# Tichocarpols A and B, Two Novel Phenylpropanoids with Feeding-Deterrent Activity from the Red Alga *Tichocarpus crinitus*

Takahiro Ishii,[†] Tatsufumi Okino,*[,†] Minoru Suzuki,[†,§] and Yuji Machiguchi[‡,⊥]

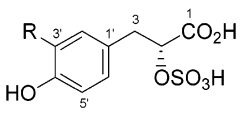
*Division of Material Science, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan, and Hokkaido National Fisheries Research Institute, Kushiro 085-0802, Japan*

*Received April 28, 2004*

Two novel phenylpropanoic acid derivatives, tichocarpol A (**1**) and tichocarpol B (**2**), were isolated along with floridoside and isethionic acid from the red alga *Tichocarpus crinitus*. The structures of these novel metabolites were elucidated using a combination of spectral (1D and 2D NMR techniques and ESIMS) and chemical methods. Compounds **1**, **2**, and floridoside exhibited feeding-deterrent activity against the sea urchin *Strongylocentrotus intermedius*.

It is well known that intense grazing by marine herbivores, such as sea urchins, can greatly reduce the abundance of some seaweeds, even driving some populations to local extinction.[1−3] This is typified by the phenomenon of "sea-urchin barren ground" or "Isoyake areas", specifically, regions where macroalgae have disappeared and crustose coralline red algae dominate extensive areas of the sublittoral rocky surfaces.[4]

To survive against competition and predation by other animals, plants, and bacteria, seaweeds are known to produce feeding deterrents, allelochemicals, and antibacterial substances. Seaweeds found in habitats subjected to high grazing impact are thought to develop chemical defense to avoid predation by herbivorous species.[5,6] For example, red algae are known to produce bromophenols and brominated diterpenes, which exhibit potent feeding-deterrent activity against sea urchins.[7,8] The red alga *Tichocarpus crinitus* is widely distributed along the coast of eastern Hokkaido, and its numbers have not been diminished from grazing by herbivorous species. In fact, we observed that sea urchins grazed on other algae (the brown alga *Laminaria longissima* and the green alga *Ulva pertusa*) and not *T. crinitus*. Thus, we investigated *T. crinitus* for feeding-deterrent compounds. A preliminary experiment showed that lipophilic metabolites of *T. crinitus* were inactive. A bioassay-guided chromatographic separation from aqueous extracts has afforded two novel phenylpropanoids, tichocarpol A (**1**) and tichocarpol B (**2**), together with two known compounds, floridoside (2-*O*-α-galactopyranosylglycerol) and isethionic acid (2-hydroxyethanesulfonic acid). Here we describe the isolation and structural elucidation of these metabolites.




**1** R=H
**2** R=OH

**Table 1.** $^{13}$C NMR (100 MHz, DEPT), $^{1}$H NMR (400 MHz), and HMBC Data[a] for Tichocarpol A (**1**)

| atom no.[b] | $\delta_C$ (mult.) | $\delta_H$ (mult., *J*/Hz) | HMBC correlations |
|---|---|---|---|
| 1 | 177.2 (C) | | |
| 2 | 80.1 (CH) | 4.69 (dd, 5.4, 6.8) | C-1, C-3, C-1′ |
| 3 | 37.8 ($CH_2$) | 3.07 (dd, 5.4, 14.2) | C-1, C-2, C-1′, C-2′, C-6′ |
| | | 2.99 (dd, 6.8, 14.2) | C-1, C-2, C-1′, C-2′, C-6′ |
| 1′ | 128.9 (C) | | |
| 2′, 6′ | 131.2 (CH) | 7.19 (d, 8.8) | C-3, C-2′, C-4′, C-6′ |
| 3′, 5′ | 115.4 (CH) | 6.84 (d, 8.8) | C-1′, C-3′, C-4′, C-5′ |
| 4′ | 154.3 (C) | | |

[a] Measured in $D_2O$. [b] Assignment was made with the aid of the HSQC spectrum.

