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(25*S*)-Cholesten-26-oic acid derivatives from an Indonesian soft coral *Minabea* sp.

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ABSTRACT

(25*S*)-3-Oxocholesta-1,4-dien-26-oic acid (**1**) and a new (25*S*)-18-acetoxy-3-oxocholesta-1,4-dien-26-oic acid (**2**) were isolated from a soft coral *Minabea* sp. (cf. *aldersladei*) collected in North Sulawesi, Indonesia, together with two known cholic-acid-type compounds, 3-oxochol-1,4-dien-24-oic acid (**3**) and 3-oxochol-4-en-24-oic acid (**4**). The structures of these compounds were determined on the basis of their spectroscopic data. The absolute stereochemistry at C-25 of **2** was determined by comparative ^{1}H NMR study using chiral anisotropic reagents [(*S*)- and (*R*)-phenylglycine methyl esters]. This is the first to report compound **1** as a natural product.

1. Introduction

Marine organisms produce steroidal components with variety of structures [1]. One of the interesting properties of structures is a high degree of oxidation, and steroidal carboxylic acids and derivatives have been detected in marine organisms more frequently than terrestrial organisms. Occurrence of steroidal carboxylic acid derivatives, which were sometimes oxygenated at various sites, has been shown in starfish, nudibranches, sponges, and, especially, soft corals. Soft corals are a rich source of biologically active natural products [1]. In the course of our studies on bioactive metabolites of marine organisms, we isolated four steroidal carboxylic acids (**1**–**4**, Fig. 1) from a soft coral *Minabea* sp. collected in Indonesia and found that **2** was a new compound and that **1** was obtained for the first time as a natural product. We describe herein the isolation and structure elucidation of compounds **1** and **2**.

2. Experimental

2.1. General procedures

NMR spectra were measured on a JEOL JNM-AL-400 or JNM-LA-600 NMR spectrometer in $CDCl_3$ (δ_H 7.24, δ_C 77.0). Mass spectra were obtained by a JEOL JMS-MS 700 mass spectrometer (EI or FAB mode). UV and IR spectra were recorded on HITACHI U-3310 and on Perkin-Elmer Spectrum One FT-IR spectrometers, respectively. Optical rotations were recorded with a JASCO DIP-370 digital polarimeter. Fetal bovine serum (FBS) was obtained from GIBCO after checking the lot, and all other reagents and chemicals for bioassays were of the highest grade available commercially.

2.2. Organism, extraction, and isolation

Minabea sp. (most likely *Minabea* cf. *aldersladei*) was collected by scuba diving at the Lembeh Strait, Indonesia. The voucher specimen is deposited at the Faculty of Fisheries and Marine Science, Sam Ratulangi University as 04-09-30 = 2-1a. The soft coral was immediately cut into small pieces and soaked in ethanol on the boat. The EtOH extract was evaporated and the residue was partitioned between *n*-hexane (200 mL) and MeOH–H_2O (9:1, 200 mL).

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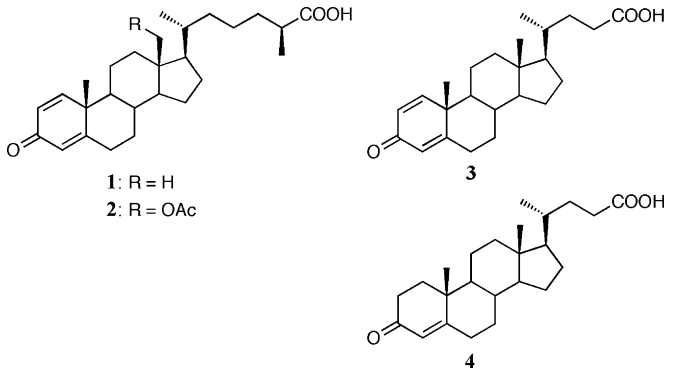

Fig. 1. Structures of compounds **1**–**4**.

The aqueous MeOH layer was concentrated, dissolved in water, and extracted successively with EtOAc and *n*-BuOH. The EtOAc extract showed activity against L1210 and V79 cells and was separated on a SiO_2 column with $CHCl_3$–MeOH (gradient elution) into six fractions. The third fraction (50 mg) was subjected to Sephadex LH-20 column chromatography with $CHCl_3$–MeOH (1:1) and then with MeOH followed by reversed-phase HPLC (ODS, 80% MeOH–H_2O containing 0.05% trifluoroacetic acid) to yield compounds **1** (1.5 mg), **2** (1.1 mg), **3** (11 mg), and **4** (1.2 mg). The same fraction also gave two known cytotoxic briareine-type diterpenes, minabein-4 (1.0 mg) and minabein-6 (1.4 mg) [2,3].

