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Chemical Constituents from the Stony Coral *Alveopora japonica*

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Abstract – Chemical investigations of the stony coral *Alveopora japonica* Eguchi (Poritidae) resulted in the isolation of four known compounds (**1** - **4**). The structures of **1** - **4** were identified as a sterol, ergosta-5,24(28)-dien-3β-ol (**1**), a mixture of monoacyl glycrols (**2**), eicosanoic acid and tetracosanoic acid, and two nucleosides, thymine (**3**) and 2'-deoxythymidine (**4**), respectively, on the basis of spectroscopic and physicochemical analyses including 1D- and 2D- NMR techniques as well as by comparison of their data with the published values. Compounds **1** - **4** were isolated from this species for the first time. Moreover, these compounds (**1** - **4**) were found in the genus *Alveopora* and the family Poritidae for the first time.
Keywords – *Alveopora japonica*, Poritidae, ergosta-5,24(28)-dien-3β-ol, nucleoside, saturated fatty acids

Introduction

Recently global warming has been associated with increases in the sea surface temperature, resulting in the changes of marin ecosystem. (Phinney *et al*., 2006) From these reason, in the sea of Jeju island, the stony coral *Alveopora japonica* Eguchi (Poritidae) has been grown as a dominant coral species. This coral has been distributed in tropical and subtropical region only. (Hyeong *et al*., 2008)

A. japonica is hemispherical to encrusting, septa short with short, and fine spines which seldom connect. (Fig. 1) The genus *Alveopora* of the Poritidae family includes over 46 species and mainly distributed in Southern Japan over to China. (van der Meij, *et al*., 2010) Previous report on the genus *Porites* has revealed the total fatty acid composition. (Imbs Andrey *et al*., 2007) However, there are no reports on the chemical investigations from this species and the Poritidae. This paper deals with the isolation and structure elucidation of **1** - **4**.

Experimental

General – UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. 1D and 2D NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA) with tetramethylsilane (TMS) as internal standard. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F254 (0.25 mm, Merck). Silica gel (230 - 400 mesh, Merck, Germany) was used for column chromatography. Preparative HPLC was run on an Acme 9000 HPLC (Young Lin, South Korea) using the YMC-pack ODS-A column and the flow rate was 1 ml/min.

Fig. 1. The photograph of stony coral *A. japonica*.

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Animal material – The stony coral *A. japonica* was collected in the Jeju island in November 2009, and were identified by Professor Jun-Im Song (Department of Life Sciences, Ewha Womans University). A voucher specimen (No. EA307) was deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

Extraction and Isolation – The freeze-dried stony corals *A. japonica* (1 kg) were chopped into small pieces and extracted with MeOH three times by sonication method under room temperature. The MeOH solutions were concentrated in vacuo to yield a dried MeOH-soluble extract (20 g). This extract was suspended in distilled water and fractionated with *n*-hexane, EtOAc, and *n*-BuOH, successively. The *n*-hexane extract (7 g) was chromatographed over a silica gel (70 g) column, eluting with a gradient solvent system of *n*-hexane-EtOAc (100 : 1 to 1 : 1), to afford 20 fractions (H1-H20). Fraction H8 (2 g) was chromatographed on a silica gel (20 g) column eluting with $CHCl_3$-MeOH (100 : 1 to 10 : 1) to afford eight subfractions (H8.1 to H8.8). Subfraction H8.3 (0.3 g) was chromatographed on a silica gel (30 g) column eluting with $CHCl_3$-MeOH (100 : 1 to 10 : 1) to yield compound **1** (3 mg). Fraction H5 (1 g) was chromatographed on a silica gel (20 g) column eluting with $CHCl_3$-MeOH (100 : 1 to 5 : 1) to afford nine subfractions (H5.1 to H5.9). Subfraction H5.5 (0.2 g) was subjected to a silica gel (30 g) column eluting with $CHCl_3$-MeOH (100 : 1 to 10 : 1) to give **2** (5 mg) and **3** (2 mg). Fraction H20 (1 g) was chromatographed on a silica gel (30 g) column eluting with $CHCl_3$-MeOH (100 : 1 to 5 : 1) to afford ten subfractions (H20.1 to H20.10). Subfraction H20.4 (0.1 g) was subjected to semi-preparative HPLC (MeOH-H_2O, 35 : 65) to yield **4** (3 mg).

