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Dytesinins A and B, New Clerodane-type Diterpenes with a Cyclopropane Ring from the Tunicate *Cystodytes* sp.

Kazutaka Shimbo,[a] Masashi Tsuda,[a] Eri Fukushi,[b] Jun Kawabata[b] and Jun'ichi Kobayashi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*
[b]*Graduate School of Agriculture, Hokkaido University, Sapporo 060-0812, Japan*

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Abstract—Two new clerodane-type diterpenes, dytesinins A (**1**) and B (**2**), with a cyclopropane ring have been isolated from the Okinawan marine tunicate *Cystodytes* sp., and the structures were elucidated on the basis of spectroscopic data including newly developed 2D NMR experiments such as CH-selected editing HSQC-NOESY and *J*-resolved HMBC.

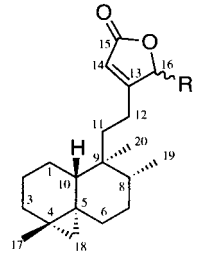
Introduction

During our continuing search for unique secondary metabolites from marine tunicates,[1–3] we have investigated extracts of the Okinawan tunicate *Cystodytes* sp., and isolated two new clerodane-type diterpenes, dytesinins A (**1**) and B (**2**), with a cyclopropane ring. CH-selected editing HSQC-NOESY, a new technique constructed by modification of editing HSQC (E-HSQC),[4,5] and *J*-resolved HMBC recently developed by Furihata and Seto[6] were applied for analysis of the relative stereochemistry. Here we describe the isolation and structure elucidation of **1** and **2**.

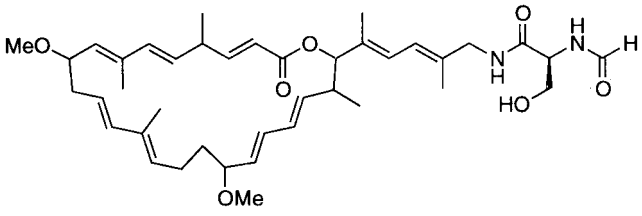
Results and Discussion

The tunicate *Cystodytes* sp. collected off Ie Island, Okinawa, was extracted with MeOH, and the EtOAc-soluble materials of the MeOH extract were subjected to silica gel column chromatography and C_{18} HPLC to afford dytesinins A (**1**, 0.00085%, wet weight) and B (**2**, 0.00019%) together with known compounds, iejimalides A (**3**), B, C and D[7,8] and rigidin (**4**) (Chart 1).[9]

Dytesinin A (**1**) was shown to have the molecular formula, $C_{20}H_{30}O_3$, by HREIMS (*m/z* 318.2222 M^+, Δ +2.7 mmu). IR absorptions at 3430 and 1740 cm^{-1} were suggestive of the presence of hydroxy and carbonyl groups, respectively. The ^{13}C NMR (Table 1) spectrum revealed carbon signals due to two sp^2 quaternary carbon, one sp^2 methine, one hemiacetal carbon, three sp^3 quaternary carbons, two sp^3 methines, eight sp^3 methylenes, and three methyls. The ^1H NMR spectrum showed signals due to a doublet methyl (δ_H 0.80, d, *J*=6.7 Hz), two singlet methyls (δ_H 1.15 and 0.77), and two protons [δ_H 0.47 (d, *J*=3.8 Hz) and 0.12 (d, *J*=3.8 Hz)] on a cyclopropane ring, although proton resonances at δ_H 1.4–1.6 were severely overlapped.



1 R = OH
2 R = H



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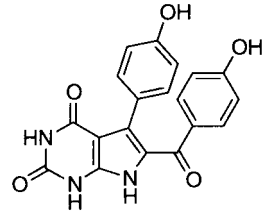

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Chart 1. Structures of dytesinins A (**1**) and B (**2**), iejimalide A (**3**), and rigidin (**4**).