The red alga *T. crinitus* was collected in June 2001 on the Katsurakoi coast of eastern Hokkaido and extracted with 60% methanol. The aqueous methanol extract, which exhibited feeding-deterrent activity against the sea urchin *Strongylocentrotus intermedius*, was fractionated by gel filtration column chromatography to yield two active fractions. These active fractions were further subjected to a combination of gel filtration column, ion exchange, and paper chromatography to yield two new compounds, tichocarpol A (**1**) and tichocarpol B (**2**), and two known compounds, floridoside and isethionic acid. The known compounds were identified by comparing their spectral data with those reported in the literature.[9−12]

Tichocarpol A (**1**), $[\alpha]^{26}_D$ +2.7° (*c* 0.4, $H_2O$), was isolated as a white solid. Negative ion HR-ESIMS gave a molecular formula of $C_9H_{10}O_7S$ ($[M - H]^-$ 261.0071). Positive ion LR-EIMS showed two peaks at *m*/*z* 182 $[M - SO_3]^+$ and *m*/*z* 164 $[M - H_2SO_4]^+$, suggesting the presence of a sulfate group. The $^{1}$H NMR spectrum (Table 1) showed three mutually coupled aliphatic protons at $\delta$ 4.69 (1H, dd, $J$ = 6.8, 5.4 Hz), 3.07 (1H, dd, $J$ = 14.2, 5.4 Hz), and 2.99 (1H, dd, $J$ = 14.2, 6.8 Hz) and four aromatic protons [$\delta$ 6.84 (2H, d), $\delta$ 7.19 (2H, d)]. Integration and coupling constants (8.8 Hz) of low-field protons resonating at $\delta$ 6.84 and 7.19 supported a *para*-substituted benzene ring. This moiety was assigned as a hydroxy phenyl moiety on the basis of $^{13}$C NMR resonances at $\delta$ 154.3, 131.2, 128.9, and 115.4. A base peak at *m*/*z* 107 $[HOC_6H_4CH_2^+$, EIMS] in the mass spectrum confirmed this moiety. HMBC correlations from H-2′ and H-6′ to C-3 indicated the aromatic ring was attached directly to the methylene (C-3). In addition, a carbonyl carbon at $\delta$177.2 correlated to the $H_2$-3 protons in HMBC, implying the presence of a $\beta$-arylpropanoic acid. The carboxyl group was thought to be adjacent to the

* To whom correspondence should be addressed. Tel: +81-11-706-4519. Fax: +81-11-706-4867. E-mail: okino@ees.hokudai.ac.jp.
[†] Hokkaido University.
[‡] Hokkaido National Fisheries Research Institute.
[§] Present address: Laboratory of Marine Natural Product Chemistry, Borneo Marine Research Institute, University Malaysia Sabah, 88999 Kota Kinabalu, Sabah, Malaysia.
[⊥] Present address: Research and Technological Guidance Division, Fisheries Agency of Japan, Kasumigaseki, Tokyo 100-0013, Japan.

10.1021/np0498509 CCC: $27.50 
Published on Web 09/25/2004

**Table 2.** $^{13}C$ NMR (100 MHz, DEPT), $^{1}H$ NMR (400 MHz), and HMBC Data[a] for Tichocarpol B (**2**)

| atom no. | $\delta_C$ (mult.) | $\delta_H$ (mult., *J*/Hz) | HMBC correlations |
|---|---|---|---|
| 1 | 175.3 (C) | | |
| 2 | 78.3 (CH) | 4.84 (dd, 5.6, 6.4) | C-3, C-1′ |
| 3 | 37.6 ($CH_2$) | 3.09 (dd, 5.6, 14.2) | C-2, C-1′, C-2′, C-6′ |
| | | 3.03 (dd, 6.4, 14.2) | C-2, C-1′, C-2′, C-6′ |
| 1′ | 128.8 (C) | | |
| 2′ | 117.6 (CH) | 6.87 (br s ) | C-3, C-1′, C-3′, C-4′, C-6′ |
| 3′ | 143.9 (C) | | |
| 4′ | 143.0 (C) | | |
| 5′ | 116.3 (CH) | 6.87 (d, 7.8) | C-1′, C-3′, C-4′ |
| 6′ | 122.3 (CH) | 6.77 (d, 7.8) | C-3, C-2′, C-4′ |

