# Gymnastatins F−H, Cytostatic Metabolites from the Sponge-Derived Fungus *Gymnascella dankaliensis*

Taro Amagata, Katsuhiko Minoura, and Atsushi Numata*

*Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094 Japan*

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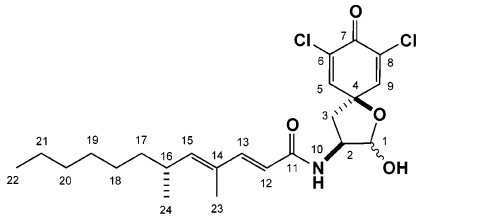
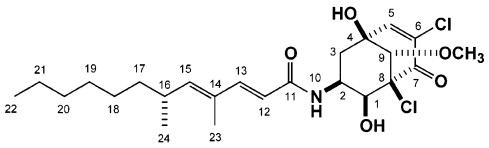
Gymnastatins F −H (**2**−**4**) and gymnamide (**5**) have been isolated from the mycelial MeOH extract of the *Halichondria* sponge-derived fungus *Gymnascella dankaliensis*. Their stereostructures have been established on the basis of spectroscopic analyses using 1D and 2D NMR techniques. The stereochemistry of gymnastatin H (**4**) was determined by its synthesis. Among these compounds, gymnastatins F (**2**) and G (**3**) possess a unique bicyclo[3.3.1]nonane ring as natural products, and they were found to exhibit potent growth inhibition against the P388 cancer cell line.

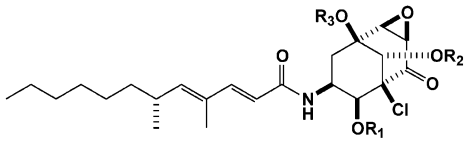
Filamentous fungi separated from marine environments have attracted our attention as a new source of bioactive compounds. In fact, a variety of structurally unique and highly bioactive compounds have been reported to date.[1] In 1989 we initiated a search for anticancer lead compounds from fungi inhabiting the ocean, and have reported a number of novel cytostatic and/or antitumor metabolites.[2−6] As part of this study, we have already reported the structures and cytostatic activities of 1-oxa-spiro[4.5]decane derivatives, gymnastatins A (**1**) and B−E,[7] and unusual steroids, gymnasterones A and B[8] and dankasterone,[9] which were isolated as cytostatic metabolites from a fungal strain of *Gymnascella dankaliensis* OUPS-N134 originally separated from the sponge *Halichondria japonica*. Further investigation on metabolites of this fungal strain led to the isolation of four additional novel metabolites, designated gymnastatins F−H (**2**−**4**), and gymnamide (**5**), of which **2** and **3** possessed a different ring system from that of gymnastatins previously reported.[7] We report herein the isolation and structure elucidation of these compounds together with their growth inhibition against murine P388 lymphocytic leukemia cells.[10]



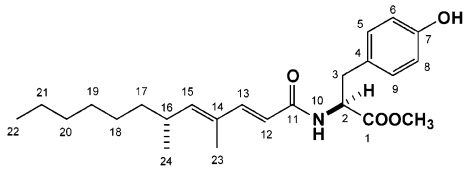
**1**



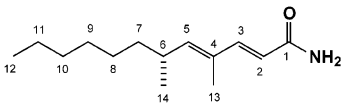
**2**



**3**: $R_1$, $R_2$, $R_3$=H
**6**: $R_1$, $R_2$=Ac, $R_3$=H
**7**: $R_1$, $R_2$, $R_3$=Ac



**4**



**5**

## Results and Discussion

The fungal strain was cultured with static conditions in a liquid medium containing 1% malt extract, 1% glucose, and 0.05% peptone in artificial seawater (pH 7.5) for 28 days at 27 °C. The MeOH extract of the mycelia was purified by bioassay (P388 cell line)-guided fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography procedures as well as reversed-phase HPLC to afford gymnastatins F−H (**2**−**4**) and gymnamide (**5**).