*2.2.1. (25S)-3-Oxocholesta-1,4-dien-26-oic acid (**1**)*

White powder. $[\alpha]_D^{25}$ + 5.3° (c 0.16, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε) 244 (4.07); IR (KBr) ν_{max} 2945, 1706, and 1662 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS (glycerol): *m*/*z* 413 $[M+H]^+$; HREIMS: *m*/*z* 412.2972 (M^+, calcd. for $C_{27}H_{40}O_3$, 412.2977).

Table 1
^1H (400 M) and ^{13}C (100 M) NMR data[a] for compounds **1** and **2** in $CDCl_3$.

	1		**2**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	156.4	7.05 d (10.0)	156.2	7.04 d (10.0)
2	127.3	6.22 dd (10.0, 2.0)	127.4	6.24 dd (10.0, 2.0)
3	186.7	–	186.7	–
4	123.7	6.07 brs	123.8	6.08 brs
5	170.0	–	169.6	–
6	33.0	2.35 m, 2.46 m	32.8	2.34 m, 2.45 m
7	33.7	1.10 m, 1.93 m	33.7	1.10 m, 1.93 m
8	35.5	1.60 m	35.7	1.59 m
9	52.4	1.02 m	52.3	1.04 m
10	43.8	–	43.7	–
11	22.9	1.60 m, 1.79 m	22.8	1.59 m, 1.70 m
12	39.5	1.14 m, 2.01 m	34.7	1.06 m, 2.40 m
13	42.7	–	45.4	–
14	55.4	0.98 m	55.0	1.15 m
15	24.4	1.17 m, 1.58 m	24.1	1.15 m, 1.64 m
16	28.1	1.23 m, 1.81 m	27.7	1.38 m, 1.86 m
17	56.0	1.10 m	56.2	1.09 m
18	12.0	0.71 s	62.6	4.22 d (11.7), 3.94 d (11.7)
19	18.7	1.21 s	18.8	1.21 s
20	35.6	1.38 m	35.7	1.42 m
21	18.5	0.88 d (6.6)	18.7	0.97 d (6.3)
22	35.7	0.99 m, 1.35 m	35.7	0.99 m, 1.35 m
23	23.7	1.15 m, 1.33 m	23.4	1.14 m, 1.34 m
24	34.0	1.37 m, 1.58 m	34.0	1.36 m, 1.58 m
25	39.1	2.44 m	39.0	2.45 m
26	180.8	–	180.5	–
27	17.0	1.16 d (6.8)	17.1	1.17 d (7.1)
OAc	–	–	171.4	–
	–	–	21.1	2.07 s

[a] Assigned by ^1H–^1H COSY, HMQC, and HMBC experiments.

*2.2.2. (25S)-18-Acetoxy-3-oxocholesta-1,4-dien-26-oic acid (**2**)*

White powder. $[\alpha]_D^{25}$ + 13.2° (c 0.11, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε) 243 (4.14); IR (KBr) ν_{max} 2945, 1737, 1600, and 1238 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 470 $[M]^+$, 410, 397, 289, 267, 173, 147, and 121. HREIMS: *m*/*z* 470.3042 (M^+, calcd. for $C_{29}H_{42}O_5$, 470.3033).

*2.3. Reaction of **2** with (S)- and (R)-phenylglycine methyl esters (PGMEs) [4,5]*

Compound **2** (0.6 mg) and (*S*)-PGME hydrochloride (5.2 mg) were dissolved in DMF (0.5 mL), and PyBOP (2.6 mg), HOBT (2.8 mg), and *N*-methylmorpholine (100 μL) were successively added to the solution at 0 °C. After stirred at room temperature for 4 h, EtOAc (10 mL) was added to the reaction mixture, and the solution was washed successively with 7% HCl (10 mL 2×), saturated $NaHCO_3$ (10 mL 2×), and saturated NaCl (10 mL 2×), dried (Na_2SO_4), and evaporated. The residue was purified by HPLC (ODS, gradient elution with 50–100% MeOH in H_2O) to give the (*S*)-PGME amide (0.2 mg).

The (*R*)-PGME amide (0.2 mg) was prepared from 0.6 mg of **2** and (*R*)-PGME hydrochloride in a similar procedure as above.