[a] Measured in $D_2O$.

oxymethine carbon. Methyl esterification of **1** with 5% HCl in MeOH for 60 min at 100 °C yielded (*p*-hydroxyphenyl)-lactic acid, which was confirmed by $^{1}H$ NMR. $^{1}H$ and $^{13}C$ NMR data for authentic (*p*-hydroxyphenyl)lactic acid and **1** were identical except for oxymethine atom shifts (H-2: $\delta$ 4.46 and 4.69, C-2: $\delta$ 74.1 and 80.1, respectively). This difference revealed that the oxygen attached to C-2 was connected to a sulfate group. Thus, the planar structure of **1** was completed.

The absolute configuration at C-2 in the (*p*-hydroxyphenyl)lactic acid, which was derived from **1** by acid hydrolysis, was assigned as *R* by comparing the observed optical rotation ($[\alpha]^{25}_D$ +10.5° (*c* 0.1, MeOH)) with the literature value ($[\alpha]^{20}_D$ +10.8° (*c* 0.52, MeOH)).[13,14] Therefore, tichocarpol A was identified as (2*R*)-3-(*p*-hydroxyphenyl)-2-sulfooxypropanoic acid.

Tichocarpol B (**2**), $[\alpha]^{28}_D$ +5.9° (*c* 0.34, $H_2O$), was also obtained as a white solid. HR-ESIMS gave a molecular formula of $C_9H_{10}O_8S$ (*m*/*z* 277.0024 $[M - H]^-$). The LR-ESIMS spectrum displayed peaks at *m*/*z* 198 $[M - SO_3]^+$ and *m*/*z* 180 $[M - H_2SO_4]^+$, which indicated the presence of a sulfate group as in **1**. Spectra from $^{1}H$ and $^{13}C$ NMR analysis revealed signals due to $\beta$-arylpropanoic acid groups ($\delta_H$ 3.03, 3.09, and 4.84), a methylene carbon ($\delta_C$ 37.6), an oxygenated methine carbon ($\delta_C$ 78.3), and a carbonyl carbon ($\delta_C$ 175.3). Chemical shifts for **2** in this region, similar to those observed for **1**, indicated a sulfate attached to an oxygen-bearing carbon at C-2. The presence of a 1,3,4-trisubstituted benzene group was demonstrated by characteristic signals in the $^{1}H$ and $^{13}C$ NMR spectra (Table 2) at $\delta_H$ 6.77 (1H, d, $J$ = 7.8 Hz), 6.87 (1H, br s), and 6.87 (1H, d, $J$ = 7.8 Hz), $\delta_C$ 143.9 (C), 143.0 (C), 122.3 (CH), 117.6 (CH), and 116.3 (CH). Thus, chemical shifts and molecular formula assigned a dihydroxy phenyl aromatic ring in **2**, and HMBC correlations (C-3/H-2′ and C-3/H-6′) confirmed the planar structure as 3-(3′,4′-dihydroxyphenyl)-2-sulfooxypropanoic acid. The stereochemistry of **2** was assumed to be the same as **1**.