Gymnastatin G (**3**) had the molecular formula $C_{23}H_{34}ClNO_6$, established by HREIMS. The IR spectrum showed bands at 3364, 3309, 1735, 1649, and 1608 $cm^{-1}$, characteristic of a hydroxyl group, a ketone, an NH and a carbonyl group of a secondary amide, and a double bond. This compound was acetylated due to its instability during the NMR measurement. Acetylation of **3** gave stable diacetate **6** and triacetate **7**, the former of which was mainly used for a structure analysis of **3**. Diacetate **6** was predicted to have a different ring system from that of gymnastatins A (**1**) and B−E on the basis of a missing characteristic hemiacetal carbon signal around $\delta_C$ 95. Inspection of the $^1H$ and $^{13}C$ NMR spectra of **6** (Table 1) based on DEPT and HSQC experiments revealed the presence of the following functional groups: three methyl groups including one primary, secondary, and vinylic methyl each, six methylenes, six $sp^3$-methines bearing one epoxy, carbamido, and methyl group each, two acetoxy groups, one disubstituted and trisubstituted double bond each, and a ketone. Assignments of the remaining two quaternary $sp^3$-carbons linked to a chlorine atom and a hydroxy group were allowed by comparison of the $^{13}C$ NMR data with those of triacetate **7**. The C-4 signal ($\delta_C$ 70.7) in diacetate **6** appeared shifted downfield by ca. 8 ppm relative to triacetate **7** ($\delta_C$ 78.4), whereas the C-8 signal ($\delta_C$ 73.7) in **6** showed almost the same chemical shift as that of **7** ($\delta_C$ 73.4). This evidence indicated that the C-4 and C-8 carbons in **6** were linked to a hydroxy group and

* Corresponding author. Tel: +81(726) 90-1000. Fax: +81(726) 90-1005. E-mail: numata@gly.oups.ac.jp.

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**Table 1.** $^1$H and $^{13}$C NMR Data of Gymnastatins G Diacetate (**6**) and Triacetate (**7**) in $CDCl_3$

| | **6** | | | | | **7** | |
|---|---|---|---|---|---|---|---|
| position | $\delta_H$[a] *J*/Hz | $\delta_C$ | $^1$H−$^1$H COSY | HMBC | NOESY | $\delta_H$ *J*/Hz | $\delta_C$ |
| 1 | 5.34 (dd, 3.1, 1.2) | 74.2 (CH) | 2 | 2, 3, 8, 9, 1-OCOCH$_3$ | 2 | 5.34 (dd, 3.1, 1.2) | 74.0 (CH) |
| 2 | 4.40 (dddd, 13.1, 7.8, 5.0, 3.1) | 45.3 (CH) | 1, 3α, 3*β*, 10 | | 1, 3α, 5, 6 | 4.40 (dddd, 13.1, 8.2, 5.0, 3.1) | 45.2 (CH) |
| 3 | α 2.42 (ddd, 13.1, 5.0, 1.2) | 36.9 (CH$_2$) | 2, 3*β* | 2, 4, 5, 9 | 2, 3*β*, 5 | α 3.12 (ddd, 13.1, 5.0, 1.2) | 33.4 (CH$_2$) |
| | *β* 2.09 (t,13.1) | | 2, 3α | 2, 4, 5 | 3α, 9 | *β* 2.39 (t,13.1) | |
| 4 | | 70.8 (C) | | | | | 78.4 (C) |
| 5 | 3.60 (dd, 3.7, 2.0) | 58.1 (CH) | 6, 9 | 3 | 2, 3α, 6 | 4.32 (dd, 3.8, 2.0) | 54.8 (CH) |
| 6 | 3.79 (d, 3.7) | 53.7 (CH) | 5 | 7, 8, 9 | 2, 5 | 3.79 (d, 3.8) | 53.6 (CH) |
| 7 | | 194.6 (C) | | | | | 194.2 (C) |
| 8 | | 73.7 (C) | | | | | 73.4 (C) |
| 9 | 5.65 (d, 2.0) | 73.9 (CH) | 6 | 4, 5, 7, 8, 9-OCOCH$_3$ | 3*β* | 5.98 (d, 2.0) | 70.7 (CH) |
| 10 | 5.60 (d, 7.8) | | 2 | 2, 11 | | 5.42 (d, 8.2) | |
| 11 | | 166.0 (C) | | | | | 165.8 (C) |
| 12 | 5.62 (d, 15.1) | 116.0 (CH) | 13 | 11, 14 | 23 | 5.60 (d, 15.1) | 116.0 (CH) |
| 13 | 7.21 (d, 15.1) | 148.2 (CH) | 12 | 11, 12, 14, 15, 23 | 15 | 7.20 (d, 15.1) | 148.3 (CH) |
| 14 | | 130.7 (C) | | | | | 130.7 (C) |
| 15 | 5.66 (d, 9.8) | 149.0 (CH) | 16, 23 | 14, 16, 23, 24 | 13, 16, 24 | 5.66 (d, 10.0) | 149.0 (CH) |
| 16 | 2.49 (m) | 33.3 (CH) | 15, 17, 24 | | 15, 23, 24 | 2.49 (m) | 33.3 (CH) |
| 17 | a 1.25 (m) | 37.2 (CH$_2$) | 16 | | | a 1.26 (m) | 37.2 (CH$_2$) |
| | b 1.33 (m) | | 16, 18 | | | b 1.33 (m) | |
| 18 | 1.20 (m) | 27.5 (CH$_2$) | 17b | 19 | | 1.22 (m) | 27.5 (CH$_2$) |
| 19 | 1.22 (m) | 29.4 (CH$_2$) | | | | 1.22 (m) | 29.4 (CH$_2$) |
| 20 | 1.22 (m) | 31.8 (CH$_2$) | | 18, 19, 21 | | 1.23 (m) | 31.8 (CH$_2$) |
| 21 | 1.26 (m) | 22.6 (CH$_2$) | 22 | | | 1.25 (m) | 22.6 (CH$_2$) |
| 22 | 0.87 (t, 6.9) | 14.1 (CH$_3$) | 21 | 20, 21 | | 0.87 (t, 6.8) | 14.1 (CH$_3$) |
| 23 | 1.73 (s) | 12.5 (CH$_3$) | 15 | 13, 14, 15 | 12, 16 | 1.73 (s) | 12.5 (CH$_3$) |
| 24 | 0.96 (d, 6.6) | 20.5 (CH$_3$) | 16 | 15, 16, 17 | 15, 16 | 0.97 (d, 6.6) | 20.5 (CH$_3$) |
| 8-OH | 3.48 (br. s) | | | | | | |
| 1-OCOCH$_3$ | | 169.4 (C) | | | | | 169.4 (C) |
| 1-OCOC*H*$_3$ | 2.25 (s) | 20.8 (CH$_3$) | | 1-COCH$_3$ | | 2.26 (s) | 20.8 (CH$_3$) |
| 4-OCOCH$_3$ | | | | | | | 169.7 (C) |
| 4-OCOC*H*$_3$ | | | | | | 2.09 (s) | 21.8 (CH$_3$) |
| 9-OCOCH$_3$ | | 171.5 (C) | | | | | 170.0 (C) |
| 9-OCOC*H*$_3$ | 2.18 (s) | 20.9 (CH$_3$) | | 9-COCH$_3$ | | 2.15 (s) | 20.7 (CH$_3$) |