Keywords: clerodane; diterpene; tunicate; 2D NMR.
* Corresponding author. Tel./fax: +81-11-706-4985;
e-mail: jkobay@pharm.hokudai.ac.jp

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Table 1. ^{1}H and ^{13}C NMR data of dytesinins A (**1**) and B (**2**) in $CDCl_3$

Position	**1**			**2**		
	δ_H		δ_C	δ_H		δ_C
1	1.17	1.49	23.5 t	1.15	1.51	23.5 t
2	0.82	1.33	20.5 t	0.84	1.33	20.6 t
3	1.55	1.61	32.6 t	1.57	1.62	32.6 t
4			17.4 s			17.4 s
5			25.9 s			24.7 s
6	1.27	1.59	33.6 t	1.26	1.56	33.8 t
7	1.47[a]		29.9 t	1.46[a]		29.9 t
8	1.57		37.0 d	1.52		37.0 d
9			40.2 s			40.1 s
10	1.53		43.3 d	1.47		43.3 d
11	1.57[a]		33.3 t	1.57[a]		33.3 t
12	2.32	2.44	20.6 t	2.27	2.32	21.6 t
13			170.4 s			171.3 s
14	5.84		117.1 d			115.0 d
15			171.2 s			177.7 s
16	6.01		98.8 d	4.75[a]		73.1 t
17	1.15[b]		22.5 q	1.03[b]		22.7 q
18	0.12	0.47	24.4 t	0.13	0.47	24.6 t
19	0.80[b]		16.3 q	0.81[b]		16.3 q
20	0.77[b]		15.3 q	0.78[b]		15.8 q

[a] 2H.
[b] 3H.

The gross structure of dytesinin A (**1**) was elucidated by extensive 2D NMR experiments including ^{1}H-^{1}H COSY, HSQC, CH_2-selected E-HSQC,[4,5] CH_2-selected editing HSQC-TOCSY,[4,5] and HMBC (Fig. 1). Detailed analyses of ^{1}H-^{1}H COSY, HSQC, CH_2-selected E-HSQC, CH_2-selected E-HSQC-TOCSY revealed four proton-connectivities from H_2-1 to H_2-3 and H-10, from H_2-6 to H_3-19, and from H_2-11 to H_2-12. The HMBC spectrum showed correlations for H_3-17/C-3, H_3-17/C-4, H_3-17/C-5, H_2-18/C-3, H_2-18/C-4, H_2-18/C-5, H_2-18/C-6, H_2-18/C-10, H_3-19/C-7, H_3-19/C-9, H_3-20/C-8, H_3-20/C-9, H_3-20/C-10, H_3-20/C-11, and H-1β (δ_H 1.17)/C-10, suggesting connecitivities of the 6/6/3 tricyclic core. The presence of a γ-hydroxybutenolide ring was indicated by HMBC correlations for H-14 (δ_H 5.84)/C-12 (δ_C 20.6), H-14/C-13 (δ_C 170.4), H-14/C-15 (δ_C 171.2), and H-16 (δ_H 6.01)/C-13. Thus the gross structure of dytesinin A was assigned as **1**.

The relative stereochemistry of the tricyclic core was elucidated mainly on the basis of NOESY correlations (Fig. 2). NOESY correlations for H-6α (δ_H 1.27)/H_3-17 (δ_H 1.15), H-6α/H-18β (δ_H 0.12), and H-2α/H-18α (δ_H 0.47) indicated that C-17 and C-18 were β-equatorially and α-axially oriented, respectively. The α-axial orientation for C-20 was

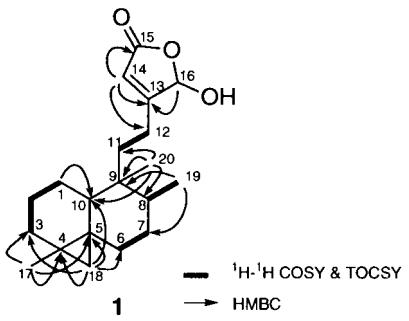

Figure 1. 2D NMR correlations for dytesinin A (**1**).

