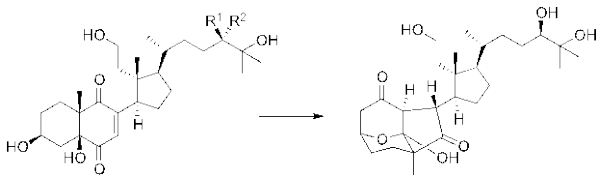
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Aplysiasecosterols B and C: two new 9,11-secosteroids with a *cis*-fused 1,4-quinone structure from the sea hare *Aplysia kurodai*

Leave this area blank for abstract info.

Masaki Kita,* Atsushi Kawamura, Hideo Kigoshi*



Aplysiasecosterol B R^1 = OH, R^2 = H
Aplysiasecosterol C R^1 = H, R^2 = OH

Aplysiasecosterol A

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Aplysiasecosterols B and C: two new 9,11-secosteroids with a *cis*-fused 1,4-quinone structure from the sea hare *Aplysia kurodai*

Masaki Kita *, Atsushi Kawamura, Hideo Kigoshi *

Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8571, Japan

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Marine natural products
Secosteroid
Isolation and structure
Biosynthesis

ABSTRACT

Two new 9,11-secosteroids with a *cis*-fused 3β,5β-dihydroxy-1,4-quinone structure, aplysiasecosterols B and C, were isolated from the sea hare *Aplysia kurodai*. Their structures were determined by 1D- and 2D-NMR spectroscopic analysis, molecular modeling studies, and a modified Mosher's method. Aplysiasecosterol B might be the biosynthetic precursor of aplysiasecosterol A, another 9,11-secosteroid with a tricyclic γ-diketone structure from *A. kurodai*, via two α-ketol rearrangements and intramolecular acetalization.

Oxygenated steroids and secosteroids are found in both terrestrial and marine organisms. They are known to be modulators of cholesterol metabolism and various other processes in living systems.[1] A structurally and functionally diverse group of natural secosteroids have been discovered, including 5,6-, 8,9-, 8,14-, 9,10-, 9,11-, and 13,17-secosteroids.[2] Among them, 9,11-secosteroids show a variety of activities, such as anti-proliferative activity, stimulation of bone formation, inhibition of protein kinase C, anti-inflammatory activity, and antifungal activity.[3] These molecules are expected to provide new insights for the discovery and development of a new class of pharmacological tools and therapeutic agents. In our continuing search for new secondary metabolites of marine origin,[4,5] aplysiasecosterol A (**1**), a 9,11-secosteroid with an unprecedented tricyclic γ-diketone structure, was isolated from the sea hare *Aplysia kurodai* (Fig. 1).[6] We proposed a biosynthetic pathway for the tricyclic γ-diketone structure of **1** starting from a cholest-7-en-3,5,6-triol, which includes two α-ketol rearrangements and an intramolecular acetalization. Further studies in search of new molecules from *A. kurodai* led to the isolation of two 9,11-secosteroids with a *cis*-1,4-quinone structure, aplysiasecosterols B (**2**) and C (**3**), and their planar structures were identical to those of the proposed biosynthetic precursors for **1**. We report here the isolation, structure determination, and bioactivity of **2** and **3**.

