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Altercrasin A, a novel decalin derivative with spirotetramic acid, produced by a sea urchin-derived *Alternaria* sp.

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ABSTRACT

Altercrasin A (**1**), a novel decalin derivative with a spiroskeleton, has been isolated from a strain of *Alternaria* sp. OUPS-117D-1 originally derived from the sea urchin *Anthocidaris crassispina*, and its structure has been elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques. In addition, the absolute configuration for **1** was established by chemical transformation and the modified Mosher's method. This compound exhibited moderate cytotoxicity against human cancer cell lines.

Marine microorganisms are potentially prolific sources of highly bioactive secondary metabolites that may serve as useful leads in the development of new pharmaceutical agents. We have focused on the potential new antitumor materials from marine-derived microorganisms that produce a number of compounds with unique structures.[1–3] As a part of this study, we have examined metabolites from the fungus *Alternaria* sp. OUPS-117D-1 originally obtained from the sea urchin *Anthocidaris crassispina*, and isolated a new compound, a cytochalasin-like decalin derivative with spirotetramic acid. The new compound was designated as altercrasin A (**1**).[4] Delaminomycins[5] isolated from *Streptomyces albulus*, lucensimycins[6–8] from *Streptomyces lucensis*, fusarisetin A[9–11] from *Fusarium* sp., and diaporthichalasin[12,13] from *Diaporthe* sp. had been reported previously as metabolites with a similar decalin derivative. The common feature of these compounds was that the hydrogen atom at the spiro moiety was too poor to supply information on stereochemistry. The stereochemistry for delaminomycins remained unknown,[5] and the relative configuration for lucensimycins was deduced by molecular dynamic simulations.[8] On the other hand, the relative configuration for diaporthichalasin was established by X-ray crystallographic analysis.[12] Determination of the stereostructure of fusarisetin A also was carried out by X-ray crystallographic analysis; however, its absolute configuration elucidated by the CD spectrum of the dibenzoate derivative was reassigned by a total synthesis.[10,11] As above, the absolute configuration for this class with spiro-γ-lactam or -lactone is experimentally unsolvable unless a good single crystal is obtained. We attempted to experimentally elucidate the absolute configuration of **1**. However, it also had stereogenic centers in the side chain, which added further difficulty. We describe herein the elucidation of the absolute stereostructure and cytotoxic activity of **1** (Fig. 1).

Alternaria sp., a microorganism from *A. crassispana*, was cultured at 27 °C for 6 weeks in a medium (70 L) containing 1% glucose, 1% malt extract, and 0.05% peptone in artificial seawater adjusted to pH 7.5. After incubation, the EtOAc extract of the culture filtrate was purified by bioassay-directed fractionation employing a stepwise combination of silica gel column and Sephadex LH-20 chromatographies, followed by reverse-phase HPLC, which afforded altercrasin **A** (**1**) (10.4 mg) as a pale yellow oil.[4]

Altercrasin **A** (**1**) had the molecular formula $C_{24}H_{33}NO_5$ as established from the $[M+Na]^+$ peak in HRFABMS. The IR spectrum exhibited absorption bands at 3345, 1730, and 1691 cm^{-1} characteristic of hydroxyl groups and carbonyls. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) using DEPT and ^1H–^{13}C correlation spectroscopy (HMQC) revealed the presence of three secondary methyls (C-17, C-21, and C-23), a tertiary methyl (C-24), three sp^3-hybridized methylenes (C-1, C-2, and C-4), eight sp^3-methines (C-3, C-5, C-8, C-10, C-13, C-16, C-19, and C-20) including two oxymethines (C-16 and C-20), two quaternary sp^3-carbons (C-9 and C-12), four sp^2-methines (C-6, C-7, C-14, and C-15), three carbonyls (C-11, C-18, and C-22) including an amide carbonyl

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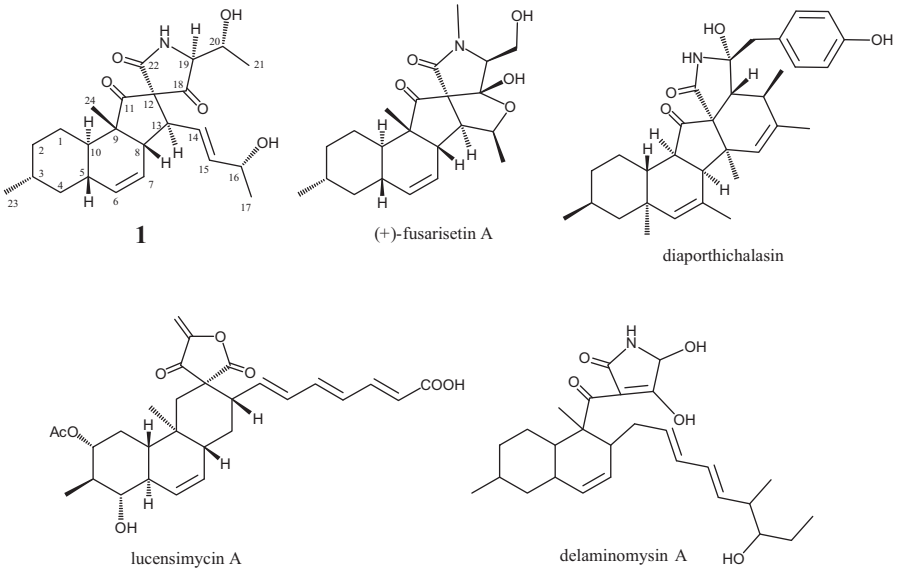

Figure 1. Altercrasin **A** (**1**) and structurally related natural products.

