Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Four new polyoxygenated gorgosterols from the gorgonian Isis hippuris

Mohammad Helal Uddin [a], Novriyandi Hanif [b], Agus Trianto [a c], Yutaka Agarie [a], Tatsuo Higa [a] & Junichi Tanaka [a]

[a] Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

[b] Department of Chemistry, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan

[c] Department of Marine Science, Diponegoro University, Semarang 50234, Indonesia

Published online: 14 Mar 2011.

To cite this article: Mohammad Helal Uddin , Novriyandi Hanif , Agus Trianto , Yutaka Agarie , Tatsuo Higa & Junichi Tanaka (2011) Four new polyoxygenated gorgosterols from the gorgonian Isis hippuris , Natural Product Research: Formerly Natural Product Letters, 25:6, 585-591, DOI: 10.1080/14786419.2010.485303

To link to this article: http://dx.doi.org/10.1080/14786419.2010.485303

Natural Product Research
Vol. 25, No. 6, March 2011, 585–591

Taylor & Francis
Taylor & Francis Group

Four new polyoxygenated gorgosterols from the gorgonian *Isis hippuris*

Mohammad Helal Uddin[a], Novriyandi Hanif[b], Agus Trianto[ac], Yutaka Agarie[a], Tatsuo Higa[a] and Junichi Tanaka[a]*

[a]*Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan;* [b]*Department of Chemistry, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan;* [c]*Department of Marine Science, Diponegoro University, Semarang 50234, Indonesia*

(*Received 30 September 2009; final version received 7 April 2010*)

Four new polyoxygenated steroids (**1**–**4**) together with four known ones (**5–8**) have been isolated from the gorgonian *Isis hippuris*. The structures of the new compounds have been elucidated by spectroscopic analysis and chemical conversion. All of the new steroids showed moderate cytotoxicity against cultured NBT-T2 cells.

Keywords: polyoxygenated steroids; gorgosterol; NBT-T2; cytotoxicity

1. Introduction

Marine invertebrates such as sponges and octocorals have been recognised as rich sources of steroids having unusual structures and various biological activities (D'Auria, Minale, & Riccio, 1993). Recent examples include cytotoxic eurysterols (Boonlarppradab & Faulkner, 2007), antitubercular parguesterols (Wei, Rodriguez, Wang, & Franzblau, 2007), anti-angiogenic cortistatins (Aoki et al., 2006, 2007; Watanabe, Aoki, Tanabe, Setiawan, & Kobayashi, 2007), proteasome-inhibiting agosterols (Aoki et al., 1998; Tsukamoto, Tatsuno, van Soest, Yokosawa, & Ohta, 2003) and 4-acetoxyplakinamine B, which inhibits acetylcholinesterase (Langjae, Bussarawit, Yuenyongsawad, Ingkaninan, & Plubrukarn, 2007).

The gorgonian *Isis hippuris*, distributed widely in the western Pacific, has yielded a number of polyoxygenated steroids, most of which can be classified into two distinct structural types: the hippuristanol class, having a spiroketal moiety (Higa, Tanaka, & Tachibana, 1981a; Higa, Tanaka, Tsukitani, & Kikuchi, 1981b; Kazlauskas, Murphy, Quinn, Wells, & Schönholzer, 1977) and the polyoxygenated gorgosterol class (Rao, Ramana, Rao, Fahy, & Faulkner, 1988; Tanaka, Higa, Tachibana, & Iwashita, 1982) and others (Sheu, Chen, Sung, Chiang, & Dai, 2000). Those of the first type were originally reported as cytotoxins and later rediscovered

*Corresponding author. Email: jtanaka@sci.u-ryukyu.ac.jp

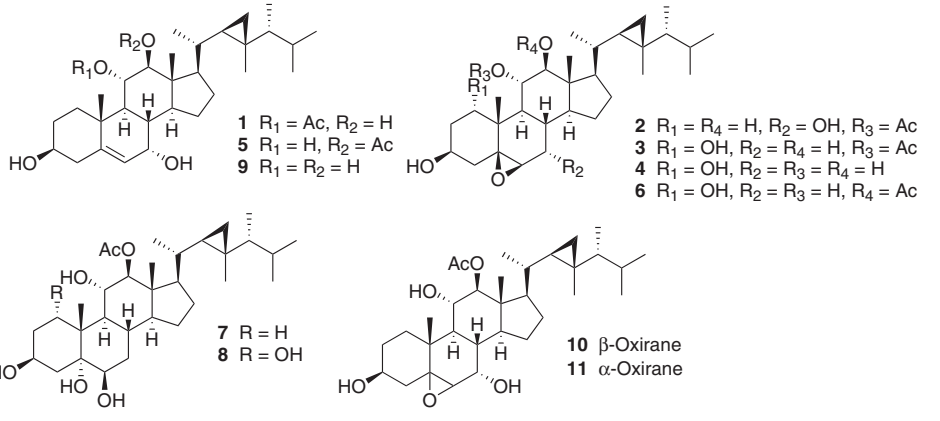
ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786419.2010.485303
http://www.informaworld.com