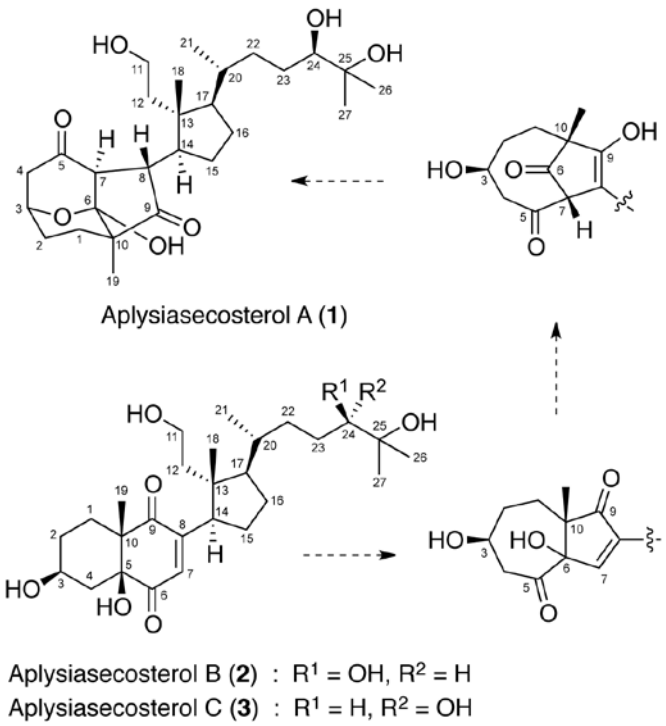


Fig. 1. Structures of aplysiasecosterols A–C and their proposed biosynthetic intermediates.

As described previously,[6] the sea hare *A. kurodai* was immersed in aqueous ethanol, and the concentrated extract was

partitioned between ethyl acetate and water. The ethyl acetate layer was further partitioned with *n*-hexane, dichloromethane, and 60% aqueous methanol. Purification of the dichloromethane layer by repeated SiO_2, Al_2O_3, and ODS column chromatography and reverse-phase HPLC afforded two minor constituents, aplysiasecosterols B (**2**) {5.1×10^{-8} %, $[\alpha]^{27}_D$ +20 (*c* 0.073, MeOH)} and C (**3**) {2.9×10^{-8} %, $[\alpha]^{27}_D$ +12 (*c* 0.073, MeOH)} as colorless oils, along with aplysiasecosterol A (**1**) (1.2×10^{-7} %). Aplysiasecosterols B and C did not show significant cytotoxicity against the human cervical carcinoma cell line HeLa S3 at 200 μM or the human myelomonocytic leukemia cell line HL-60 at 50 μM. Since aplysiasecosterol A shows a moderate growth-inhibitory effect toward HL-60 cells (IC_{50} = 16 μM), the tricyclic γ-diketone structure of **1** was suggested to be important for its cytotoxicity.

The molecular formulae of **2** and **3** were established to be both $C_{27}H_{44}O_7$ by HR-ESIMS ($[M+Na]^+$, *m/z* 503.2965, Δ –1.4 mmu for **2** and *m/z* 503.2964, Δ –1.5 mmu for **3**, respectively), which was identical to that of **1**. Based on a consideration of 1D- and 2D-NMR data, the planar and relative stereostructures of aplysiasecosterols B and C were found to be identical, except for the C-24 oxymethine configuration (Tables S1 and S2). The ^1H, ^{13}C NMR, DEPT135 and HSQC spectra in $CDCl_3$ showed that **2** had four singlet methyl groups (δ_H 0.70, 1.13, 1.17, 1.22), one doublet methyl group (δ_H 0.99), and two carbonyl carbons (δ_C 200.8, 201.9). The IR ($CHCl_3$) spectrum of **2** showed absorption bands for hydroxy groups (3567 cm^{-1}) and unsaturated carbonyl groups (1681 cm^{-1}).

A detailed analysis of the COSY spectrum of aplysiasecosterol B (**2**) allowed us to construct three partial structures: C-1–C-4, C-7–C-24, and C-11–C-12 (Fig. 2). HMBC correlations between Me-18/C-12, C-13, C-14 and C-17 revealed that the C-7–C-24 unit was linked to the C-11–C-12 unit via the sp^3 quaternary carbon C-13. Similarly, HMBC correlations between Me-19/C-1, C-5, C-9, and C-10 indicated that C-1, C-5, C-9, and C-19 were each connected to the sp^3 quaternary carbon C-10. The C-8–C-9 connectivity was established based on HMBC correlations between H-7 and H-14/C-9. Further HMBC correlations between H-4 and H-7/C-5, H-7 and 5-OH/C-6, and H-4/C-10 established an α-hydroxy unsaturated ketone structure at C-6. Thus, the results showed that **2** has a bicyclic dihydroxy-1,4-quinone structure that corresponds to a typical steroid AB ring. Furthermore, the connectivity of two singlet methyl groups (C-26, 27) and a 1,2-diol moiety was clarified based on HMBC correlations between Me-26/C-24, C-25, and C-27, and Me-27/C-24, C-25, and C-26. Based on the molecular formula and the degree of unsaturation, **2** was shown to contain five hydroxy groups. For aplysiasecosterol C (**3**), COSY, HMQC and HMBC correlations were almost the same as those described above for **2**. Therefore, the planar structures of **2** and **3** were established.

