C1), 115.01 (d, C2), and 119.1 (d, C6); δ 4.26 (H-β)/δ 129.0; δ 6.67 (1 H, d, *J*=1.8 Hz, H-2)/δ 82.8; δ 6.54 (1 H, dd, *J*=8.0 and 1.8 Hz, H-6)/δ 82.8], thus completing 1,1,2-trimethoxy-2-(3,4-dihydroxyphenyl)ethane. Although there was a chiral center (Cα), the specific rotation was 0°.

Since compound **7** was likely to be an artefact produced during isolation, we extracted the sponge both with EtOH and acetone; moreover we avoided using MeOH during purification.

The ether soluble portion of the EtOH extract was subjected to silica gel column chromatography with 5 % EtOH-$CHCl_3$ and HPLC on silica gel with acetone-hexane (2:3) to afford **8** instead of **7**. Compound **8** had a molecular formula of $C_{14}H_{22}O_5$ as established by the HRFAB mass spectrum (negative) [*m/z* 269.1380, Δ -0.9 mmu, $(M-H)^-$ for $C_{14}H_{21}O_5$]. The ^{1}H NMR spectrum in $CDCl_3$ showed three ethoxy groups at δ 0.99, 1.14, and 1.22 (each 3 H, t, *J*=7.1 Hz, 3 x $-OCH_2CH_3$) and δ 3.25, 3.37, 3.43, 3.55, 3.60, and 3.69 (each 1 H, dq, *J*=9.4 and 7.1 Hz, 6 x $-OCH_2CH_3$) in place of three methoxy groups in **7**. Other spectral features were quite similar to those of **7**. Thus, compound **8** was 1,1,2-triethoxy-2-(3,4-dihydroxyphenyl)ethane (Fig. 1).

The ether soluble portion of the acetone extract was chromatographed on silica gel with acetone-hexane (1:1) and then subjected to gel-filtration on Sephadex LH-20 with acetone to yield **9**. Compound **9** had a molecular formula of $C_{16}H_{12}O_4$ as determined by the HRFAB mass spectrum (negative) [*m/z* 267.0639, Δ -1.9 mmu, $(M-H)^-$ for $C_{16}H_{11}O_4$]. The ^{1}H NMR spectrum in acetone-d_6 showed eight aromatic protons [δ 6.91 (1 H, d, *J*=8.2 Hz, H-5'), 7.07 (1 H, dd, *J*=8.2 and 2.0 Hz, H-6'), 7.19 (1 H, s, H-5), 7.20 (1 H, d, *J*=2.0 Hz, H-2'), 7.24 (1 H, s, H-8), 7.45 (1 H, dd, *J*=8.4 and 2.0 Hz, H-3), 7.62 (1 H, d, *J*=8.4 Hz, H-4), and 7.75 (1 H, d, *J*=2.0 Hz, H-1)] and four D_2O-exchangeable protons [δ 8.17 (4 H, br. s, 6-, 7-, 3'-, and 4'-OH)]. Interpretation of the 2D NMR spectra inferred the presence of a 6,7-dihydroxynaphthyl group [δ 123.7 (d, C1), 136.9 (s, C2), 123.4 (d, C3), 127.2 (d, C4), 129.2 (s, C4a), 110.2 (d, C5), 147.1 and 147.5 (each s, C6 and C7), 110.7 (d, C8), and 130.8 (s, C8a)] and a 3,4-

dihydroxyphenyl group [δ 134.5 (s, C1'), 114.8 (d, C2'), 146.3 (s, C3'), 145.4 (s, C4'), 116.5 (d, C5'), and 119.3 (d, C6')]. HMBC cross peaks; H-2'/C2 and H-1/C1' suggested that C2 of the 6,7-dihydroxynaphthyl group was directly attached to C1' of the 3,4-dihydroxyphenyl group (Fig. 1), which was also confirmed by NOESY cross peaks at H-1/H-2', H-1/H-6', H-3/H-2', and H-3/H-6'.

Thus, compounds **7-9** were apparently artefacts.

Structure of Compound 6.