The feeding-deterrent activity of each compound was evaluated by the cellulose plate method (see Experimental Section). Tichocarpols A and B and floridoside exhibited strong activity, while isethionic acid was inactive and found to stimulate feeding activity. Unfortunately, some impurities (probably saccharide-related compounds) were observed in the $^{1}H$ NMR spectrum of tichocarpol A even after several purification attempts. However, a fraction consisting mainly of such impurities showed no activity, confirming tichocarpol A as the real active compound. Details of the bioassay will be published elsewhere.[15] It is interesting to note that in a recent study the floridoside−isethionic acid complex released by the red alga *Delisea pulchra* was demonstrated to act as an ecologically relevant cue for the induction of metamorphosis in the sea urchin *Holopneustes purpurascens*.[16] In the present study, floridoside as well as tichocarpols A and B exhibited feeding-deterrent properties.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP-140 polarimeter and HORIBA SEPA-300 high sensitive polarimeter. $^{1}H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectra were measured in $D_2O$ solution using a JEOL-JNM-EX-400 spectrometer. EIMS were obtained on a JEOL JMS-FABmate spectrometer. HRESIMS were obtained on a JEOL JMS-700Tz spectrometer. Column liquid chromatography was performed using Toyopearl HW-40F (TOSOH Co., Japan) and Sephadex LH-20 (Pharmacia). Filter paper (Toyo Advantec 50) was used for preparative paper chromatography. Ion exchange chromatography was performed using AG 50W-X8 (20−50 mesh, $H^+$, BioRad) and AG 1-X8 (20−50 mesh, $OH^-$, BioRad). A sample of DL-(*p*-hydroxyphenyl)lactic acid was purchased from Tokyo Kasei, Japan.

**Plant Material.** *Tichocarpus crinitus* (Gmelin) Ruprecht was collected at a depth of 1−2 m on the Katsurakoi coast, Kushiro, Hokkaido, on June 4, 2001. Voucher specimens were deposited in the Graduate School of Environmental Earth Science, Hokkaido University. Plant material was stored at −20 °C until processed.

**Extraction and Isolation.** Partially dried algae (340 g) were extracted with 60% methanol for 24 h, and the extract was concentrated after filtration. The aqueous methanol extract, which demonstrated feeding-deterrent activity, was fractionated by gel filtration column chromatography (Toyopearl HW-40F) with $H_2O$. The active fraction (53.5 mg) was rechromatographed on a Sephadex LH-20 column (3 × 56 cm) with $H_2O$ as the eluent to afford three fractions (A−C) and isethionic acid (4.8 mg). Fraction B (6.2 mg) was further separated by paper chromatography with a combination of *n*-butanol/EtOH/$H_2O$ (7:2:1) and *n*-butanol/AcOH/$H_2O$ (4:2:1) and detected by spraying with phenol reagent to yield tichocarpol A (**1**) (1.8 mg) and tichocarpol B (**2**) (1.4 mg). Fraction C was passed successively through AG 50W-X8 (200 mL, $H^+$) and AG 1-X8 (200 mL, $OH^-$) columns using ion-exchanged water (500 mL). The neutral effluent was evaporated to dryness and recrystallized with hot EtOH to yield floridoside as a white crystal (7.0 mg).

**Tichocarpol A (1):** white solid; $[\alpha]^{26}_D$ +2.7° (*c* 0.4, $H_2O$); $^{1}H$ NMR, $^{13}C$ NMR data, Table 1; LR-EIMS *m*/*z* 182 $[M - SO_3]^+$ (2), 164 $[M - H_2SO_4]^+$ (67), 119 $[M - H_2SO_4 - COOH]^+$ (3), 107 $[HOC_6H_4CH_2]^+$ (100), 91 $[C_6H_5CH_2]^+$ (10), 77 $[C_6H_5]^+$ (13); LR-ESIMS *m*/*z* 261 $[M - H]^-$; HR-ESIMS *m*/*z* 261.0071 $[M - H]^-$ (calcd for $C_9H_9O_7S$, 261.0069).