[a] $^1$H chemical shift values (*δ*/ppm from TMS) followed by multiplicity and then the coupling constant (*J*/Hz).

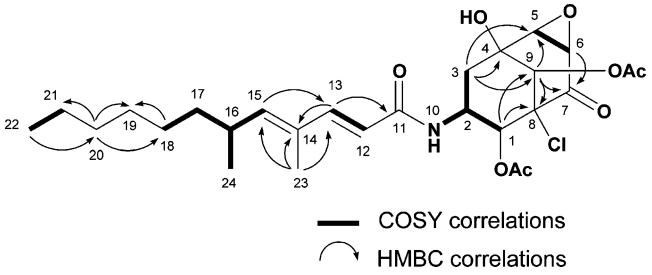



**Figure 1.** 2D NMR correlations for **6**.

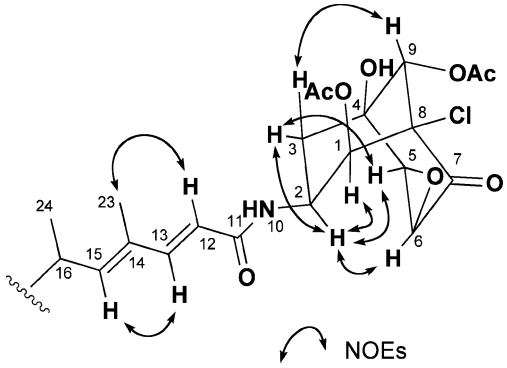



**Figure 2.** NOE correlations for **6**.

a chlorine atom, respectively. The $^1$H−$^1$H COSY analysis of **6** led to five partial structural units as shown by boldfaced lines in Figure 1, which were supported by HMBC correlations (Table 1). The connection of these units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Figure 1, and the planar structure of **6** was elucidated.