In addition to 3,4-dihydroxystyrenes, an hypoxanthin base has been obtained as an active compound. The ^{1}H NMR spectrum exhibited three methyl signals at δ 3.57, 3.89, and 4.12 (each 3 H, s) and two low-field protons at δ 8.73 and 9.55 (each 1 H, s). HMBC cross peaks [δ 8.73 (1 H, s, H-2)/δ 146.2 (s, C4), 152.8 (s, C6), and 33.9 (q, N1-Me); δ 9.55 (1 H, s, H-8)/δ 146.2 and 113.9 (s, C5); δ 3.57 (3 H, s, Me-1)/δ 152.8; δ 4.12 (3 H, s, Me-7)/δ 113.9 and 141.6 (d, C8); δ 3.89 (3 H, s, Me-9)/δ 141.6 and 146.2] led to connectivities of -C5-N7Me-C8-N9Me-C4-N-C2-N1Me-C6-. The positive FAB mass spectrum revealed an ion peak at *m/z* 179, indicating a formula of $C_8H_{11}N_4O$ (*m/z* 179.0931, Δ -0.2 mmu). Considering the molecular formula and chemical shifts of C4, C5, and C6, structure of **6** was assigned to 1,7,9-trimethylhypoxanthinium ion (Fig. 3).

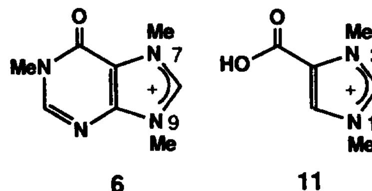

Fig. 3 Structures of **6** and **11**

Larval metamorphosis-inducing activity of 1-7 and 9 on H. roretzi.

(*Z*)-Narain (**2**) as well as the anion[2d] alone induced larval metamorphosis on *H. roretzi* at a concentration of 5 μM, while no larvae in the control group underwent metamorphosis. However, *N,N*-dimethylguanidine sulfate was inactive at a concentration of 50 μM. (*E*)-Narain (**1**), its anion, **3**, **4**, and **7** were active at a concentration of 50 μM; **5** and **6** were much less active, while **9** did not show any effect. Therefore, the anion moiety, especially the sulfate group, may play an important role as the metamorphosis-inducer. Obviously, the stereochemistry of the double bond is important for activity. 4-Carboxy-1,3-dimethylimidazolium ion (norzooanemonin,[4] **11**) (Fig. 3), which is biogenetically related to **6** and was isolated from the marine sponge *Cacospongia scalaris* as a metamorphosis-inducing compound in our laboratory,[5] showed the same degree of activity as **6**.

CONCLUSION

We have successively isolated 3,4-dihydroxystyrenes **3-5** and **7** (Fig. 1) along with (*E*)- (**1**) and (*Z*)-narains (**2**) from the MeOH extract of a marine sponge *Jaspis* sp. as metamorphosis-inducers of ascidian *Halocynthia roretzi* larvae. The EtOH and acetone extracts afforded **8** and **9**, respectively, instead of **7**. It is noteworthy that this is the first report of the isolation of cycloaddition products such as **3-5** and **9**. Although **9** was a new metabolite, an *O*-methyl derivative of **9** has been produced from 3,4-dimethoxyphenylacetoaldehyde upon acid treatment,[6] which may indicate that **9** has arisen from 3,4-dihydroxyphenylacetoaldehyde (**10**). In fact, (*E*)-narain (**1**) was converted to **10** by treatment with

sulfatase in a yield of 20 %.[7] However, **10** was not converted into **7** or **9** in either MeOH or acetone, respectively, at room temperature. Thus, it is likely that the sponge originally contained a catechol derivative bearing a C_2-unit [C], which was transferred during isolation. Obviously, compounds **7** and **8** were derived from the putative compound [C] with addition of each extraction solvent. In contrast, compounds **3-5** and **9** seem [4 + 2] cycloaddition products: compounds **3-5** arose from addition of a C_2-unit to hydroxy groups of another [C] (*head-to-tail* orientation), while compound **9** from addition of two molecules of [C] in *head-to-head* orientation. Narains (**1** and **2**) may correspond to conjugated sulfates derived from enol isomer of **10**. At present we have no evidence for structure of compound [C]. Though **6** has been synthesized,[8] this is the first example from natural sources.

No abnormal effects on larvae were observed when exposed to (*Z*)-narain (**2**), indicating that **2** will be a good tool to study metamorphosis mechanism of ascidian larvae. Biochemical events in larvae during metamorphosis induced by narains are now under investigation.

EXPERIMENTAL

General.