as selective inhibitors against the translation factor eIF4A (Bordeleau et al., 2006; Lindqvist et al., 2008). Some of the second types were reported to show cytotoxicity or a reversal of multidrug resistance activity (Tanaka et al., 2002). In the course of further studies on the bioactive metabolites of the gorgonian, we found four new gorgosterols (**1**–**4**), whose structures and biological activities are described herein.

2. Results and discussion

An extract of the gorgonian was subjected to a series of chromatographic processes guided by cyclopropyl signals in ^{1}H-NMR spectra to furnish four new polyoxygenated gorgosterols (**1**–**4**) and four known members (**5**–**8**) (Tanaka et al., 2002).



The molecular formula of compound **1**, $C_{32}H_{52}O_5$, indicated seven degrees of unsaturation, which can be accounted for by a steroidal nucleus, a trisubstituted double bond (δ 5.66dd; δ 146.1s, 124.0d), an acetoxy group (δ 2.10; δ 21.9, 172.8 and 1713 cm^{-1}) and a cyclopropyl ring (δ −0.11dd, 0.41td and 0.47dd), as observed for the previously discovered members (Sheu et al., 2000; Wei et al., 2007). Judging from four oxymethine signals (δ 3.38dd, 3.56m, 3.88brs and 5.25dd; δ 76.8, 64.8, 70.9 and 82.8), the molecule was suggested to have three hydroxyls and one acetoxy group. By tracking COSY cross peaks, we have H-9 (δ 1.78m)/H-11 (δ 5.25dd), H-11/H-12 (δ 3.38dd), H-12/12-OH (δ 2.00d), H-3/H-2,4 and H-6/H-7; these functionalities were elucidated to be at C-3, -7, -11 and -12. The acetoxy group was placed at C-11 by a HMBC correlation (H-11/C=O). Coupling constants ($J_{9,11} = 10.0$ Hz and $J_{11,12} = 8.8$ Hz) and NOEs (H-11/H-18,19) indicated that both H-11 and H-12 are axially oriented. The configuration at C-7 was determined by an NOE between H-7 and H-19. Treatment of **1** with NaOMe in MeOH gave tetrol **9**, which showed the same ^{1}H-NMR spectrum as that obtained from **5**. Therefore, compound **1** is elucidated as gorgost-5-en-3β,7α,11α,12β-tetrol 11-acetate.

A crystalline molecule **2** was found to have a molecular formula of $C_{32}H_{52}O_6$ with an additional oxygen atom on **1**. Compound **2** contained one acetoxy (δ 2.12s, 5.05t; δ 172.7s, 22.2q and 77.5d; 1712 cm^{-1}) and three hydroxyl groups (δ 4.12brs, 3.74m and 3.27dd; δ 67.2d, 68.9d and 82.6d; 3422 cm^{-1}). Analysis of COSY correlations [H-9 (δ 1.67m)/H-11 (δ 5.05), H-11/H-12 (δ 3.27dd), H-12/12-OH

Table 1. IC_{50} values for cytotoxicity against NBT-T2 cells.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Latrunculin A
IC_{50} ($\mu g\,mL^{-1}$)	11	1.8	3.5	7.5	13	2.3	13	12	0.15

(δ 2.03d) and H-3/H-2,4] indicated that **2** has the same A and C rings. The remaining oxygen atom can be placed to an epoxide (δ 3.10d; δ 63.7s and 64.0d) in the place of a double bond in **1**. Since the epoxy signals are much closer to those (δ 3.10d; δ 64.3s and 64.4d) observed for β-epoxy steroid **10** than those (δ 3.25d; δ 69.4s and 62.8d) for α-epoxide **11** (Tanaka et al., 2002), the structure is assigned as 5β,6β-epoxygorgostan-3β,7α,11α,12β-tetrol 11-acetate.