The relative stereochemistry of the bicyclo[3.3.1]nonane ring in **6** was established by a combination of coupling constants between vicinal protons ($J_{2,3\beta}$ 13.1 and $J_{1,2}$ 3.1 Hz), a *W*-type long-range coupling (H-5/H-9 and H-1/H-3α), and NOEs (H-1/H-2, H-2/H-3α, H-2/H-5, H-2/H-6, H-3α/H-5, and H-3*β*/H-9), shown in Figure 2. The geometry of the dienes in the side chain was deduced from the large coupling constant between H-12 and H-13 ($J_{12,13}$ 15.1 Hz), a chemical shift ($\delta_C$ 12.5) of the $^{13}$C NMR signal of a vinylic methyl,[11] and NOEs (H-12/H-23 and H-13/H-15) (Figure 2). The relative configuration of the chiral center (C-16) in the side chain was deduced from the fact that the NMR chemical shifts of C-16 ($\delta_H$ 2.49, m, $\delta_C$ 33.3) and C-24 ($\delta_H$ 0.96, d, 6.6 Hz, $\delta_C$ 20.5) in **6** were completely identical with those of gymnastatin A (**1**).[7] The absolute configurations of C-16 and C-2 in **6** have not been established independently, but are assumed to be the same as those of gymnastatin A (**1**) by a consideration of biosynthesis of compound **3**. It is most likely that gymnastatin G (**3**) is biogenetically synthesized via gymnastatin A (**1**) (Scheme 1), which is composed of L-tyrosine and 4,6*R*-dimethyldodeca-2*E*,4*E*-dienoic acid (**8**). As shown in Scheme 1, the bicyclo[3.3.1]nonane ring of compound **3** is considered to be formed by an Aldol condensation between the two different carbonyl groups (C-1 and C-7) in aldehyde **9**. On the basis of this consideration, it was assumed that the absolute configuration of C-2 and C-16 in acetate **6** and consequently gymnastatin G (**3**) is *S* and *R*, respectively, and hence the absolute stereostructures of these compounds are represented as **6** and **3**.

Gymnastatin F (**2**) was assigned the molecular formula $C_{24}H_{35}Cl_2NO_5$ deduced from HREIMS. The general features of the $^1$H and $^{13}$C NMR spectra (Table 2) of **2** closely resembled those of **6** except that the signals for two acetoxy groups and the epoxide in