**Tichocarpol B (2):** white solid; $[\alpha]^{28}_D$ +5.9° (*c* 0.34, $H_2O$); $^{1}H$ NMR, $^{13}C$ NMR data, Table 2; LR-EIMS *m*/*z* 198 $[M - SO_3]^+$ (15), 180 $[M - H_2SO_4]^+$ (56), 135 $[M - H_2SO_4 - COOH]^+$ (65), 123 $[(HO)_2C_6H_3CH_2]^+$ (100), 107 $[C_6H_5CH_2]^+$ (36), 77 $[C_6H_5]^+$ (60); LR-ESIMS *m*/*z* 277 $[M - H]^-$; HR-ESIMS *m*/*z* 277.0024 $[M - H]^-$ (calcd for $C_9H_9O_8S$, 277.0018).

**Conversion (Acid Hydrolysis) of 1 into (2*R*)-3-(*p*-Hydroxyphenyl)lactic Acid.** A solution of compound **1** (5 mg) in aqueous 0.5 M HCl (1 mL) was allowed to react at 100 °C for 3 h. The hydrolysate was extracted with EtOAc and then washed with $H_2O$, dried over anhydrous $Na_2SO_4$, and evaporated to yield (2*R*)-3-(*p*-hydroxyphenyl)lactic acid.

**Bioassay.** A simple and convenient feeding-deterrent assay was performed using the cellulose plate method with cellulose TLC sheets according to a protocol previously described.[8]

**Acknowledgment.** This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and by the Northern Advancement Center for Science & Technology. The authors thank H. Matsumoto and S. Oka, Center for Instrumental Analysis, Hokkaido University, for performing the NMR and ESIMS measurements.

**Supporting Information Available:** $^{1}H$ and $^{13}C$ NMR of tichocarpol A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Himmelman, J. H.; Steele, D. H. *Mar. Biol.* **1971**, *9*, 315−322.
(2) Breen, P. A.; Mann, K. H. *Mar. Biol.* **1976**, *34*, 137−142.
(3) Lang, C.; Mann, K. H. *Mar. Biol.* **1976**, *36*, 321−326.
(4) Breitburg, D. L. *Ecology* **1984**, *65*, 1136−1143.
(5) Taniguchi, K.; Kurata, K.; Suzuki, M. *Nippon Suisan Gakkaishi* **1991**, *57*, 2065−2071.
(6) Taniguchi, K.; Akimoto, Y.; Kurata, K.; Suzuki, M. *Nippon Suisan Gakkaishi* **1992**, *58*, 571−575.
(7) Kurata, K.; Taniguchi, K.; Takashima, K.; Hayashi, I.; Suzuki, M. *Phytochemistry* **1997**, *45*, 485−487.
(8) Kurata, K.; Taniguchi, K.; Agatsuma, Y.; Suzuki, M. *Phytochemistry* **1998**, *47*, 363−369.
(9) Meng, J.; Rosell, K.; Srivastava, L. M. *Carbohyd. Res.* **1987**, *161*, 171−180.
(10) Barrow, K. D.; Karsten, U.; King, R. J. *Phytochemistry* **1993**, *34*, 1429−1430.
(11) Simon-Colin, C.; Kervarec, N.; Pichon, R.; Deslandes, E. *Carbohyd. Res.* **2002**, *337*, 279−280.
(12) Simon-Colin, C.; Kervarec, N.; Pichon, R.; Bessières, M.; Deslandes, E. *Phycol. Res.* **2002**, *50*, 125−128.
(13) Valls, N.; Vallribera, M.; López-Canet, M.; Bonjoch, J. *J. Org. Chem.* **2002**, *67*, 4945−4950.
(14) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *Chem. Eur. J.* **2001**, *7*, 3446−3460.
(15) Ishii, T.; Okino, T.; Suzuki, M.; Kuramata, K.; Machiguchi, Y. *Mar. Biotechnol.*, in press.
(16) Williamson, J. E.; de Nys, R.; Kumar, N.; Carson, D. G.; Steinberg P. D. *Biol. Bull.* **2000**, *198*, 332−345.

NP0498509