Compound **3** was shown to be isomeric to **2** by HRESI–MS and to retain the same functionalities as in **2**: an epoxide (δ 3.12d; δ 62.2s and 63.6d), three hydroxyl (δ 3.86brs, 4.16m and 3.30d; δ 74.0d, 63.7d and 82.9d; 3411 cm^{-1}) and one acetoxy (δ 2.11s, 5.07dd; δ 21.9q, 172.3s and 77.4d; 1719 cm^{-1}) groups. COSY connectivity studies revealed the presence of two hydroxyl groups on the A ring [H-1 (δ 3.86)/H-2$\alpha\beta$ (δ 1.68 and 2.09), H-2$\alpha\beta$/H-3 (δ 4.16)], an acetate at C-11 and a hydroxyl at C-12 [H-11 (δ 5.07)/H-9, 12 (δ 3.30)], as in **2**, and no hydroxyl group on the B ring [H-6 (δ 3.12)/H-7β (δ 2.06), H-7α (δ 1.36)/H-7β,8 (δ 1.59)]. Since we observed almost the same chemical shifts from H-2 to H-8, C-1 to C-8 and C-14 to C-30 between **3** and **6**, their structural difference was believed to be the position of the acetate. Therefore, the structure of **3** was elucidated as 5β,6β-epoxygorgostan-1α,3β,11α,12β-tetrol 11-acetate. As compound **3** was suspected to be an artefact of the isolation process, we treated **6** with several conditions but failed to detect **3**. In addition, we isolated **2** in a good amount without an isomeric molecule having an acetate at C-12, indicating that the specimen contained 11-acetoxy series. These facts may support compound **3** being a natural product.

Compound **4**, $C_{30}H_{50}O_5$ by HRESI–MS, contained four hydroxyl groups (δ 4.36brs, 4.14m, 3.59dd and 3.19d; δ 83.6d, 73.8d, 73.8d and 63.4d; 3385 cm^{-1}) and an epoxide (δ 3.10d; δ 63.3s and 64.5d). 2D-NMR analysis indicated that it was a desacetyl derivative of **3**. Both compounds **3** and **6** could be converted to **4**, which showed identical ^{1}H-NMR signals and thin layer chromatography (TLC) behaviour to those of a natural sample of **4**.

The cytotoxicity of compounds **1**–**8** were evaluated using NBT-T2 rat bladder epithelial cells. The IC_{50} values for **1**–**8** (Table 1) indicated somewhat enhanced activity, with the compounds **2**–**4** and **6** having an epoxy function.

3. Experimental

3.1. *General experimental procedures*

Optical rotations were measured on a Jasco P-1010 polarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Jeol A500 NMR spectrometer. The chemical shifts are given in δ (ppm) and coupling constants are in Hz. ESI–MS data were obtained on a PE Q-STAR mass spectrometer and IR spectra were obtained on a Varian FTIR FTS3000 spectrometer. HPLC was performed on a Hitachi L-6000

pump equipped with a Shodex RI-101 monitor and a Hitachi L-4000 UV detector using a Cosmosil 5C18AR-II or a Mightysil RP-18 column. Merck silica gel was used for vacuum flash chromatography and normal phase column chromatography. All solvents used were of reagent grade. A Tecan Sunrise microplate reader was used for cytotoxicity testing.

3.2. *Animal material*

A specimen of the gorgonian *I. hippuris* was collected using SCUBA at a depth of 10 m from Izena Island in Okinawa and was kept frozen until extraction. As we have studied the metabolites of *I. hippuris* previously (Bordeleau et al., 2006; Higa et al., 1981a, 1981b; Lindqvist et al., 2008; Tanaka et al., 1982, 2002), the species was identified by one of us (JT).