**Scheme 1.** Plausible Biosynthetic Pathway for **2**−**4**

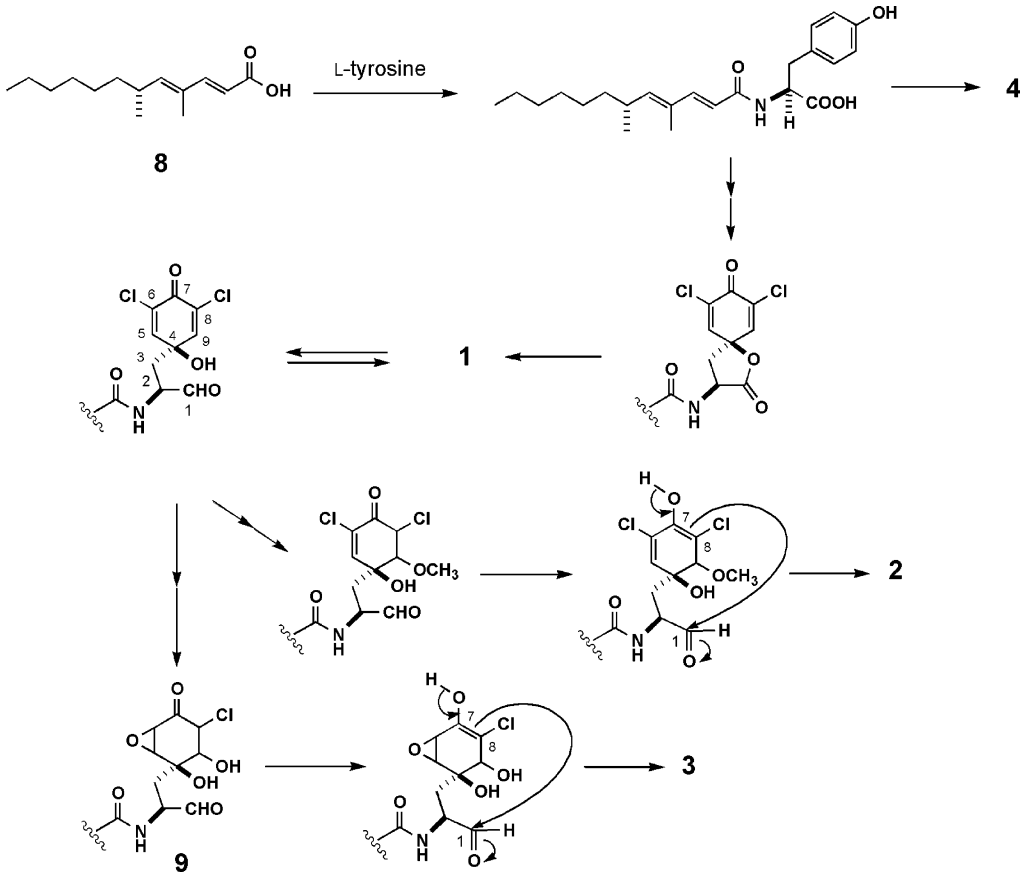


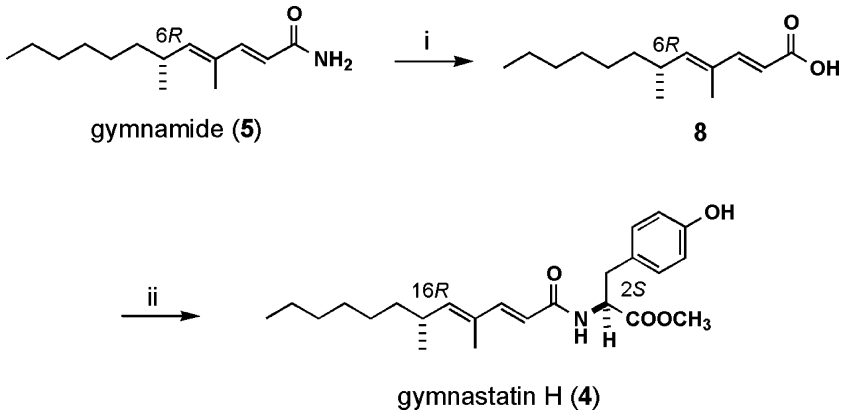

**Table 2.** $^{1}H$ and $^{13}C$ NMR Data of Gymnastatins F (**2**) and H (**4**) in $CDCl_3$

| | **2** | | | | | **4** | |
|---|---|---|---|---|---|---|---|
| position | $\delta_H$[a] J/Hz | $\delta_C$ | $^{1}H-^{1}H$ COSY | HMBC | NOESY | $\delta_H$ J/Hz | $\delta_C$ |
| 1 | 3.88 (br t, 3.0) | 73.0 (CH) | 2 | 9 | 2, 10, 1-OH | | 172.3 (C) |
| 2 | 4.20 (dddd, 13.1, 8.9, 5.1, 3.0) | 45.5 (CH) | 1, 3α, 3$\beta$ | | 1, 3α, 10 | 4.97 (dt, 7.8, 5.8) | 53.3 (CH) |
| 3 | α 2.03 (dd, 13.1, 5.1) | 33.2 ($CH_2$) | 2, 3$\beta$ | 1, 2, 5, 9 | 2, 3$\beta$, 5 | a 3.06 (dd, 14.1, 5.8) | 37.3 ($CH_2$) |
| | $\beta$ 2.14 (t, 13.1) | | 2, 3α | 1, 2, 5, 9 | 3α, 9, 10 | b 3.13 (dd, 14.1, 5.8) | |
| 4 | | 74.0 (C) | | | | | 127.8 (C) |
| 5 (5, 9) | 6.92 (d, 2.4) | 147.4 (CH) | 9 | 6, 7, 9 | 2, 3α | 6.97 (2H, d, 8.5) | 130.5 (CH) |
| 6 (6, 8) | | 131.2 (C) | | | | 6.74 (2H, d, 8.5) | 115.5 (CH) |
| 7 | | 183.2 (C) | | | | | 154.8 (C) |
| 8 | | 81.8 (C) | | | | | |
| 9 | 3.97 (d, 2.4) | 88.7 (CH) | 5 | 1, 5, 7, 8, 9-$OCH_3$ | 3$\beta$, 9-$OCH_3$ | | |
| 10 | 6.03 (d, 8.9) | | 2 | 11 | 1, 2, 3α | 5.94 (d, 7.8) | |
| 11 | | 165.4 (C) | | | | | 166.2 (C) |
| 12 | 5.71 (d, 15.1) | 116.9 (CH) | 13 | 11, 13 | 23 | 5.73 (d, 15.3) | 117.1 (CH) |
| 13 | 7.20 (d, 15.1) | 147.3 (CH) | 12 | 11, 12, 14, 15 | 15 | 7.24 (d, 15.3) | 147.2 (CH) |
| 14 | | 130.8 (C) | | | | | 130.9 (C) |
| 15 | 5.65 (d, 9.3) | 148.3 (CH) | 16, 23 | 13, 16, 17, 23, 24 | 13, 16, 24 | 5.64 (d, 9.8) | 148.2 (CH) |
| 16 | 2.49 (m) | 33.3 (CH) | 15, 24 | 24 | 15, 23, 24 | 2.51 (m) | 33.2 (CH) |
| 17 | a 1.26 (m) | 37.2 ($CH_2$) | | | | a 1.26 (m) | 37.3 ($CH_2$) |
| | b 1.33 (m) | | | | | b 1.33 (m) | |
| 18 | 1.22 (m) | 27.5 ($CH_2$) | | | | 1.22 (m) | 27.5 ($CH_2$) |
| 19 | 1.22 (m) | 29.4 ($CH_2$) | | | | 1.22 (m) | 29.4 ($CH_2$) |
| 20 | 1.23 (m) | 31.8 ($CH_2$) | | | | 1.23 (m) | 31.8 ($CH_2$) |
| 21 | 1.25 (m) | 22.6 ($CH_2$) | | | | 1.25 (m) | 22.7 ($CH_2$) |
| 22 | 0.87 (t, 6.8) | 14.1 ($CH_3$) | | 20, 21 | | 0.88 (t, 6.7) | 14.1 ($CH_3$) |
| 23 | 1.75 (s) | 12.5 ($CH_3$) | 15 | 13, 14, 15 | 12, 16 | 1.75 (s) | 12.5 ($CH_3$) |
| 24 | 0.97 (d, 6.6) | 20.5 ($CH_3$) | 16 | 15, 16, 17 | 15, 16 | 0.97 (d, 6.6) | 20.6 ($CH_3$) |
| 1-OH | 3.09 (br s) | | 1 | | | | |
| 4-OH | 3.27 (br s) | | | | | | |
| 7-OH | | | | | | 5.18 (br s) | |
| 9-$OCH_3$ | 3.69 (s) | 63.0 ($CH_3$) | | 9 | | | |
| 1-$OCH_3$ | | | | | | 3.74 (s) | 52.4 ($CH_3$) |