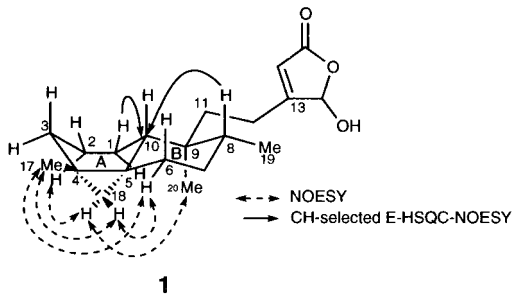

Figure 2. NOESY and CH-selected E-HSQC-NOESY correlations and relative stereochemistry of tricyclic core of dytesinin A (**1**).

deduced from the NOESY correlation for H-18α/H_3-20. Any evidence of β-axial orientations for H-8 and H-10, however, could not be provided from the NOESY spectrum, since the methine proton signals were very close to each other, and other proton signals such as H-1β, H_2-3, H-6β, H_2-7, and H_2-11 were severely overlapped with those of H-8 and H-10. To separate H-8 and H-10 using well resolved carbon chemical shifts, we demonstrated the CH-selected E-HSQC-NOESY experiment (Fig. 3). Since **1** had only four methine carbons whose chemical shifts were relatively separated, reduction of the data points of the F_1 axis (t_1 increments, 16) and increase of the numbers of transients (scan numbers, 3k) can expect a sufficient signal-to-noise ratio. In the CH-selected E-HSQC-NOESY spectrum, correlations for C-10/H-8 and C-10/H-1β were observed, thus indicating that H-8 and H-10 were both β-axially oriented. The chair form of the ring B was implied by analyses of $^{1}J_{CH}$ coupling constants obtained from *J*-resolved HMBC spectrum[6] incorporating *J*-scaling pulse sequence[10–12] (n=30) (Fig. 4). The large coupling constants for H-6α/C-10 and H-6α/C-8 were determined to be both $^{1}J_{CH}$ 6.2 Hz based on the observed values (30× J_{CH}=186 Hz) in the *J*-resolved HMBC spectrum. Therefore, relationships between H-6α and C-10 and between H-6α and C-8 were suggested to be both antiperiplanar arrangements from the Newman projections of C-6–C-5 and C-6–C-7 bonds (Fig. 5).[13] On the other hand, the rather smaller $^{1}J_{CH}$ value (3.1 Hz) for H-6α/C-18 was obtained from the magnitude of the coupling constant (30× J_{CH}=93 Hz), indicating *gauche* relationship between H-6α and C-18.[13] The stereochemistry of C-16 remained unresolved, since epimerization of C-16

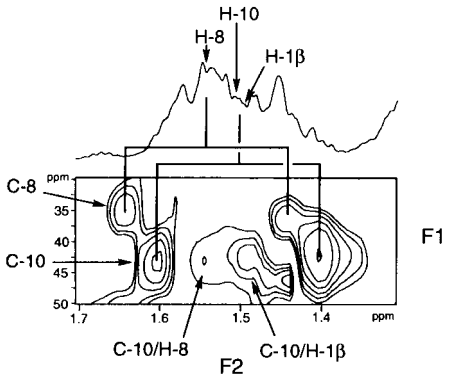

Figure 3. CH_2-selected E-HSQC-NOESY spectrum (part) of dytesinin A (**1**).

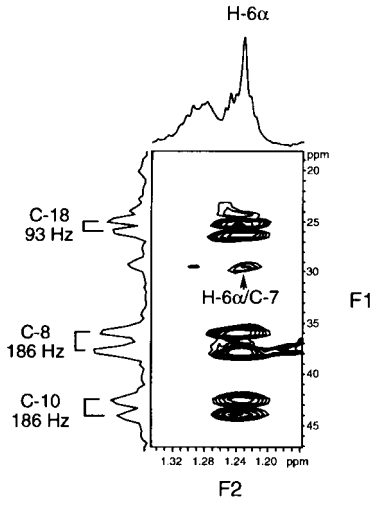

Figure 4. *J*-resolved HMBC spectrum (part) of dytesinin A (**1**).

was observed. Thus the relative stereochemistry of dytesinin A (**1**) was concluded as shown in Fig. 2.

HREIMS data (*m/z* 302.2263 M^+, +1.7 mmu) of dytesinin B (**2**) was revealed to possess the molecular formula, $C_{20}H_{30}O_2$, corresponding to those of the deoxy form of dytesinin A (**1**). Though the ^1H and ^{13}C NMR data (Table 1) were close to those of **1**, differences were found for the butenolide moiety (C-13–C-16). Dytesinin A (**1**) possessed a hemiacetal group at C-16, while the ^1H and ^{13}C NMR data of **2** showed signals due to an oxymethylene [δ_H 4.75 (2H, s), δ_C 73.1, t]. Therefore, the structure of dytesinin B (**2**) was elucidated to be the deoxy form at C-16 of dytesinin A (**1**).