Ergosta-5,24(28)-dien-3β-ol (1) – White powder, $[\alpha]_D^{20}$ –32.0° (*c* 0.2, MeOH) [literature value: –36.0° (*c* 0.15, MeOH) (Lu *et al*., 2004)]; IR ν_{max} (KBr) 3350, 1650 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD): δ 0.68 (3H, s, CH_3-18), 0.95 (3H, d, *J* = 6.8 Hz, CH_3-21), 1.00 (3H, s, CH_3-19), 1.03 (3H, d, *J* = 7.0 Hz, CH_3-26), 1.03 (3H, d, *J* = 7.0 Hz, CH_3-27), 3.51 (1H, m, H-3), 4.65 (1H, d, *J* = 1.6 Hz, H-28a), 4.71 (1H, d, *J* = 1.6 Hz, H-28b), 5.35 (1H, dd, *J* = 2.0, 3.2 Hz, H-6); ^{13}C-NMR (100 MHz, CD_3OD): δ 37.4 (C-1), 32.1 (C-2), 71.9 (C-3), 56.9 (C-4), 140.9 (C-5), 121.8 (C-6), 32.1 (C-7), 31.9 (C-8), 50.3 (C-9), 36.7 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 18.9 (C-19), 35.9 (C-20), 19.6 (C-21), 34.9 (C-22), 34.0 (C-23), 157.0 (C-24), 31.1 (C-25), 22.0 (C-26), 22.2 (C-27), 106.1 (C-28); CIMS *m/z* 397 $[M-H]^-$.

Monoacyl glycerols (2) – White powder, IR ν_{max} (KBr) 3400 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, t, *J* = 6.8 Hz, CH_3-24), 1.31 (2H, m, H-23), 2.34 (2H, d, *J* = 7.6 Hz, H-2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 178.8 (C-1), 33.8 (C-2), 29.0-29.7 (C-3 to C-21), 31.9 (C-22); CIMS *m/z* eicosanoic acid: 313 $[M+H]^+$ and tetracosanoic acid: 369 $[M+H]^+$.

Thymine (3) – Colorless needle. IR ν_{max} (KBr) 1660 cm^{-1}; UV λ_{max} (log ε) (MeOH) 254 (3.54) nm; ^{1}H-NMR (400 MHz, CD_3OD): δ 1.84 (3H, d, *J* = 1.2 Hz, CH_3), 7.21 (1H, d, *J* = 1.2 Hz, H-6); ^{13}C-NMR (100 MHz, CD_3OD): δ 150.0 (C-2), 163.5 (C-4), 110.4 (C-5), 139.1 (C-6), 12.1 (CH_3); EIMS *m/z* 126 $[M]^+$.

2'-Deoxythymidine (4) – Colorless needle, $[\alpha]_D^{20}$ –48.0° (*c* 0.2, MeOH) [literature value: –53.7° (*c* 0.15, MeOH) (Kitajima *et al*., 1999)]; UV (MeOH) λ_{max} (log ε) 256 (3.98) nm; IR ν_{max} (KBr) 3250, 1670 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.81 (1H, d, *J* = 0.8 Hz, H-6), 6.27 (1H, t, *J* = 6.4 Hz, H-1'), 2.22 (2H, m, H-2'), 3.89 (1H, m, H-3'), 4.39 (1H, m, H-4'), 3.77 (2H, dddd, *J* = 3.2, 4.0, 12.0, 12.0 Hz, H-5'), 1.87 (3H, d, *J* = 0.8 Hz, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 152.4 (C-2), 166.4 (C-4), 111.5 (C-5), 138.2 (C-6), 86.2 (C-1'), 41.2 (C-2'), 72.2 (C-3'), 88.8 (C-4'), 62.8 (C-5'), 12.4 (CH_3); EIMS *m/z* 242 $[M]^+$.

Results and Discussion

Repeated chromatography of the EtOAc-soluble fraction of the MeOH extract from *A. japonica* on silica gel, YMC-pack RP-C_{18} columns led to the isolation of four compounds (**1 - 4**). The isolated compounds were identified as ergosta-5,24(28)-dien-3β-ol (**1**), (Lyu *et al*., 2007), monoacyl glycerols (**2**) (Yao *et al*., 2007), thymidine (**3**) (Thureau *et al*., 2006), and 2'-deoxythymidine (**4**) (Kitajima *et al*., 1999). Compounds **1 - 4** were isolated for the first time from this species. (Fig. 2) Moreover, these compounds (**1 - 4**) were found in the genus *Alveopora* and the family Poritidae for the first time.

Compound **1** was obtained as white powder and it showed a molecular ion peak at *m/z* 397 $[M-H]^-$ in the CIMS. The IR spectrum showed the presence of hydroxyl at 3350 cm^{-1} and terminal methylene group at 1650 cm^{-1}. The ^{1}H- and ^{13}C-NMR and DEPT spectra showed five methyl groups at δ_H 0.68 (3H, s, CH_3-18), 0.95 (3H, d, *J* = 6.8 Hz, CH_3-21), 1.00 (3H, s, CH_3-19), 1.03 (3H, d, *J* = 7.0 Hz, CH_3-26), and 1.03 (3H, d, *J* = 7.0 Hz, CH_3-27), an oxygenated methine proton at δ_H 3.51 (1H, m)/δ_C 71.9 (C-3), and two types of olefinic protons at δ_H 4.65 (1H, d, *J* = 1.6 Hz, H-28a) and 4.71 (1H, d, *J* = 1.6 Hz, H-