[a] $^{1}H$ chemical shift values ($\delta$/ppm from TMS) followed by multiplicity and then the coupling constant (J/Hz).

the bicyclononane ring of **6** were replaced by those of hydroxy and methoxy groups, and a trisubstituted double bond with a chlorine atom, respectively. The planar structure of **2** deduced from this evidence was confirmed by $^{1}H-^{1}H$ COSY (H-1/H-2, H-2/H-10, H-2/H-3α, H-2/H-3$\beta$, etc.) and HMBC (H-1/C-9, H-3/C-5, H-3/C-9, H-5/C-6, H-5/C-7, H-5/C-9, H-9/C-7, H-9/C-8, H-9/9-O$CH_3$) correlations. The stereostructure of the bicyclononane ring of **2** was deduced from a combination of the proton vicinal coupling constants

**Scheme 2.** Synthesis of Gymnastatin H (**4**) from Gymnamide (**5**)[a]




[a] (i) 20% KOH; (ii) L-tyrosine methyl ester, WSC.

($J_{2,3\alpha}$ 13.1 and $J_{1,2}$ 3.0 Hz) and NOEs (H-1/H-2, H-2/H-3α, H-3α/H-5, H-3β/H-9). The geometry of the diene and the relative configuration of C-16 in the side chain of **2** were deduced from the fact that the NMR data of **2** including the coupling constant (H-12/H-13), NOEs (H-12/H-23 and H-13/H-15), and the $^1$H and $^{13}$C chemical shifts of C-16, C-23, and C-24 were identical with those of **6**. The biosynthetic pathway of **2** (Scheme 1) via gymnastatin A (**1**) with the 2*S* and 16*R* configurations led to the absolute stereostructure of **2** for gymnastatin F.

Gymnamide (**5**) was shown to have a molecular formula of $C_{14}H_{25}NO$ by HREIMS. Its UV and IR spectra suggested the presence of a conjugated amide moiety. The general features of the $^1$H and $^{13}$C NMR spectra (Table 2) closely resembled those of the side chain in gymnastatins. This compound was hydrolyzed by an aqueous KOH solution to give 4,6*R*-dimethyldodeca-2*E*,4*E*-dienoic acid (**8**), which was identified by comparison of spectral data including optical rotation with published values.[12] This evidence allowed assignment of stereostructure **5** to gymnamide.

Gymnastatin H (**4**) had the molecular formula $C_{24}H_{35}NO_4$ established by HREIMS. Its UV and IR spectra exhibited absorption bands characteristic of hydroxyl and/or amino groups, an ester, an amide, a double bond, and an aromatic ring. The $^1$H and $^{13}$C NMR spectra (Table 2) of **4** suggested the presence of a tyrosine methyl ester moiety (C-1−C-9) and the same side chain (C-11−C-24) as that of other gymnastatins. In addition, the $^1$H and $^{13}$C chemical shifts ($\delta_H$ 4.97, $\delta_C$ 53.3) of C-2 in **4** revealed that 4,6-dimethyldodeca-2*E*,4*E*-dienoic acid was linked as an amide to C-2 of tyrosine methyl ester, and the planar structure **4** of gymnastatin H was thus elucidated. The absolute chemistry of **4** was determined by its synthesis (Scheme 2). L-Tyrosine methyl ester was treated with acid **8** derived from **5** in the presence of water-soluble carbodiimide (WSC) to furnish the desired molecule **4**. The spectral data and optical rotation of this product were identical with those of the natural product. This evidence led to stereostructure **4** for gymnastatin H.[13]