Optical rotations were determined with a JASCO DIP-140 polarimeter. CD spectrum was recorded with a JASCO J-720W spectropolarimeter in MeOH. UV spectra were recorded on a Hitachi U-2000 spectrometer in MeOH. Infrared spectra were measured on a JASCO IR-700 spectrometer. NMR spectra were recorded on a Bruker ARX-500 NMR spectrometer at 27 °C in DMSO-d_6, acetone-d_6, or chloroform-d. Residual CHD_2SOCD_3 (2.49 ppm), CD_3SOCD_3 (39.5 ppm), CHD_2COCD_3 (2.05 ppm), CD_3COCD_3 (29.8 ppm), $CHCl_3$ (7.26 ppm), and $CDCl_3$ (77.0 ppm) signals were used as internal standards. FAB mass spectra were measured on a JEOL SX-102 mass spectrometer.

Bioassay.

Fifteen newly hatched larvae were incubated in 4 mL of artificial seawater (460 mM NaCl, 10.1 mM KCl, 9.2 mM $CaCl_2$, 35.9 mM $MgCl_2 \cdot H_2O$, 17.5 mM $MgSO_4 \cdot 7H_2O$, 10 mM Tris-HCl, pH 8.2) at 13.2 °C in the dark. The sample to be assayed was added to the larvae in aqueous solution. For organic soluble compounds **7** and **9** DMSO (10 µL) was used as solvent, and to the control group 10 µL of DMSO was added. The larvae which had undergone metamorphosis, *i.e.*, shortening of tail, were counted under a microscope.

Isolation of 1-7.

A sponge sample (1.1 kg, wet weight) collected off the Izu Peninsula was extracted with MeOH (1 L x 3). The extract was concentrated under reduced pressure at 40 °C. The aqueous residue (500 mL) was extracted with ether (700 mL x 3), then *n*-BuOH (700 mL x 3). The fractions were monitored by metamorphosis-inducing activity of the tadpole larvae of *H. roretzi*: the *n*-BuOH fraction (6.19 g) was more active than the ether fraction (3.49 g). The *n*-BuOH fraction was subjected to ODS flash column chromatography (ODS-A 60-400/230, 8 x 13 cm, YMC Co., Ltd.) using aqueous MeOH. The eluate with 30 % MeOH-H_2O, which showed strong activity, was partially chromatographed on ODS (Lobar

LiChroprep RP-18, 40-63 µm, 25-310 mm, Merck) with 15 % MeOH-H_2O (0.01 % TFA; flow rate, 3.0 ml/min) using RI detector to give five active fractions. The first and second fractions were further purified by HPLC (Asahipak ODP-50, 5 µm, 10 x 250 mm, Asahi Chemical Industry Co., Ltd.; 5 % CH_3CN-H_2O; 2.0 mL/min) to afford (*E*)- (**1**; yield, 8.2 x 10^{-3} %, wet weight) and (*Z*)-narain (**2**; 6.7 x 10^{-3} %), respectively. The third fraction was gel-filtered on Toyopearl HW-40 (1.1 x 40 cm, Tosoh Corporation) with 50 % MeOH-H_2O (1.5 mL/min) to furnish **3** (1.3 x 10^{-3} %). The fourth fraction was purified on Sephadex LH-20 (2.2 x 100 cm, Pharmacia) using 90 % MeOH-H_2O (2.0 mL/min) to afford **4** (6.6 x 10^{-3} %). The fifth fraction was separated by HPLC (YMC-Pack Ph, 5 µM, 20 x 250 mm, YMC Co., Ltd.) with 10 % CH_3CN-H_2O (0.01 % TFA; 3.0 mL/min) to yield **6** (4.0 x 10^{-3} %) and with 20 % CH_3CN-H_2O (0.01 % TFA; 3.0 ml/min) then **5** (6.1 x 10^{-4} %). The ether layer was subjected to silica gel column chromatography (Wakogel C-200, 4.4 x 20 cm, Wako Purechemical Industries, Ltd.) with MeOH-$CHCl_3$ (7 mL/min). The eluent with 5 % MeOH-$CHCl_3$ was purified by silica gel column chromatography (Wakogel C-200, 1.1 x 40 cm) with acetone-hexane (1:3, 3.0 mL/min) to afford **7** (5.1 x 10^{-3} %).