*2.3.1. (S)-PGME amide of **2***

^1H NMR data (600 MHz, $CDCl_3$): δ 7.01 (1H, d, *J* = 10.2 Hz, H-1), 6.21 (1H dd, *J* = 10.2, 1.8 Hz, H-2), 6.05 (1H, s, H-4), 4.22 (1H, d, *J* = 11.7 Hz, H-18a), 3.93 (1H, d, *J* = 11.7 Hz, H-18b), 2.07 (3H, s, -OAc), 1.20 (3H, s H_3-19), 1.10 (3H, d, *J* = 6.6 Hz, H_3-27), 0.96 (3H, d, *J* = 6.2 Hz, H_3-21), 7.25–2.35 (5H, m, Ph of PGME), 6.37 (1H, d, *J* = 7.3 Hz, NH of PGME), 5.56 (1H, d, *J* = 7.3 Hz, CH of PGME), 3.71 (3H, s, OMe of PGME).

*2.3.2. (R)-PGME amide of **2***

^1H NMR data (600 MHz, $CDCl_3$): δ 7.01 (1H, d, *J* = 10.3 Hz, H-1), 6.21 (1H dd, *J* = 10.3, 2.2 Hz, H-2), 6.05 (1H, s, H-4), 4.20 (1H, d, *J* = 11.7 Hz, H-18a), 3.90 (1H, d, *J* = 11.7 Hz, H-18b), 2.05 (3H, s, -OAc), 1.20 (3H, s H_3-19), 1.13 (3H, d, *J* = 7.0 Hz, H_3-27), 0.89 (3H, d, *J* = 6.6 Hz, H_3-21), 7.26–2.34 (5H, m, Ph of PGME), 6.40 (1H, d, *J* = 7.0 Hz, NH of PGME), 5.57 (1H, d, *J* = 7.0 Hz, CH of PGME), 3.71 (3H, s, OMe of PGME).

2.4. Antimicrobial assay

Compounds **1**–**4** were tested for antimicrobial activity against *Staphylococcus aureus* IAM 12544T, *Escherichia coli* IAM 12119T, *Saccharomyces cerevisiae* IAM 14383T, and *Mucor hiemalis* IAM 6088. Test compounds were dissolved in methanol or ethanol and 40 μL of each solution was absorbed on a disk (8 mm in diameter). After incubation, diameters of the inhibition zones were measured.

2.5. Cytotoxicity testing

Cytotoxicity of compounds **1**–**4** was tested against Chinese hamster V79 and murine leukemia L1210 cell lines. V79 cells were grown as a monolayer culture in Eagle's MEM (Nissui Seiyaku Co., Ltd., Tokyo, Japan) with 10% heat-inactivated FBS. The relative plating efficiencies against V79 cells were determined as the ratio of the number of colonies in various concentrations of samples to that in the sample-free control, as described in previous papers [6,7]. Two hundred cells were seeded on a 60/15-mm plastic plate with 4 mL culture medium and incubated overnight at 37 °C. After each sample in DMSO (4 μL) was added to the culture medium, cells were further cultured for four days. The numbers of colonies in the sample plates were counted and compared with those in the control cultures.

Growth inhibitory activity of compounds **1**–**4** against L1210 cells was tested in 96-well plastic plates by XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] (cell proliferation kit II®). Compounds were dissolved in MeOH and 10 μL of each sample solution was poured in a well and the solvent evaporated in a clean bench. The suspension of L1210 cells in RPMI 1640 medium (4×10^4 cells/mL, 100 μL) was added into each well and the number of vital cells in the sample wells after 72 h was compared with those in the control (MeOH) wells.

3. Results and discussion

As a part of our investigation on biologically active metabolites from marine micro- and macro-organisms, an Indonesian soft coral *Minabea* sp. was studied since the EtOAc extract showed cytotoxicity against L1210 and V79 cells. HPLC separation of the active fraction afforded two bioactive known compounds, minabein-4 and minabein-6 [2,3], and four steroidal carboxylic acids **1**–**4** (Fig. 1). Structures of two known compounds **3** and **4** were assigned on the basis of their spectroscopic data and comparison with the reported values for 3-oxochol-1,4-dien-24-oic acid (**3**) [8,9] and 3-oxochol-4-en-24-oic acid (**4**) [10,11].