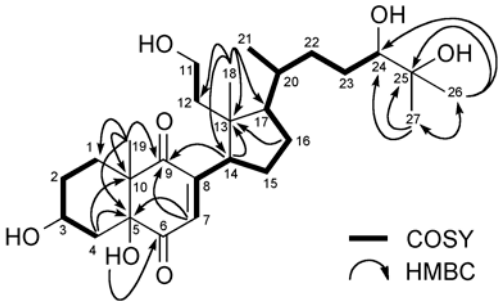


Fig. 2. Planar structure of aplysiasecosterols B (**2**) and C (**3**) determined by 2D-NMR analysis (arrows, selected HMBC correlations).

The relative stereochemistry around the cyclopentane ring in **2** and **3** was determined from the ROESY spectrum (Fig. 3). For compound **2**, ROEs were observed for H-11a/H-14, H-11b/H-14, H-12a/H-14, H-14/H-17, H-17/Me-21, H17/H22b, H-12a/Me-21, and Me-18/H-20. These data strongly indicated that H-12, H-14, H-17, and Me-21 were oriented in one face, and Me-18 and H-20 were oriented in the other face of the cyclopentane ring. As a result, the relative stereochemistry of the cyclopentane ring part in **2** and **3** was identical to those of typical 9,11-secosteroids and aplysiasecosterol A (**1**) (Fig. S1).[6–9]

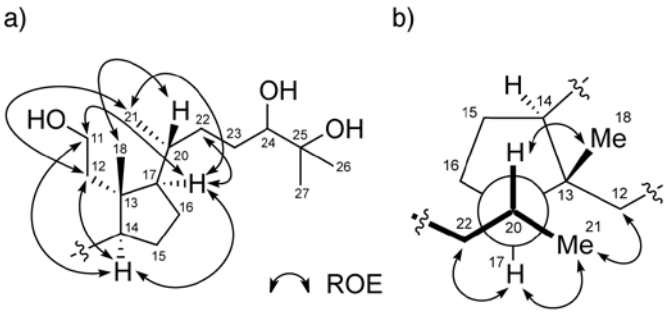
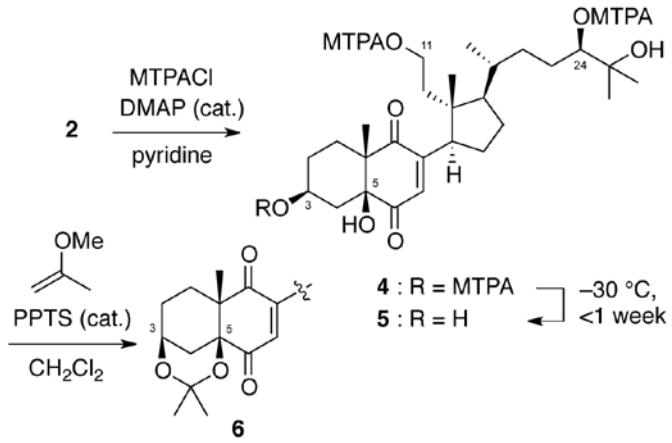


Fig. 3. (a) Relative stereochemistry around the cyclopentane rings of aplysiasecosterols B (**2**) and C (**3**) determined from the ROESY spectrum. (b) A Newman projection with a view along the C20–C17 bond.

To establish the relative stereochemistry of the cyclohexane ring part for aplysiasecosterols B (**2**) and C (**3**), MTPA esters were then prepared (Scheme 1). While 3,11,24-tris-(*S*) and (*R*)-MTPA esters **4** were tentatively obtained from **2** in analytically pure form, the C-3 ester moiety was selectively hydrolyzed at –30 °C for less than one week to give 11,24-bis-(*S*) and (*R*)-MTPA esters **5**. This result strongly suggested the presence of neighboring group participation of the C-5 tertiary alcohol moiety via strong hydrogen bonding. The multiplicity of H-3 (dtt, *J* = 9.1, 2.6, 2.6 Hz) and 3-OH (d, *J* = 9.1 Hz) in both compounds **2** and **3** suggested that H-3 was oriented in an equatorial position with respect to the cyclohexane ring with a chair conformation. Furthermore, treatment of triol **5** with 2-methoxypropene afforded acetonide **6**. These results established that both 3-OH and 5-OH could be oriented in axial positions concerning the A ring with a *syn*-arrangement.