3. $[\alpha]_D^{20}$ +0.13° (*c* 0.35, 90 % MeOH-H_2O). IR ν_{max} (KBr) 3400, 1260, and 1040 cm^{-1}. UV λ_{max} (MeOH) 215.5 (ε 17200), 263.5 (7700), 281.5 (5300, sh.), and 302 nm (2500, sh.). ^1H and ^{13}C NMR see Table 1. HMBC cross peaks: H-2/C3, C8a, C1', C2', and C6'; H-3/C2 and C4a; H-5/C4a, C7, C8a, and C1"; H-7/C8a and C1"; H-8/C4a, C6, and C8a; H-2'/ C2, C3', C4', and C6'; H-5'/C1', C3', and C4'; H-6'/C2, C2', and C4'; H-1"/C5, C7, and C2"; H-2"/C6 and C1". NOESY cross peaks: H-2/H-3, H-2', and H-6'; H-3/H-2' and H-6'; H-5/H-2"; H-7/H-8, H-1", and H-2"; H-5'/H-6'. FABMS (negative, TEA matrix) *m/z* 483 $(M+Na)^-$ and 381 $(M-SO_3Na+H)^-$. HRFABMS (negative, PEGsul matrix) *m/z* 482.9674 (calcd for $C_{16}H_{12}O_{12}S_2Na$, Δ +0.6 mmu).

4. $[\alpha]_D^{23}$ 0.0° (*c* 0.72, 90 % MeOH-H_2O). IR ν_{max} (KBr) 3430, 1250, and 1030 cm^{-1}. UV λ_{max} (MeOH) 215.5 (ε 32500, sh.), 261.0 (13800), 280.0 (8700, sh.), and 300.0 nm (500, sh.). ^1H and ^{13}C NMR see Table 1. HMBC cross peaks: H-2/C3 and C8a; H-3/C2, C4a, C1', C2', and C6'; H-5/C4a, C7, C8a, and C1"; H-7/C5 and C8a; H-8/C4a and C6; H-2'/C3 and C6'; H-5'/C1', C3', and C4'; H-6'/C3, C2', C3', and C4'; H-1"/C5, C7, and C2"; H-2"/C6 and C1". NOESY cross peaks: H-2/H-3, H-2', and H-6'; H-3/H-2' and H-6'; H-5/H-2"; H-7/H-8, H-1", and H-2"; H-5'/H-6'. FABMS (negative, TEA matrix) *m/z* 483 $(M+Na)^-$ and 381 $(M-SO_3Na+H)^-$. HRFABMS (negative, TEA matrix) *m/z* 482.9653 (calcd for $C_{16}H_{12}O_{12}S_2Na$, Δ -1.5 mmu).

5. $[\alpha]_D^{23}$ 0.0° (*c* 0.15, MeOH). IR ν_{max} (KBr) 3340, 1680, 1600, and 1260 cm^{-1}. UV λ_{max} (MeOH) 204.5 (ε 28100, sh.), 228.5 (13400), 271.5 (7700), and 306.0 nm (4600, sh.). ^1H and ^{13}C NMR see Table 1. HMBC cross peaks: H-2/C3 and C8a; H-3/C2, C4a, C1', C2', and C6'; H-5/C4a, C7, C8a, and C1"; H-7/C5, C8a, and C1"; H-8/C4a, C6, and C8a; H-2'/C3, C3', C4', and C6'; H-5'/C1', C3', and C4'; H-6'/C3, C2', and C4'; H-1"/C5, C6, and C7; 3'-OH/C2', C3', and C4': 4'-OH/C3', C4', and C5'. NOESY cross peaks: H-2/H-3, H-2', and H-6'; H-3/H-2' and H-6'; H-5/H-1"; H-7/H-8 and H-1"; H-5'/H-6'. FABMS (negative, TEA matrix) *m/z* 367 M^-. HRFABMS (negative, TEA-PEGsul matrix) *m/z* 367.0132 (calcd for $C_{15}H_{11}O_9S$, Δ +0.8 mmu).