All of the isolated compounds (**2**−**5**) were evaluated for cancer cell growth inhibition against the P388 lymphocytic leukemia cell line. 5-Fluorouracil ($ED_{50}$ 0.073 μg/mL) was used as a positive control. The results showed that both gymnastatins F (**2**) and G (**3**) exhibited significant growth inhibition ($ED_{50}$ 0.13 and 0.030 μg/mL, respectively), whereas gymstatin H (**4**) and gymnamide (**5**) were inactive (>10 μg/mL, each). The inhibitory activity of compound **3** was more potent than that of **2**, implying that an α,β-epoxyketone system is more important than an α,β-unsaturated ketone system for enhancement of the activity in gymnastatin analogues.

## Experimental Section

**General Experimental Procedures.** Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter. CD spectra were recorded on a JASCO J-500A spectrometer. UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin-Elmer 1720X FT-IR spectrometer. 1D and 2D NMR spectra were recorded at 27 °C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125.7 MHz for $^1$H and $^{13}$C, respectively, with TMS as an internal reference. EIMS was determined using a Hitachi M-4000H mass spectrometer. Liquid chromatography over Si gel (mesh 230−400) was performed at medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R401) and Shim-pack PREP-ODS (250 mm × 20 mm i.d.). Analytical TLC was performed on precoated Merck aluminum sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent $CH_2Cl_2$−MeOH (19:1), and compounds were viewed under UV lamp and sprayed with 10% $H_2SO_4$ followed by heating.

**Biological Materials.** The fungus was initially isolated from the sponge *Halichondria japonica*, collected in the Osaka Bay of Japan on April, 1994. The fungal strain (OUPS-N134) was identified as *Gymnascella dankaliensis* by Dr. T. Ito, Institute for Fermentation, Osaka, Japan, on the basis of the analysis of its fruiting body.

**Culture Conditions and Extraction.** The fungal strain was grown in a liquid medium (90 L) containing 1% malt extract, 1% glucose, and 0.05% peptone in artificial seawater adjusted to pH 7.5 for 28 days at 27 °C. The culture was filtered under suction, and the mycelium collected was extracted 3× with MeOH. The combined extracts were evaporated in vacuo to give the crude extracts (11.0 g).

**Isolation of Pure Compounds.** The $CH_2Cl_2$−MeOH (1:1) soluble portion of the crude extract was passed through Sephadex LH-20 using $CH_2Cl_2$−MeOH (1:1) as eluent. The second fraction (F1; 7.0 g), in which the activity was concentrated, was chromatographed on a Si gel column with an *n*-hexane−$CH_2Cl_2$−MeOH gradient as eluent to give three active fractions [F2 (558.7 mg) eluted with MeOH−$CH_2Cl_2$ (1:99), F3 (212.4 mg) and F4 (144.0 mg) eluted with MeOH−$CH_2Cl_2$ (1:49)]. F2 was further chromatographed on a Si gel column with a $CH_2Cl_2$−MeOH gradient as eluent, and the MeOH−$CH_2Cl_2$ (1:99) eluate (164.9 mg) was twice purified by RP HPLC using acetone−$H_2O$ (4:1 and 3:2, respectively) to afford **2** (1.3 mg) and **4** (1.4 mg). F3 and F4 afforded **5** (7.4 mg) and **3** (8.8 mg), respectively, after purification by RP HPLC using acetone−$H_2O$ (7:3).

**Gymnastatin F (2):** colorless powder; $[\alpha]^{26}_D$ −77.7 (*c* 0.16, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 263 nm (4.25); IR (KBr) $\nu_{max}$ 3360, 3303, 1703, 1653, 1608 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; HREIMS *m*/*z* 487.1888 [M]$^+$ (calcd for $C_{24}H_{35}Cl_2NO_5$, 487.1891).

**Gymnastatin G (3):** colorless powder; $[\alpha]^{26}_D$ −53.1 (*c* 1.47); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 266 nm (4.63); IR (KBr) $\nu_{max}$ 3364, 3309, 1735, 1649, 1608 cm$^{-1}$; HREIMS *m*/*z* 455.2085 [M]$^+$ (calcd for $C_{23}H_{34}ClNO_6$, 455.2072).

**Acetylation of 3.** To a solution of **3** (18.8 mg) in pyridine (0.2 mL) was added $Ac_2O$ (0.2 mL), and the reaction mixture was left at rt overnight. The solution was concentrated to dryness under reduced pressure, and the residue was purified by RP HPLC using MeOH−$H_2O$ (9:1) to afford diacetate **6** (7.3 mg) and triacetate **7** (2.7 mg).