Compound **1** showed the $[M+H]^+$ ion at *m*/*z* 413 in the FABMS, and the molecular formula $C_{27}H_{40}O_3$ was determined from HREIMS and NMR data. The ^{1}H NMR spectrum of **1** revealed four methyl signals at δ 0.71 (3H, s), 0.88 (3H, d, J = 6.6 Hz), 1.16 (3H, d, J = 6.8 Hz), and 1.21 (3H, s) ascribable to methyl groups of a steroidal compound. A 1,4-dien-3-one structure at the A ring was deduced from the analysis of NMR data for **1** (Table 1) and confirmed by comparison of IR, UV, and NMR data for **1** with those for **3**. Compound **1** had three more carbons than **3**, and the difference was observed at the side chain. The presence of an α-methylcarboxylic acid moiety in **1** was determined from ^{1}H–^{1}H COSY and HMBC spectra of **1**. The cholestane structure was assigned from NOE correlations, which were detected between H-4/H-6, H_3-19/H-1, H_3-19/H-8, H_3-19/H-11, H_3-18/H-8, H_3-18/H-11, H_3-18/H-20, and H_3-21/H-17 in the NOESY spectrum of **1**. The structure of **1** was, therefore, elucidated as 3-oxocholesta-1,4-dien-26-oic acid. The Me ester of **1** has been isolated from an Antarctic soft coral *Anthomastus bathyproctus* [12], and the reported ^{13}C NMR data for this compound were very similar to those for **1** ($\Delta\delta$: −0.1 to +0.6 ppm) except for the signal due to C-26, which was observed at δ_C 180.8 ($\Delta\delta$: +3.4 ppm) in the spectrum of **1**. Compound **1** was obtained as one of degradation products from cholesterol by *Pseudomonas* sp. NCIB 10590 under aerobic conditions, but NMR data and the stereochemistry at C-25 were not reported [8]. This is the first instance to show **1** as a natural product.

Compound **2** gave the $[M]^+$ ion at *m*/*z* 470 in the EIMS, and the molecular formula was deduced as $C_{29}H_{42}O_5$ from HREIMS and NMR data. The structure of **2** was elucidated by interpretation of IR, UV, and NMR data and comparison with those for **1** and **3**. ^{1}H and ^{13}C NMR data for **2** (Table 1) was similar to those for **1** except that the signals ascribed to an acetoxymethyl group were observed at δ_H 4.22 (1H, d, J = 11.7 Hz), 3.94 (1H, d, J = 11.7 Hz), and 2.07 (3H, s) and at δ_C 62.6, 21.1, and 171.4 in the spectra of **2** instead of the C-18 methyl group (δ_H 0.71, δ_C 12.0) in **1**. Position of the –OAc group was confirmed by the HMBC spectrum of **2**, which showed correlations from H_2-18 (δ 4.22 and 3.94) to C-12 (δ 34.7), C-14 (55.0), and C-17 (56.2). The signals for C-12 and C-13 were shifted as expected from β and γ effects. Thus, the structure of **2** was assigned as 18-acetoxy-3-oxocholesta-1,4-dien-26-oic acid, which was a new compound.

Stereochemistry at C-25 could not be determined from NMR data of **1** and **2**, because both (25*S*)- and (25*R*)-26-oic acids showed very similar chemical shifts [13]. Therefore, diastereomeric amide derivatives of **2** were prepared with (*S*)- and (*R*)-PGMEs [4,5,14]. The ^{1}H NMR shift differences ($\Delta\delta = \delta_{(S)} - \delta_{(R)}$) between (*S*)- and (*R*)-PGME amide derivatives of **2** were calculated following the Kusumi's method [4,5], and the $\Delta\delta$ value of −0.03 was detected for the H_3-27 signals. On the contrary, signals due to H-18a, H-18b, OAc, and H_3-21 showed $\Delta\delta$ values of +0.02, +0.03, +0.02, and +0.07, respectively. Consequently, the absolute configuration at the C-25 position of **2** was determined as (*S*), and on the usual biosynthetic precedents, **1** was assigned to have the (25*S*)-configuration.

Antimicrobial activity against Gram-positive (*S. aureus*) and negative bacteria (*E. coli*), yeast (*S. cerevisiae*), and a filamentous fungus (*M. hiemalis*) and cytotoxicity against V79 and L1210 cells were examined, and compounds **1**–**4** showed no apparent activity at 100 μg/disk (antimicrobial), 10 μM (V79 cells), and 50 μg/mL (L1210).

Marine organisms will attract our attention as a rich source of novel steroidal components with interesting biological activities [1].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2009.04.002.

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