3.3. *Extraction and isolation*

The gorgonian (15.6 kg, wet) was cut into pieces and extracted by steeping in acetone (27 L) for one day; the extraction process was then repeated three times. After concentration under vacuum, the resulting aqueous suspension was extracted with EtOAc to give 163 g of crude oil. The oil was passed through a bed of CHP-20P polystyrene gel by eluting first with MeOH and then with EtOAc. The MeOH eluate was concentrated to give an oil (53.7 g) which was separated by vacuum flash chromatography on silica gel to give 13 fractions. The 12th fraction (7.00 g) was chosen for further separation on silica gel as its ^{1}H-NMR spectrum exhibited cyclopropyl signals characteristic of gorgosterols. Of the nine subfractions obtained after open column chromatography (OCC) on silica, the fourth subfraction (1.19 g) was subjected to repeated separation on RP–HPLC (MeOH : MeCN : H_2O, 6 : 1 : 1) to give new sterols **1** (9.3 mg), **2** (21.2 mg), **3** (4.1 mg) and **4** (3.5 mg), with two known sterols **5** (14.4 mg) and **6** (48.4 mg). Similarly, the fifth subfraction (756.3 mg) yielded three sterols, **6** (34.3 mg), **7** (3.6 mg) and **8** (33.7 mg).

3.3.1. *Gorgost-5-en-3β,7α,11α,12β-tetrol 11-acetate (**1**)*

White amorphous powder; $[\alpha]_D^{26}$ −44 ($c = 0.22$, MeOH); IR (neat) 3419, 2957, 1713 and 1256 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.66 (dd, $J = 1.7$, 5.6 Hz, H-6), 5.25 (dd, $J = 8.8$, 10.0 Hz, H-11), 3.88 (brs, H-7), 3.56 (m, H-3), 3.38 (dd, $J = 7.3$, 8.8 Hz, H-12), 1.12 (s, H-19), 1.11 (d, $J = 6.6$ Hz, H-21), 0.94 (d, $J = 6.6$ Hz, H-28), 0.93 (d, $J = 6.6$ Hz, H-27), 0.91 (s, H-29), 0.87 (d, $J = 6.6$ Hz, H-26), 0.77 (s, H-18), 0.47 (dd, $J = 4.1$, 4.4 Hz, H-30a), 0.41 (td, $J = 5.4$, 6.6 Hz, H-22), 0.27 (m, H-24) and −0.11 (dd, $J = 4.4$, 5.4 Hz, H-30b); ^{13}C-NMR ($CDCl_3$) δ 172.8s (Ac), 146.1s (C-5), 124.0d (C-6), 82.8d (C-12), 76.8d (C-11), 70.9d (C-3), 64.8d (C-7), 57.8d (C-17), 50.5d (C-24), 47.5s (C-13), 46.7d (C-14), 45.5d (C-9), 42.4t (C-4), 39.0s (C-10), 37.5t (C-1), 37.0d (C-8), 33.5d (C-20), 32.1d (C-25), 31.6t (C-2), 30.3d (C-22), 27.9t (C-16), 25.3s (C-23), 23.6t (C-15), 22.3q (C-21), 22.2q (C-27), 21.9q (Ac), 21.7t (C-30), 21.4q (C-26), 18.0q (C-19), 15.3q (C-28), 13.7q (C-29) and 9.1q (C-18); HRESI–MS m/z 539.3715 $[M + Na]^+$ (Calcd for $C_{32}H_{52}O_5Na$ 539.3712).

3.3.2. *5β,6β-Epoxygorgostan-3β,7α,11α,12β-tetrol 11-acetate (**2**)*

Colourless plates (MeOH); $[\alpha]_D^{26}$ −14 ($c = 0.15$, MeOH); IR (neat) 3422, 2954, 1712 and 1249 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.05 (t, $J = 9.0$ Hz, H-11), 4.12 (brs, H-7), 3.74 (m, H-3), 3.27 (dd, $J = 8.3$, 9.0 Hz, H-12), 3.10 (d, $J = 3.4$ Hz, H-6), 1.09 (d, $J = 6.6$ Hz, H-21), 1.03 (s, H-19), 0.94 (d, $J = 6.8$ Hz, H-28), 0.93 (d, $J = 6.8$ Hz, H-27), 0.90 (s, H-29), 0.86 (d, $J = 6.8$ Hz, H-26), 0.74 (s, H-18), 0.47 (dd, $J = 4.1$, 4.6 Hz, H-30a), 0.39 (td, $J = 5.4$, 6.6 Hz, H-22), 0.27 (m, H-24) and −0.12 (dd, $J = 4.6$, 5.4 Hz, H-30b); ^{13}C-NMR ($CDCl_3$) δ 172.7s (Ac), 82.6d (C-12), 77.5d (C-11), 68.9d (C-3), 67.2d (C-7), 64.0d (C-6), 63.7s (C-5), 58.0d (C-17), 50.5d (C-24), 47.7s (C-13), 46.9d (C-14), 45.0d (C-9), 42.2t (C-4), 37.7t (C-1), 35.1s (C-10), 33.3d (C-20), 32.8d (C-8), 32.1d (C-25), 30.9t (C-2), 30.2d (C-22), 27.7t (C-16), 25.3s (C-23), 22.9t (C-15), 22.4q (C-21), 22.2q (Ac), 21.9q (C-27), 21.7t (C-30), 21.4q (C-26), 16.3q (C-19), 15.3q (C-28), 13.7q (C-29) and 9.1q (C-18); HRESI-MS *m/z* 555.3663 $[M + Na]^+$ (Calcd for $C_{32}H_{52}O_6Na$ 555.3662).