Dytesinins A (**1**) and B (**2**) are new clerodane-type diterpenes with a cyclopropane ring at C-4–C-5, although isolation of diterpenes from marine tunicates is very rare.[14–16] In this paper it was demonstrated that CH-selected E-HSQC-NOESY and *J*-resolved HMBC are very useful tools for stereochemical analysis. Although HSQC-NOESY experiment seems to be suitable to assign NOEs between two closely resonated protons, whose NOEs cannot be discriminated in the NOESY spectrum, the sensitivity of this method is very low. On the other hand, editing HSQC-NOESY affords a sufficient sensitivity within a reasonable measuring time, since selecting only methine or methylene carbons enables reduction of data points and increase of the numbers of transients.

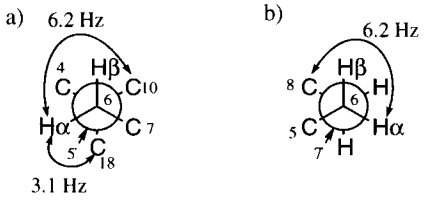

Figure 5. Newman projections for (a) C-6–C-5 and (b) C-6–C-7 bonds in dytesinin A (**1**). $^1J_{CH}$ coupling constants are given in hertz.

Experimental

Extraction and isolation

The tunicate *Cystodytes* sp. (TN-514, 1.85 kg, wet weight) was collected off Ie Island, Okinawa, and kept frozen until used. The tunicate was extracted with MeOH (1 L×2), and the extract was partitioned between EtOAc (500 mL) and 1 M NaCl aq. Parts (490 mg) of the EtOAc-soluble materials (1.22 g) were subjected to silica gel column chromatography ($CHCl_3$→$CHCl_3$:MeOH, 98:2) to give two crude fractions containing the diterpenes. The fraction eluted with 2% $CHCl_3$/MeOH was purified by C_{18} HPLC (Mightysil RP-18, 5 mm, Kanto Chemical Co. Inc., 10×250 mm; eluent, CH_3CN/H_2O, 90:10; flow rate, 3.0 mL/min; UV detection at 220 nm) to afford dytesinin A (**1**, 0.00085%, wet weight, t_R 14.8 min). The fraction eluted with $CHCl_3$ was separated by C_{18} HPLC (Mightysil RP-18, 5 mm, eluent, CH_3CN:H_2O, 80:20; flow rate, 3.0 mL/min; UV detection at 220 nm) to yield dytesinin B (0.00019%, t_R 38.4 min).

Dytesinin A (1). Colorless amorphous solid; $[\alpha]_D^{25}$=~0° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3430, 2925, 1740 and 1630 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 95, 189 191, 300 $(M-H_2O)^+$, and 318 (M^+); HREIMS *m/z* 318.2222 [calcd for $C_{20}H_{30}O_3$, (M^+): 318.2195].

Dytesinin B (2). Colorless amorphous solid; $[\alpha]_D^{25}$=−37° (*c* 0.25, $CHCl_3$); IR (KBr) ν_{max} 2925 and 1715 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 95, 189, 191, 274 $(M-2CH_3)^+$, 287 $(M-CH_3)^+$, and 302 (M^+); HREIMS *m/z* 302.2263 [calcd for $C_{20}H_{30}O_2$, (M^+): 302.2246].

NMR experiments

Dytesinin A (**1**, 5.5 mg) or B (**2**, 1.2 mg) was dissolved in 200 or 80 μL (for 500 or 600 MHz spectrometer) of 99.96% deuterium-labeled chloroform ($CDCl_3$). ^1H NMR, ^1H–^1H COSY, NOESY, HMQC, and HMBC spectra were measured at 300 K with a Bruker ARX-500 spectrometer equipped with 5 mm diameter H/X inverse probe. *J*-resolved HMBC spectrum was measured at 300 K with a Bruker AMX-500 spectrometer equipped with 5 mm diameter *Z*-gradient H/C/N inverse probe. ^{13}C NMR, CH_2-selected E-HSQC, CH_2-selected E-HSQC-TOCSY, and CH-selected E-HSQC-NOESY spectra were recorded at 300 K on a Bruker AMX-600 spectrometer equipped with 2.5 mm Z-gradient C/H dual (^{13}C NMR) or H/X inverse probe. 5 mm symmetrical thin-wall micro cells for $CDCl_3$ and 2.5 mm symmetrical micro cells for $CDCl_3$ (Shigemi Co. Ltd.) were used as NMR tubes for 500 and 600 MHz spectrometer, respectively.