Table 1
NMR spectral data for **1** in $CDCl_3$

Position	δ_H[a]		J/Hz	δ_C		Position	δ_H[a]		J/Hz	δ_C	
1α	1.55	dq	12.8 (1β), 3.8 (2α, 2β, 10)	25.9	(t)	13	3.16	dd	11.8 (8), 9.0 (14)	52.8	(d)
1β	1.12	qd	12.8 (1α, 2α, 10), 3.8 (2β)			14	5.62	ddd	15.5 (15), 9.0 (13), 1.8	124.3	(d)
2α	0.90	m		36.0	(t)	15	5.73	dd	15.5 (14), 5.5 (16)	141.9	(d)
2β	1.74	ddt	12.8 (2α), 5.3 (3), 3.8 (1α, 1β)			16	4.21	m		68.8	(d)
3	1.48	m		33.5	(d)	17	1.16	d	6.8 (16)	24.2	(q)
4α	0.82	q	12.8 (3, 4β, 5)	42.6	(t)	18				207.1	(s)
4β	1.87	ddd	12.8 (4α), 5.5 (4), 3.2 (5)			19	3.66	d	7.0 (20)	70.4	(d)
5	1.92	br t	12.8 (4α, 10)	37.4	(d)	20	3.99	m		67.8	(d)
6	5.55	d	11.3 (7)	133.2	(d)	21	1.25	d	6.2 (20)	20.4	(q)
7	5.70	ddd	11.3 (6), 4.8 (8), 2.5	125.3	(d)	22				170.7	(s)
8	2.65	ddt	11.8 (13), 4.8 (7), 1.8	49.9	(d)	23	0.91	d	6.5 (3)	22.6	(q)
9				52.9	(s)	24	0.95	s		16.0	(q)
10	1.49	td	12.8 (1β, 5), 3.8 (1α)	38.7	(d)	16-OH	3.79	d	5.0 (16)		
11				211.6	(s)	20-OH	4.17	d	5.5 (20)		
12				74.0	(s)	NH	8.01	br s			

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (J/Hz). Figures in parentheses indicate the proton coupling with that position.

(C-22), and two hydroxyl groups (16-OH and 20-OH). The ^1H–^1H COSY analysis of **1** led to three partial structures including hydroxyl butylene groups (H-13/H-14, H-14/H-15, H-15/H-16, H-16/16-OH, and H-16/H-17) and hydroxyl ethyl groups (H-19/H-20, H-20/20-OH, and H-20/H-21), as shown by bold-faced lines in Figure 2. The connection of these three units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Figure 2, and the planar structure of **1** was elucidated. The geometrical configuration of an olefin moiety in the side chain (C-14–C-15) was revealed as *trans* from the large coupling constant in ^1H NMR signals ($J_{14,15}$ = 15.5 Hz) (Table 1).

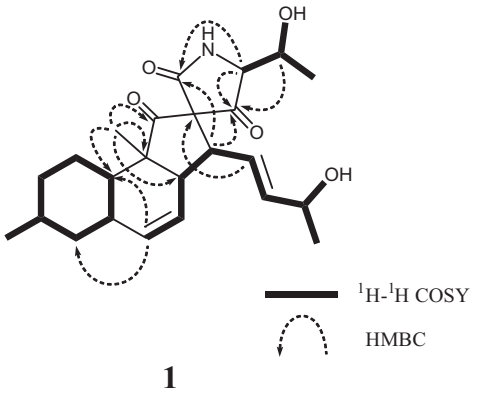

Figure 2. Typical 2D NMR correlations in **1**.

The relative stereochemistry of **1** was deduced from the NOESY experiments (Fig. 3). NOESY correlations (H-1β/H-3, H-1β/H-5, H-3/H-5, H-2α/H-4α, H-2α/H-10, and H-4α/H-10) suggested that the A ring existed in a chair conformation with H-1β, H-3, and H-5 in coaxial arrangements. In the B ring, the observed correlations (H-10/H-13, H-8/H-24, and H-5/H-24) demonstrated a half chair conformation, and showed that the methyl group C-24 was oriented *cis* to H-5 in a quasi-axial arrangement, and the ring linkage with the C ring was *cis* in an α-orientation. In the spiro-γ-lactam, there was no information from the NOESY experiments except for the correlation between H-14 and H-19; therefore, the relative configuration at C-12 and C-19 in the D ring could not be determined. In order to obtain further information on the connectivity between C and D rings, the reduction of **1** was carried out