3.3.3. *5β,6β-Epoxygorgostan-1α,3β,11α,12β-tetrol 11-acetate (**3**)*

White amorphous powder; $[\alpha]_D^{25}$ +6.5 ($c = 0.18$, MeOH); IR (neat) 3411, 2955, 1719 and 1253 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.07 (dd, $J = 8.8$, 9.0 Hz, H-11), 4.16 (m, H-3), 3.86 (brs, H-1), 3.30 (d, $J = 8.8$ Hz, H-12), 3.12 (d, $J = 3.4$ Hz, H-6), 1.08 (d, $J = 6.6$ Hz, H-21), 1.06 (s, H-19), 0.94 (d, $J = 6.6$ Hz, H-28), 0.92 (d, $J = 6.6$ Hz, H-27), 0.90 (s, H-29), 0.86 (d, $J = 6.6$ Hz, H-26), 0.73 (s, H-18), 0.47 (dd, $J = 4.2$, 4.9 Hz, H-30a), 0.39 (td, $J = 5.4$, 5.6 Hz, H-22), 0.27 (m, H-24) and −0.12 (dd, $J = 4.9$, 5.6 Hz, H-30b); ^{13}C-NMR ($CDCl_3$) δ 172.3s (Ac), 82.9d (C-12), 77.4d (C-11), 74.0d (C-1), 63.7d (C-3), 63.6d (C-6), 62.2s (C-5), 58.0d (C-17), 52.7d (C-14), 50.5d (C-24), 48.0s (C-13), 44.5d (C-9), 42.2t (C-4), 40.7s (C-10), 37.9t (C-2), 33.3d (C-20), 32.1d (C-25), 31.8t (C-7), 30.2d (C-22), 27.7t (C-16), 27.7d (C-8), 25.2s (C-23), 23.6t (C-15), 22.4q (C-21), 22.2q (C-27), 21.9q (Ac), 21.6t (C-30), 21.4q (C-26), 16.0q (C-19), 15.3q (C-28), 13.7q (C-29) and 9.0q (C-18); HRESI-MS *m/z* 555.3666 $[M + Na]^+$ (Calcd for $C_{32}H_{52}O_6Na$ 555.3662).

3.3.4. *5β,6β-Epoxygorgostan-1α,3β,11α,12β-tetrol (**4**)*

White amorphous powder; $[\alpha]_D^{25}$ +3.8 ($c = 0.26$, MeOH); IR (neat) 3385, 2955 and 1264 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.36 (brs, H-1), 4.14 (m, H-3), 3.59 (dd, $J = 8.8$, 10.0 Hz, H-11), 3.19 (d, $J = 8.8$ Hz, H-12), 3.10 (d, $J = 3.4$ Hz, H-6), 1.13 (d, $J = 6.6$ Hz, H-21), 1.11 (s, H-19), 0.94 (d, $J = 6.6$ Hz, H-28), 0.93 (d, $J = 6.6$ Hz, H-27), 0.90 (s, H-29), 0.86 (d, $J = 6.6$ Hz, H-26), 0.67 (s, H-18), 0.47 (dd, $J = 4.4$, 4.6 Hz, H-30a), 0.31 (m, H-22) 0.26 (m, H-24) and −0.09 (dd, $J = 4.6$, 5.1 Hz, H-30b); ^{13}C-NMR ($CDCl_3 : CD_3OD$, 5 : 1) δ 83.6d (C-12), 73.8d (C-1), 73.8d (C-11), 64.5d (C-6), 63.4d (C-3), 63.3s (C-5), 58.3d (C-17), 53.4d (C-14), 50.7d (C-24), 47.3s (C-13), 45.6d (C-9), 42.1t (C-4), 40.8s (C-10), 37.6t (C-2), 33.5d (C-20), 32.2t (C-7), 32.0d (C-25), 30.6d (C-22), 28.1t (C-16), 28.0d (C-8), 25.4s (C-23), 23.8t (C-15), 22.5q (C-21), 22.3q (C-27), 21.7t (C-30), 21.5q (C-26), 15.8q (C-19), 15.3q (C-28), 13.8q (C-29) and 9.1q (C-18); HRESI-MS *m/z* 513.3592 $[M + Na]^+$ (Calcd for $C_{30}H_{50}O_5Na$ 513.3556).