Scheme 1. Preparation of the MTPA esters of aplysiasecosterol B (**2**) and their degradation.

The absolute stereochemistry at C24 was determined by a modified Mosher's method.[10] For compound **2**, positive Δδ values of bis-MTPA esters **5** were found for protons on the C18–C23 side, while negative Δδ values were found for protons on the C25–C27 side, which established a 24*R* configuration for aplysiasecosterol B (**2**) (Fig. 4). Similarly, the opposite Δδ patterns led to the assignment of a 24*S* configuration for aplysiasecosterol C (**3**).

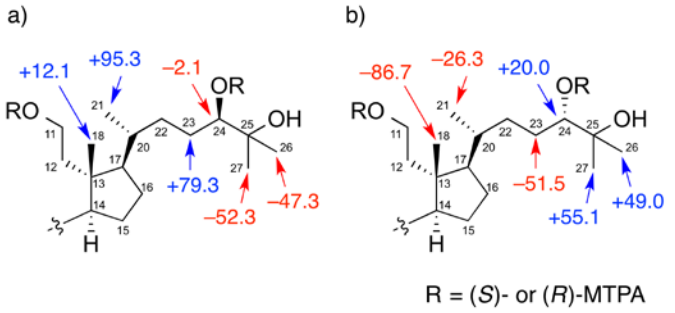


Fig. 4. Δδ values ($\delta_S - \delta_R$) for the 11,24-bis-MTPA esters **5** prepared from (a) aplysiasecosterol B (**2**) and (b) aplysiasecosterol C (**3**) in Hz (600 MHz, CD_3OD).

To determine whether **2** and **3** have *cis*- or *trans*-fused 1,4-quinone structures, we next performed molecular modeling studies using a Merck molecular force field 94x (MMFF94x) in an implicit solvation model ($CHCl_3$) for model compounds **7** (*cis*-fused) and **8** (*trans*-fused), in which the substituent on C-8 was replaced with a methyl group (Fig. 5). Compound **7** had two conformers (i) and (ii), while compound **8** had only one conformer (iii) within 7 kcal/mol of the lowest energy conformation. Geometry optimization with the density functional theory (DFT) method for these three conformers was conducted using the B3LYP/6-31G+(d,p) level of theory in implicit solvation model (CPCM, $CHCl_3$). As a result, the *cis*-fused 3,5-diaxial conformer (i) was lower in energy by 4.7 kcal/mol than the 3,5-diequatorial conformer (ii). This high stability of conformer (i) might be due to the hydrogen bonding among the 3-OH, 5-OH, and C-6 carbonyl groups.

The calculated distances of H-1a/Me-19, H-1b/Me-19, H-2a/H-3, H-3/H-4b, and 5-OH/Me-19 in conformer (i) were 2.4–3.1 Å, and this model satisfied all of the key ROEs observed for **2**. The calculated dihedral angles for the vicinal protons H-3/H-2a, H-2b, H-4a, and H-4b were 49.9–66.1°, which is reasonable for the relatively small magnitudes of their *J* values (~2.6 Hz) as gauche arrangements in both **2** and **3**. Meanwhile, in conformer (iii), H-1b was positioned antiperiplanar to Me-19, which did not match the observed ROE correlations. The protons H-2b, H-4b, and Me-19 in conformer (iii) were in a 1,3-diaxial arrangement with respect to each other, and their calculated distances were less than 2.8 Å, but these ROE correlations were not observed in the natural compounds. Due to the close chemical shifts, we could not observe the long-range (W-shape) coupling of H-2a/H-4a or the ROE correlation between H-2b/H-4b. Still, the conformer (i) of model **7** was thought to be most appropriate for natural **2** and **3**. Finally, based on the high structural similarities, the absolute configuration of C-19 methyl and the cyclopentane ring part were estimated to be identical to those of typical steroids. For the above reason, the absolute stereochemistry of aplysiasecosterols B (**2**) and C (**3**) was elucidated as shown in Figure 1.