6. IR ν_{max} (KBr) 1690 cm^{-1}. UV λ_{max} (MeOH) 204.5 (ε 6700) and 256.0 nm (3200). ^{1}H NMR (DMSO-d_6) δ 3.57 (3 H, s, Me-1), 3.89 (3 H, s, Me-9), 4.12 (3 H, s, Me-7), 8.73 (1 H, s, H-2), and 9.55 (1 H, s, H-8). ^{13}C NMR (DMSO-d_6) δ 31.7 (q, N9-Me), 33.9 (q, N1-Me), 35.7 (q, N7-Me), 113.9 (s, C5), 141.6 (d, C8), 146.2 (s, C4), 152.6 (d, C2), and 152.8 (s, C6). HMBC crosspeaks: H-2/C4, C6, and N1-Me; H-8/C4 and C5; Me-1/C6; Me-7/C5 and C8; Me-9/C4 and C8. FABMS (positive, Gly matrix) *m/z* 179 M^+ and 165 $(M-14)^+$. HRFABMS (positive, Gly matrix) *m/z* 179.0931 (calcd for $C_8H_{11}N_4O$, Δ -0.2 mmu).

7. $[\alpha]_D^{23}$ 0.0° (*c* 0.95, 10 % MeOH-$CHCl_3$). IR ν_{max} (KBr) 3300, 2910, 2850, 1640, 1610, 1520, 1450, 1280, 1190, and 1100 cm^{-1}. UV λ_{max} (MeOH) 222.0 (ε 6000, sh.) and 282.0 nm (2100). ^{1}H NMR (DMSO-d_6) δ 3.06 (3 H, s, OMe-α), 3.09 (3 H, s, OMe-β), 3.30 (3 H, s, OMe'-β), 3.92 (1 H, d, *J*=6.5 Hz, H-α), 4.26 (1 H, d, *J*=6.5 Hz, H-β), 6.54 (1 H, dd, *J*=8.0 and 1.8 Hz, H-6), 6.67 (1 H, d, *J*=1.8 Hz, H-2), 6.67 (1 H, d, *J*=8.0 Hz, H-5), and 8.78 (2 H, s, 3- and 4-OH). ^{13}C NMR (DMSO-d_6) δ 53.6 (q, β-OMe'), 54.6 (q, β-OMe), 55.8 (q, α-OMe), 82.8 (d, Cα), 105.8 (d, Cβ), 115.01 (d, C2), 115.19 (d, C5), 119.1 (d, C6), 129.0 (s, C1), 144.77 (s, C3), and 144.83 (s, C4). HMBC crosspeaks: H-2/C1, C3, C4, C6, and Cα; H-5/C2, C3, C4, and C5; H-6/C2, C4, C5, and Cα; H-α/C1, C2, C6, Cβ, and OMe-α; H-β/C1, OMe-β, and OMe'-β; OMe-α/Cα; OMe-β/Cβ; OMe'-β/Cβ. FABMS (negative, TEA matrix) *m/z* 227 $(M-H)^-$. HRFABMS (negative, TEA matrix) *m/z* 227.0913 (calcd for $C_{11}H_{15}O_5$, Δ -0.6 mmu).

*Isolation of **8**.*

The EtOH extract (100 mL x 3) of the sponge *Jaspis* sp. (20 g, wet weight) was concentrated to a small aqueous residue (20 mL) and partitioned into ether (20 mL x 3). The ether extract was subjected to silica gel colum chromatography (Wakogel C-200, 1.1 x 30 cm) with 2 % EtOH-$CHCl_3$ (2.0 mL/min) and silica gel HPLC (LiChrosorb SI-60, 5 µm, 10 x 250 mm, Merck; acetone-hexane 2:3; 1.2 mL/min) to afford **8** (3.0 x 10^{-3} %).

8. ^{1}H NMR ($CDCl_3$) δ 0.99, 1.14, and 1.22 (each 3 H, t, *J*=7.1 Hz, 3 x $-OCH_2CH_3$), 3.25, 3.37, 3.43, 3.55, 3.60, 3.69 (each 1 H, dq, *J*=9.4 and 7.1 Hz, 6 x $-OCH_2CH_3$), 4.15 and 4.42 (each d, *J*=6.1 Hz, H-α and H-β), 5.31 and 5.77 (each 1 H, br. s, 3- and 4-OH), 6.67 (1 H, dd, *J*=8.2 and 1.5 Hz, H-6), 6.80 (1H, d, *J*=8.2 Hz, H-5), and 6.96 (1 H, d, *J*=1.5 Hz, H-2). FABMS (negative, Gly matrix) *m/z* 539 $(2M-H)^-$ and 269 $(M-H)^-$. HRFABMS (negative, PEGsul matrix) *m/z* 269.1380 (calcd for $C_{14}H_{21}O_5$, Δ -0.9 mmu).