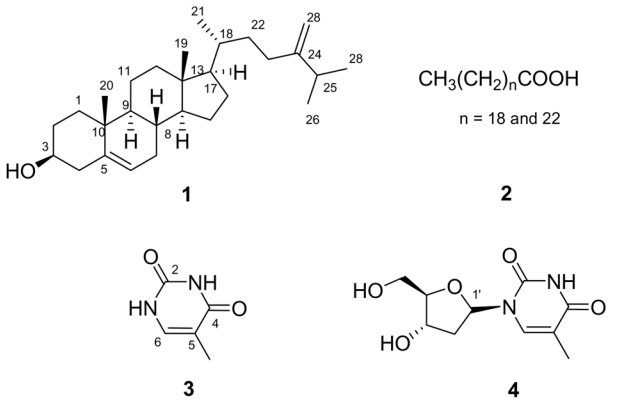

Fig. 2. Chemical structures of compounds **1** - **4** from *A. japonica*.

28b) and at δ_H 5.35 (1H, dd, J = 2.0, 3.2 Hz, H-6), which were characteristics for a sterol with terminal methylene group protons in the alkyl chain. The location of the terminal methylene group was established by the HMBC correlations observed between H-28/CH_3-26/CH_3-27 and C-24/C-25. Therefore, compound **1** was identified as ergosta-5,24(28)-dien-3β-ol, which had previously been isolated from the fruit body of *Phellinus linteus* (Lyu *et al*., 2007).

Compound **2** was obtained as white powder. The IR spectrum showed the presence of hydroxyl group at 3400 cm^{-1}. The ^{1}H- and ^{13}C-NMR and DEPT signals for a carboxylic OH proton at δ_H 10.38 (1H, br s), a terminal methyl group at δ_H 0.80 (3H, t, J = 6.8 Hz)/δ_C 26.5, an acid carbon at δ_C 178.8, and a long hydrocarbon chain at δ_H 1.25 [br s, $(CH_2)_n$]/δ_C 29.0-29.7 (C-2 to C-20) were indicative of the long chain fatty acid. The CIMS spectrum showed molecular ion peaks at *m/z* 313 and 369 $[M+H]^+$, respectively, suggested that **2** was mixture of long chain fatty acids. Thus, compound **2** was confirmed as the mixture of eicosanoic acid and tetracosanoic acid, which had previously been isolated from *Adenophora tetraphylla*. (Yao *et al*., 2007).

Compound **3** was obtained as colorless needle and it showed a molecular ion peak at *m/z* 126 $[M]^+$ in the EIMS. The IR spectrum showed the presence of olefinic group at 1665 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **3** showed the methyl group at δ_H 1.84 (3H, d, J = 1.2 Hz)/δ_C 12.1, an olefinic group at δ_H 7.21 (1H, d, J = 1.2 Hz, H-6)/δ_C 139.1 (C-6), a quaternary carbon at 110.4 (C-5), and two upfield shifted carbonyl carbon at δ_C 150.0 (C-2) and 163.5 (C-4), were indicative of the presence of a nucleoside. Comparison of the above data with those in the literature (Thureau *et al*., 2006) led to the identification of compound **3** as thymine.

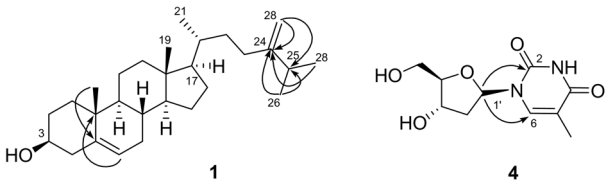

Fig. 3. Key HMBC correlations of **1** and **4**.

Compound **4** was obtained as colorless needle and it showed a molecular ion peak at *m/z* 242 $[M]^+$ in the EIMS. The IR spectrum showed the presence of hydroxyl group at 3250 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **4** showed similar patterns with those of **3** except for the deoxyribosyl moiety at [6.27 (1H, t, J = 6.4 Hz, H-1')/86.2 (C-1'), 2.22 (2H, m, H-2')/41.2 (C-2'), 3.89 (1H, m, H-3')/72.2 (C-3'), 4.39 (1H, m, H-4')/88.8 (C-4'), 3.77 (2H, dddd, J = 3.2, 4.0, 12.0, 12.0 Hz, H-5')/62.8 (C-5')]. The connectivity of thymine and deoxyribosyl moiety was supported by the HMBC spectrum (Fig. 3), which showed the correlation between δ_H 6.27 (H-1') and δ_C 152.4 (C-2)/138.2 (C-6), suggested that deoxyribosyl moiety was attached at N (position 1) atom of thymine molecule. On the basis of the above evidence, compound **4** was identified as 2'-deoxythymidine, which had previously been isolated from fennel. (Kitajima *et al*., 1999).

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