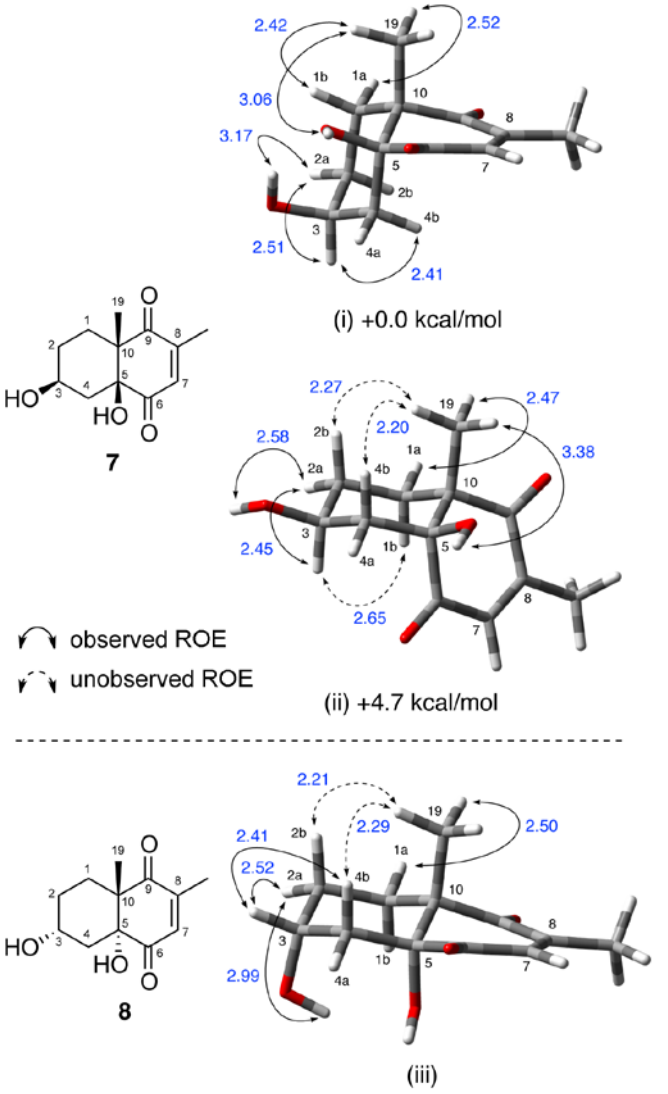


Fig. 5. Structures of the *cis*- and *trans*-fused 1,4-quinones **7** and **8** and their optimized structures at the B3LYP/6-31G+(d,p) level of theory in implicit solvation model (CPCM, $CHCl_3$). The relative energy of conformers (i) and (ii) is shown below. Observed and unobserved ROEs in aplysiasecosterol B (**2**) are shown as solid and dashed arrows, respectively. Values in blue represent the calculated distances between two selected protons in Å.

Recently, two 9,11-secosteroids that are structurally-related to aplysiasecosterols B and C were isolated from the Korean marine sponge *Ircinia* sp., which have a *trans*-fused 3β,5α-dihydroxy-1,4-quinone moiety.[7,3] As noted by Yang *et al.*, ^{13}C NMR data for the *trans*-fused compound (δ_{C3} 66.6 and δ_{C5} 80.5) were close to those of a 3β,5α-dihydroxy-6-one steroid derivative (δ_{C3} 66.8 and δ_{C5} 80.3).[11] Similarly, ^{13}C NMR data for aplysiasecosterols B (**2**) and C (**3**) (δ_{C3} 65.1 and δ_{C5} 81.0) coincided with those for a 3β,5β-dihydroxy-6-one steroid derivative (δ_{C3} 65.5 and δ_{C5} 81.9), rather than a 5α isomer. It is noted that both 3β,5α- and 3β,5β-dihydroxy-1,4-quinones could be biosynthetic precursors for the tricyclic γ-diketone structure of aplysiasecosterol A (**1**).