*Isolation of **9**.*

The acetone extract (100 mL x 3) of the sponge *Jaspis* sp. (40.38 g, wet weight) was concentrated. The aqueous residue (20 mL) was partitioned into ether (20 mL x 3). The ether layer was subjected to silica gel colum chromatography (Wakogel C-300, 1.1 x 40 cm) with acetone-hexane (1:1, 3.0 mL/min) and gel-filtration on Sephadex LH-20 (1.1 x 40 cm) with acetone (1.5 mL/min) to give **9** (6.3 x 10^{-3} %).

9. IR ν_{max} (KBr) 3280, 1610, 1520, 1410, 1290, 1230, 1180, 1110, 869, and 800 cm^{-1}. UV λ_{max} (MeOH) 230.5 (ε 29000), 259.0 (33200), 299.0 (12000), and 336.0 nm (4300, sh.). ^{1}H NMR (acetone-d_6) δ 6.91 (1 H, d, J=8.2 Hz, H-5'), 7.07 (1 H, dd, J=8.2 and 2.0 Hz, H-6'), 7.19 (1 H, s, H-5), 7.20 (1 H, d, J=2.0 Hz, H-2'), 7.24 (1 H, s, H-8), 7.45 (1 H, dd, J=8.4 and 2.0 Hz, H-3), 7.62 (1 H, d, J=8.4 Hz, H-4), 7.75 (1 H, d, J=2.0 Hz, H-1), and 8.17 (4 H, br. s, 6-, 7-, 3'-, and 4'-OH). ^{13}C NMR (DMSO-d_6) δ 110.2 (d, C5), 110.7 (d, C8), 114.8 (d, C2'), 116.5 (d, C5'), 119.3 (d, C6'), 123.4 (d, C3), 123.7 (d, C1), 127.2 (d, C4), 129.2 (s, C4a), 130.8 (s, C8a), 134.5 (s, C1'), 136.9 (s, C2), 145.4 (s, C4'), 146.3 (s, C3'), 147.1 and 147.5 (each s, C6 and C7). HMBC cross peaks: H-1/C3, C8, C4a, and C1'; H-3/C1 and C4a; H-4/C2, C5, and C8a; H-5/C4, C8a, C2', and C3'; H-8/C1, C4a, C2', and C3'; H-2'/C2, C4', and C6'; H-5'/C1', C3', and C4'; H-6'/C2' and C4'. NOESY cross peaks: H-1/H-8, H-2', and H-6'; H-3/H-4, H-5, H-2', and H-6'; H-5'/H-6'. FABMS (negative, Gly matrix) m/z 267 $(M-H)^-$. HRFABMS (negative, PEGsul matrix) m/z 267.0639 (calcd for $C_{16}H_{11}O_4$, Δ -1.9 mmu).

Enzymatic hydrolysis of (E)-narain (1) with sulfatase.

To a buffer solution of (*E*)-narain (**1**; 10 mg in 400 μL of 0.2 *N* NaOAc-HOAc adjusted to pH 5.0) was added a solution of sulfatase (Type H-1: from *Helix pomatia*, SIGMA; 100 units in 600 μL of the buffer). The mixture was kept at 37 °C for 2.5 h, and the reaction mixture was partitioned between water (5 mL) and ether (5 mL x 3). The ether layer was dried to furnish 3,4-dihydroxyphenylacetoaldehyde (**10**; 1.0 mg). TLC of the aqueous layer showed the disappearance of **1**.

10. ^{1}H NMR ($CDCl_3$) δ 3.55 (2 H, d, J=2.4 Hz, H_2-α), 5.17 and 5.27 (each 1 H, s, 3- and 4-OH), 6.64 (1 H, dd, J=8.1 and 1.9 Hz, H-6), 6.72 (1 H, d, J=1.9 Hz, H-2), 6.84 (1 H, d, J=8.1 Hz, H-5), and 9.68 (1 H, t, J=2.4 Hz, H-β). FABMS (negative, Gly matrix) m/z 151 $(M-H)^-$. HRFABMS (negative, PEGsul matrix) m/z 151.0385 (calcd for $C_8H_7O_3$, Δ -1.0 mmu).

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