3.4. *Chemical conversion*

3.4.1. *Saponification of steroids **5** and **1** to give **9***

A methanolic (400 μL) solution of compound **5** (1.2 mg) was stirred with 50 μL of 28% NaOMe/MeOH at room temperature for 1 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine and dried over Na_2SO_4. After concentration, the crude product was separated on HPLC (RP-18, MeOH : H_2O, 10 : 1) to yield **9** (0.8 mg, 72%). Similar treatment of **1** (1.1 mg) also yielded **9** (0.8 mg, 79%), showing identical behaviour on TLC and ^{1}H-NMR. Compound **9**: ^{1}H-NMR ($CDCl_3$) δ 5.67 (1H, dd, $J = 1.7$, 5.6 Hz), 3.86 (1H, brs), 3.77 (1H, dd, $J = 9.3$, 8.5 Hz), 3.59 (1H, m), 3.26 (1H, d, $J = 8.5$ Hz), 0.94 (3H, d, $J = 6.6$ Hz), 0.93 (3H, d, $J = 6.6$ Hz), 0.91 (3H, s), 0.86 (3H, d, $J = 6.6$ Hz), 0.73 (3H, s), 0.51 (1H, dd, $J = 4.4$, 4.1 Hz), 0.38 (1H, m), 0.27 (1H, m) and −0.08 (1H, dd, $J = 4.4$, 5.1 Hz); ^{13}C-NMR ($CDCl_3$) δ 146.6s, 123.6d, 83.5d, 73.9d, 71.4d, 64.9d, 57.7d, 50.6d, 47.6d, 47.0d, 45.0s, 42.3t, 38.8s, 38.5t, 36.8d, 33.7d, 32.1d, 31.5t, 30.7d, 28.3t, 25.5s, 23.5t, 22.3q, 22.2q, 21.7t, 21.5q, 17.8q, 15.4q, 13.7q and 9.2q; ESI-MS m/z 497 ($[M + Na]^+$).

3.4.2. *Saponification of steroids **6** and **3** to give **4***

A methanolic (400 μL) solution of **6** (1.2 mg) was stirred with 50 μL of 28% NaOMe in MeOH at room temperature for 1 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give a product (0.9 mg, 72%) that was identical to natural **4** (TLC and ^{1}H-NMR). Similar treatment of **3** (1.1 mg) gave a product (0.8 mg, 79%) that was also identical to compound **4** (TLC and ^{1}H-NMR).

3.5. *Cytotoxicity assay*

The cell NBT-T2 (BRC-1370) was purchased from Riken and cultured under a standard protocol using Dulbecco's modified Eagle's medium (DMEM). Cultured cells were inoculated into each well (96-well plate) with 200 μL of the medium. After preincubation (24 h), aliquots of test compounds in MeOH were added to the culture wells. After incubation for 2 days, the toxic effect of each sterol was observed under a microscope. The IC_{50} values were measured by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric method. MTT solution (15 μL, 5 mg mL^{-1} in phosphate buffered saline) was added to each well and incubated for 3 h. The medium was removed by aspiration and the residual formazan was dissolved in 100 μL of DMSO. The absorbance was measured at 560 nm with a Tecan Sunrise microplate reader.

Acknowledgements

This work was supported in part by the twenty-first century COE programme from the University of Ryukyus and grant-in-aid (no. 18032061) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References

Aoki, S., Watanabe, Y., Sanagawa, M., Setiawan, A., Kotoku, N., & Kobayashi, M. (2006). Cortistatins A, B, C, and D, anti-angiogenic steroidal alkaloids, from the marine sponge *Corticium simplex*. *Journal of the American Chemical Society, 128*, 3148–3149.

Aoki, S., Watanabe, Y., Tanabe, D., Arai, M., Suna, H., Miyamoto, K., et al. (2007). Structure–activity relationship and biological property of cortistatins, anti-angiogenic spongean steroidal alkaloids. *Bioorganic and Medicinal Chemistry, 15*, 6758–6762.