In summary, the structures and bioactivities of aplysiasecosterols B (**2**) and C (**3**), two new 9,11-secosteroids with a new *cis*-fused 1,4-quinone structure, were established. Since both aplysiasecosterols A and B have 24*R* configuration, aplysiasecosterol B (**2**) might be the biosynthetic precursor of aplysiasecosterol A (**1**). Meanwhile, the 24*S* isomer of **1** and its proposed biosynthetic precursors derived from aplysiasecosterol C (**3**) were not detected in the sea hare extracts. Further

biological, ecological, and biosynthetic studies on aplysiasecosterols including their antibacterial activity and antioxidant effects are in progress.

Acknowledgments

This work is supported in part by JSPS grants (25702047 to M.K. and 26242073 to H.K.) and by a Grant-in-Aid for Scientific Research on Innovative Areas from MEXT (Japan), "Chemical Biology of Natural Products." Support was also provided by the Naito Foundation.

References and notes

1. (a) Schroepfer, G. J. Jr. *Physiol. Rev*. **2000**, *80*, 361; (b) Kandutsch, A. A.; Chen, H. W.; Heiniger, H. J. *Science* **1978**, *201*, 498.
2. (a) Sica, D.; Musumeci, D. *Steroids* **2004**, *69*, 743; (b) Penov Gasi, K.; Sakac, M.; Jovanovic-Santa, S.; Djurendic, E. *Curr. Org. Chem.* **2014**, *18*, 216.
3. Yang, I. H.; Choi, H.; Nam, S. –J.; Kang, H. *Arch. Pharm. Res.* **2015**, *38*, 1970, and references are therein.
4. (a) Yamada, K.; Ojika, M.; Ishigaki, T.; Yoshida, Y.; Ekimoto, H.; Arakawa, M. *J. Am. Chem. Soc.* **1993**, *115*, 11020; (b) Ojika, M.; Kigoshi, H.; Ishigaki, T.; Tsukada, I.; Tsuboi, T.; Ogawa, T.; Yamada, K. *J. Am. Chem. Soc.* **1994**, *116*, 7441; (c) Yamada, K.; Ojika, M.; Kigoshi, H.; Suenaga, K. *Nat. Prod. Rep.* **2009**, *26*, 27; (d) Yamada, K.; Ojika, M.; Kigoshi, H.; Suenaga, K. *Proc. Jpn. Acad., Ser. B* **2010**, *86*, 176.
5. (a) Kita, M.; Kigoshi, H. in *Handbook of Anticancer Drugs from Marine Origin* (Ed. Kim, S. K.), Springer International Publishing, Switzerland, **2015**, pp. 701–740; (b) Kita, M.; Hirayama, Y.; Yoneda, K.; Yamagishi, K.; Chinen, T.; Usui, T.; Sumiya, E.; Uesugi, M.; Kigoshi, H. *J. Am. Chem. Soc*. **2013**, *135*, 18089; (c) Kita, M.; Kigoshi, H. *Nat. Prod. Rep*. **2015**, *32*, 534.
6. Kawamura, A.; Kita, M.; Kigoshi, H. *Angew. Chem. Int. Ed.* **2015**, *54*, 7073.
7. Yang, I. H.; Choi, H.; Won, D. H.; Nam, S. –J.; Kang, H. *Bull. Korean Chem. Soc.* **2014**, *35*, 3360.
8. Anta, C.; Gonzalez, N.; Rodriguez, J.; Jimenez, C. *J. Nat. Prod.* **2002**, *65*, 1357.
9. Su, J. –H.; Tseng, Y. –J.; Huang, H. –H.; Ahmed, A. F.; Lu, C. –K.; Wu, Y. –C.; Sheu, J. –H. *J. Nat. Prod.* **2006**, *69*, 850.
10. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
11. Kovganko, N. V.; Chernov, Y. G. *Chem. Nat. Compounds* **2001**, *37*, 351.

Footnote

* Corresponding authors. tel.: +81 29 853 4526; fax: +81 29 853 4313 (M.K.); tel./fax: +81 29 853 4313 (H.K.); e-mail addresses: mkita@chem.tsukuba.ac.jp (M.K.), kigoshi@chem.tsukuba.ac.jp (H.K.).