CH_2-selected E-HSQC and CH_2-selected E-HSQC-TOCSY spectra were measured by the sequence described before.[5] For CH_2-selection, the editing flip angle β and the delay τ were π and 3.7 ms ($^1J_{CH}$=135 Hz), respectively. The delays RD (repetition delay), BD (BIRD delay), and Δ were 2.0 s, 0.3 s, and 3.7 ms, respectively. A trim (2.5 ms) and an MLEV-17 composite pulses (mixing time; 60 ms) were used for measurement of the CH_2-selected E-HSQC-TOCSY spectrum. The F_1 and F_2 spectral widths were

5319 and 6024 Hz, respectively. The CH_2-selected E-HSQC spectrum was measured in 1k data points using 16 transients (with four dummy scans) for each $512t_1$ increments of F_1 spectral widths. On the other hand, the CH_2-selected E-HSQC-TOCSY spectrum was obtained in 1k data points using 64 transients (with four dummy scans) for each $216t_1$ increments of F_1 spectral widths. Zero-filling to 1k for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. Total measuring times for the E-HSQC and the E-HSQC-TOCSY spectra were ca. 3 and 12 h, respectively.

The CH-selected E-HSQC-NOESY experiments was carried out using the following pulse sequence; RD-BIRD[$90°x(^1H)-\Delta-180°_y(^1H,^{13}C)-\Delta-90°_{-x}(^1H)-$BD] $-90°x(^1H)-\Delta/2-180°x(^1H,^{13}C)-\Delta/2-90°_{\Phi1}(^1H)-90°_{\Phi2}$ $(^{13}C)-$editing$[\tau/2-\beta°_x(^1H)-180°_{\Phi3}(^{13}C)-\tau/2]-t_1/2-180°_y$ $(^1H)-t_1/2-90°_x(^1H,^{13}C)-\Delta/2-180°_y(^1H,^{13}C)-\Delta/2-90°_x$ $(^1H)-\tau_m-90°_x(^1H)-AQ_{\Phi4}(^1H$-decoupling); $\Phi1=2(y)$, $2(-y)$; $\Phi2=x$, $2(-x)$, x; $\Phi3=4(x)$, $4(y)$, $4(-x)$, $4(-y)$; $\Phi4=2(x, -x)$, $2(-x, x)$. For CH-selection, the editing flip angle β and the delay τ were $\pi/2$ and 7.2 ms ($^1J_{CH}=139$ Hz). The delays RD (repetition delay), BD (BIRD delay), Δ, and τ_m were 2.0 s, 0.3 s, 3.6 ms, and 0.8 s, respectively. The F_1 and F_2 spectral widths were 10638 and 6024 Hz, respectively. For each $16t_1$ increments, 3k transients (with eight dummy scans) were accumulated in 1k data points. Zero-filling to 64 for F_1 and multiplication with squared sine-bell windows shifted by $\pi/4$ and $\pi/8$ in the F_1 and F_2 dimensions, respectively, were performed prior to 2D Fourier transformation. The resulting data matrix was 0.5k×32. The total measuring time was ca. 48 h.

The *J*-resolved HMBC spectrum was measured using the sequence with ^1H-^1H decoupling reported by Furihata and Seto.[6] The *J*-scaling factors *n* and *m* were set to 30 and 31, respectively. The delays nt_1 max and the constant time for long-range J_{CH} evaluation were 333 and 3.57 ms, respectively. The F_1 and F_2 spectral widths were 23809 and 4854 Hz, respectively. For each $256t_1$ increments, 512 transients (with two dummy scans) were accumulated in 1k data points. Zero-filling to 512 for F_1 and Lorenz-Gauss transformation ($GB=0.3$, LB −10) in F_2 and multiplication with squared cosine-bell windows in the F_1 dimensions were performed prior to 2D Fourier transformation. The resulting data matrix was 0.5k×512. The total measuring time was ca. 77 h.

Acknowledgements

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