Aoki, S., Yoshioka, Y., Miyamoto, Y., Higuchi, K., Setiawan, A., Murakami, N., et al. (1998). Agosterol A, a novel polyhydroxylated sterol acetate reversing multidrug resistance from a marine sponge of *Spongia* sp. *Tetrahedron Letters, 39*, 6303–6306.

Boonlarppradab, C., & Faulkner, D. (2007). Eurysterols A and B, cytotoxic and antifungal steroidal sulfates from a marine sponge of the genus *Euryspongia*. *Journal of Natural Products, 70*, 846–848.

Bordeleau, M.-E., Mori, A., Oberer, M., Lindqvist, L., Chard, L.S., Higa, T., et al. (2006). Functional characterization of IRESes by an inhibitor of the RNA helicase eIF4A. *Nature Chemical Biology, 2*, 213–220.

D'Auria, M.V., Minale, L., & Riccio, R. (1993). Polyoxygenated steroids of marine origin. *Chemical Reviews, 93*, 1839–1895.

Higa, T., Tanaka, J., & Tachibana, K. (1981a). 18-Oxygenated polyfunctional steroids from the gorgonian *Isis hippuris*. *Tetrahedron Letters, 22*, 2777–2780.

Higa, T., Tanaka, J., Tsukitani, Y., & Kikuchi, H. (1981b). Hippuristanols, cytotoxic polyoxygenated steroids from the gorgonian *Isis hippuris*. *Chemistry Letters, 10*, 1647–1650.

Kazlauskas, R., Murphy, P.T., Quinn, R.J., Wells, R.J., & Schönholzer, P. (1977). Hippurin-1, an unusual steroid from the gorgonian *Isis hippuris*. *Tetrahedron Letters, 18*, 4439–4441.

Langjae, R., Bussarawit, S., Yuenyongsawad, S., Ingkaninan, K., & Plubrukarn, A. (2007). Acetylcholinesterase-inhibiting steroidal alkaloid from the sponge *Corticium* sp. *Steroids, 72*, 682–685.

Lindqvist, L., Oberer, M., Reibarkh, M., Cencic, R., Bordeleau, M.-E., Vogt, E., et al. (2008). Selective pharmacological targeting of a DEAD box RNA helicase. *PLoS ONE, 3*, e1583.

Rao, C.B., Ramana, K.V., Rao, D.V., Fahy, E., & Faulkner, D.J. (1988). Metabolites of the gorgonian *Isis hippuris* from India. *Journal of Natural Products, 51*, 954–958.

Sheu, J.H., Chen, S.P., Sung, P.J., Chiang, M.Y., & Dai, C.F. (2000). Hippuristerone A, a novel polyoxygenated steroid from the gorgonian *Isis hippuris*. *Tetrahedron Letters, 41*, 7885–7888.

Tanaka, J., Higa, T., Tachibana, K., & Iwashita, T. (1982). Gorgost-5-ene-3β,7α,11α,12β-tetrol-12-monoacetate, a new marine sterol from the gorgonian *Isis hippuris*. *Chemistry Letters, 11*, 1295–1296.

Tanaka, J., Trianto, A., Musman, M., Issa, H.H., Ohtani, I.I., Ichiba, T., et al. (2002). New polyoxygenated sterols exhibiting reversal of multidrug resistance from the gorgonian *Isis hippuris*. *Tetrahedron, 58*, 6259–6266.

Tsukamoto, S., Tatsuno, M., van Soest, R.W.M., Yokosawa, H., & Ohta, T. (2003). New polyhydroxy sterols: proteasome inhibitors from a marine sponge *Acanthodendrilla* sp. *Journal of Natural Products, 66*, 1181–1185.

Watanabe, Y., Aoki, S., Tanabe, D., Setiawan, A., & Kobayashi, M. (2007). Cortistatins E, F, G, and H, four novel steroidal alkaloids from marine sponge *Corticium simplex*. *Tetrahedron, 63*, 4074–4079.

Wei, X., Rodriguez, A.D., Wang, Y., & Franzblau, S.G. (2007). Novel ring B abeo-sterols as growth inhibitors of *Mycobacterium tuberculosis* isolated from a Caribbean Sea sponge, *Svenzea zeai*. *Tetrahedron Letters, 48*, 8851–8854.