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

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ABSTRACT

Two new 9,11-secosteroids with a cis-fused 3β,5β-dihydroxy-1,4-quinone structure, alysiasecosterols 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 alysiasecosterol 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 alysiasecosterol A (1), a 9,11-secosteroid with an unprecedented tricyclic γ-diketone structure, was isolated from the sea hare Aplysia kurodai (Fig. 1).5 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, alysiasecosterols 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 alysiasecosterols 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 SiO2, Al2O3, and ODS column chromatography and reverse-phase HPLC afforded two minor constituents, aplysiasecosterols B (2) \(\{1.1 \times 10^{-4} \text{ %, } [\alpha]_D^20+20 \text{ (c 0.073, MeOH)}\}\) and C (3) \(\{2.9 \times 10^{-5} \text{ %, } [\alpha]_D^20+12 \text{ (c 0.073, MeOH)}\}\) as colorless oils, along with aplysiasecosterol A (1) \(\{1.2 \times 10^{-7} \text{ %}\}\). 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 (IC50 = 16 µM), the tricyclic 3-γ-ketone structure of 1 was suggested to be important for its cytotoxicity.

The molecular formulae of 2 and 3 were established to be both \(C_{25}H_{44}O_7\) by HR-ESIMS (\([M+Na]^+\), \(m/z\) 503.2965, \(\Delta -1.4 \text{ mmu}\) for 2 and \(m/z\) 503.2964, \(\Delta -1.5 \text{ mmu}\) 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 \(^1H\), \(^13C\) NMR, DEPT135 and HSQC spectra in CDCl3 showed that 2 had four singlet methyl groups (\(\delta_0 0.70, 1.13, 1.17, 1.22\)), two doublet methyl group (\(\delta_0 0.99\)), and two carbonyl carbons (\(\delta_C 200.8, 201.9\)). The IR (CHCl3) 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 and 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 C-24 oxymethine configuration (Tables S1 and S2). The \(^1H\), \(^13C\) NMR, DEPT135 and HSQC spectra in CDCl3 showed that 2 had four singlet methyl groups (\(\delta_0 0.70, 1.13, 1.17, 1.22\)), one doublet methyl group (\(\delta_0 0.99\)), and two carbonyl carbons (\(\delta_C 200.8, 201.9\)). The IR (CHCl3) spectrum of 2 showed absorption bands for hydroxy groups (3567 cm–1) and unsaturated carbonyl groups (1681 cm–1).

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

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, \(J = 9.1, 2.6, 2.6 \text{ Hz}\)) and 3-OH (d, \(J = 9.1 \text{ Hz}\)) 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 acetondie 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. For compound 2, positive $\Delta\delta$ values of bis-MTPA esters 5 were found for protons on the C18–C23 side, while negative $\Delta\delta$ values were found for protons on the C25–C27 side, which established a 24R configuration for aplysiasecosterol B (2) (Fig. 4). Similarly, the opposite $\Delta\delta$ patterns led to the assignment of a 24S configuration for aplysiasecosterol C (3).

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 (CHCl3) 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, CHCl3). 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, and C-6 were 49.9–66.1°, which is reasonable. The calculated dihedral angles for the vicinal protons 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 aplysiasecosterol B (2) and aplysiasecosterol C (3) in Hz (600 MHz, CD3OD).

Figure 1. 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, CHCl3). 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. As noted by Yang et al., 13C NMR data for the trans-fused compound (δC 66.6 and δC 80.5) were close to those of a 3β,5α-dihydroxy-6-one steroid derivative (δC 66.8 and δC 80.3), Similarly, 13C NMR data for aplysiasecosterols B (2) and C (3) (δC 65.1 and δC 81.0) coincided with those for a 3β,5β-dihydroxy-6-one steroid derivative (δC 65.5 and δC 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 elucidated. Since both aplysiasecosterols A and B have 24R configuration, aplysiasecosterol B (2) might be the biosynthetic precursor of aplysiasecosterol A (1). Meanwhile, the 24S isomer of I 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.

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References and notes


Footnote

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