**Diacetate 6:** colorless powder; $[\alpha]^{22}_D$ −46.2 (*c* 0.68); IR (KBr) $\nu_{max}$ 3283, 1748, 1734, 1657, 1613 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; HREIMS *m*/*z* 539.2288 [M]$^+$ (calcd for $C_{27}H_{38}ClNO_8$, 539.2283).

**Triacetate 7:** colorless powder; $[\alpha]^{22}_D$ −15.9 (*c* 0.72); IR (KBr) $\nu_{max}$ 3357, 1760, 1753, 1743, 1735, 1658, 1613 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; HREIMS *m*/*z* 581.2392 $[M]^+$ (calcd for $C_{29}H_{40}ClNO_9$, 581.2389).

**Gymnastatin H (4):** colorless oil; $[\alpha]^{26}_D$ +42.3 (*c* 0.14); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 266 (4.51), 238 nm (4.18); IR (KBr) $\nu_{max}$ 3384, 3276, 1740, 1656, 1615, 1517, 1457 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 2; HREIMS *m*/*z* 401.2566 $[M]^+$ (calcd for $C_{24}H_{35}NO_4$, 401.2564).

**Gymnamide (5):** colorless oil; $[\alpha]^{26}_D$ −47.6 (*c* 0.76); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 261 (4.24), 228 nm (3.71 sh); IR (KBr) $\nu_{max}$ 3336, 3191, 1661, 1600 $cm^{-1}$; $^1H$ NMR $\delta$ ppm ($CDCl_3$) 7.24 (1H, d, *J*=15.3 Hz, H-3), 5.79 (1H, d, *J*=15.3 Hz, H-2), 5.66 (1H, d, *J*=9.8 Hz, H-5), 5.61 (2H, br. s. $CONH_2$), 2.51 (1H, m, H-6), 1.78 (3H, s, H-13), 1.37 (2H, m, H-7), 1.23 (8H, m, H-8−H-11), 0.97 (3H, d, *J* = 6.6 Hz, H-14), 0.87 (3H, t, *J* = 6.7 Hz, H-12); $^{13}C$ NMR $\delta$ ppm ($CDCl_3$) 168.8 (C-1), 148.4 (C-5), 147.8 (C-3), 130.8 (C-4), 116.1 (C-2), 37.3 (C-7), 33.2 (C-6), 31.8 (C-10), 29.4 (C-9), 27.5 (C-8), 22.6 (C-11), 20.5 (C-14), 14.1 (C-12), 12.5 (C-13); HREIMS *m*/*z* 223.1222 $[M]^+$ (calcd for $C_{14}H_{25}NO$, 223.1235).

**Hydrolysis of Gymnamide (5).** To a solution of gymnamide (**5**, 30 mg) in MeOH (0.5 mL) was added 20% aqueous KOH (2.5 mL), and the reaction mixture was heated under reflux for 1.5 h. After the reaction mixture was washed with EtOAc, the aqueous phase was acidified with diluted HCl and then extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by Si gel chromatography using *n*-hexane−EtOAc (1:99) as eluent to afford acid **8** (2.5 mg) as a colorless oil, which was identified by comparison of spectral data including optical rotation with published values:[12] $[\alpha]^{24}_D$ −39.2 (*c*, 0.55); IR (KBr) $\nu_{max}$ 1687, 1618 $cm^{-1}$; $^1H$ NMR $\delta$ ppm ($CDCl_3$) 7.31 (1H, d, *J* = 15.3 Hz, H-3), 5.80 (1H, d, *J* = 15.3 Hz, H-2), 5.72 (1H, d, *J* = 9.8 Hz, H-5), 2.54 (1H, m, H-6), 1.79 (3H, s, H-13), 1.22−1.43 (10H, m, H-7−H-11), 0.98 (3H, d, *J* = 6.6 Hz, H-14), 0.87 (3H, t, *J* = 6.9 Hz, H-12); EIMS *m*/*z* 224 $[M]^+$.

**Synthesis of Gymnastatin H (4).** To a solution of acid **8** (12 mg) in $CH_2Cl_2$ (10 mL) were added l-tyrosine methyl ester (13 mg) and WSC [water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride] (10 mg). After stirring at rt overnight, the reaction mixture was washed with 1 N HCl, $NaCO_3$(aq), and brine, dried over anhydrous $Na_2SO_4$, and then evaporated under reduced pressure. The residue was purified by Si gel chromatography using *n*-hexane−EtOAc (3:2) as eluent to give gymnastatin H (**4**, 7.3 mg) as a colorless oil, which was identified by direct comparison with the natural product.

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**Note Added after ASAP Publication.** References 10−13 have changed in the version posted on October 4, 2006.

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