Martefragin A, a Novel Indole Alkaloid Isolated from Red Alga, Inhibits Lipid Peroxidation

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Martefragin A (1), a novel indole alkaloid, was isolated from a red alga, *Martensia fragilis*, by repeated column chromatography. The structure of 1 was elucidated on the basis of spectral analysis of its methyl ester (2), including ^{1}H- and ^{13}C-NMR, ^{1}H–^{1}H correlation spectroscopy (COSY), and ^{13}C–^{1}H COSY. A single crystal X-ray analysis of the hydrochloride of 1 confirmed the assignment. Martefragin A (1) showed inhibitory activity on NADPH-dependent lipid peroxidation in rat liver microsomes. The IC_{50} values of 1, α-tocopherol and ascorbic acid were 2.8, 87 and 200 μM, respectively.

Key words martefragin A; *Martensia fragilis*; lipid peroxidation

In recent years, marine resources have attracted much attention in the search for lead compounds to develop new drugs. They have been shown to contain various compounds with novel structures, probably because of the uniqueness of the living environment.[3)] Up to the present time, a variety of bioactive compounds have been found from them.[4)]

We have been searching for bioactive compounds from extracts of marine resources, especially marine algae. Free radical-mediated lipid peroxidation has been proposed to play a critical role in several diseases.[5)] This prompted us to search for inhibitors of lipid peroxidation. This paper describes the isolation and identification of a new oxazolylindole compound having inhibitory activity against lipid peroxidation from a marine red alga, *Martensia fragilis* (=*denticulata*) HARVEY.

Results and Discussion

The chloroform–methanol extract of *Martensia fragilis* was partitioned between BuOH and water. The BuOH fraction, which showed marked inhibition of lipid peroxidation, was further fractionated with methanol and acetone, then separated twice by solvent partition, and purified by repeated column chromatography on silica gel and Sephadex LH-20, to give a new compound, which we named martefragin A.

Structure of Martefragin A Martefragin A (**1**) showed a quasi-molecular ion at *m/z* 356 $[M+H]^+$ in the positive FAB-MS, and at *m/z* 354 $[M-H]^-$ in the negative FAB-MS, indicating that its molecular weight is 355. A carbon signal at δ 168.9 and a fragment ion at *m/z* 310 in the negative FAB-MS suggested the presence of a carboxyl group. As martefragin A showed line broadening in ^{1}H-NMR, it was methylated with diazomethane to give a methyl ester (**2**), which showed sharper ^{1}H-NMR signals.

Methyl ester (**2**) displayed the molecular formula $C_{21}H_{27}N_3O_3$ as determined by high-resolution (HR) EI-MS (*m/z* 369.2058), indicating it to be a monomethyl ester. The ^{13}C-NMR spectrum of **2** showed one methoxycarbonyl group (δ 52.0 and 163.5) formed in the methylation reaction (Fig. 1, (a)). ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{13}C–^{1}H COSY, together with correlation spectroscopy *via* long-range coupling (COLOC) spectra (data not shown), revealed the presence of a 1-dimethylamino-3-methylpentyl moiety (b) and an indole moiety (c).

The rest of the structure, C_3NO, which shows carbon signals at δ 122.7, 154.9 and 159.4, was deduced to be a tri-substituted oxazole (d). However, from the spectral data, no information was obtained about the substitution pattern of the partial structures (a), (b), (c), on the oxazole ring (d).

From *Martensia fragilis*, several compounds having an indole moiety have been isolated (Fig. 2).[6,7)] Denticins A, B and C contain a moiety derived from tryptophan, and others have a moiety derived from tryptamine (designated by bold lines). These compounds, except for denticin A, also have a moiety derived from homoisoleucine (also indicated by bold lines in Fig. 2). Therefore, it can be postulated that the oxazole ring of martefragin A is derived from an amide of tryptophan and homoisoleucine by an oxidative cyclization. Thus, the structure of the methyl ester was determined as **2** shown in Fig. 3, and thus, the structure of martefragin A, having a carboxylate instead of the methoxycarbonyl, as **1** shown in Fig. 4.

Finally, the structure **1** was confirmed by a single crystal X-ray analysis. The hydrochloric acid salt of martefragin A, which crystallized from MeOH–EtOAc as pale yellow prisms, was subjected to X-ray crystal analysis. Although the conventional *R* value was large ($R=0.178$) because of large thermal movement of the terminal alkyl moiety, the result indicated the structure of martefragin A to be **1**, including the relative stereochemistry (Fig. 4). The absolute configuration of **1** has not been determined yet. Natural products with an

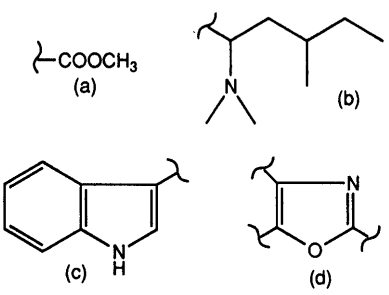

Fig. 1. Partial Structures of **2**

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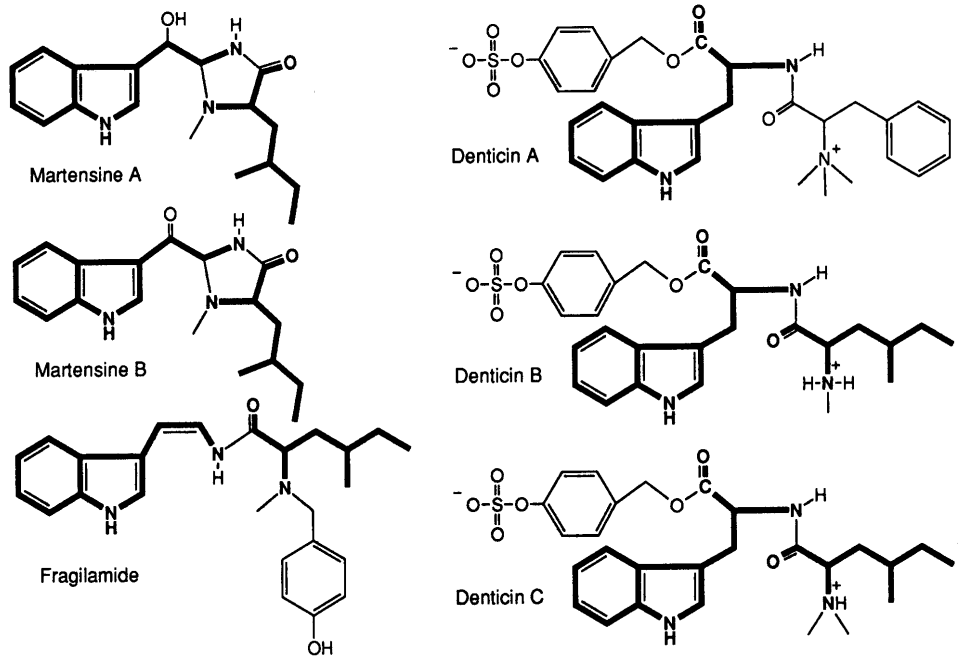

Fig. 2. Structurally Related Known Compounds Isolated from *Martensia fragilis*

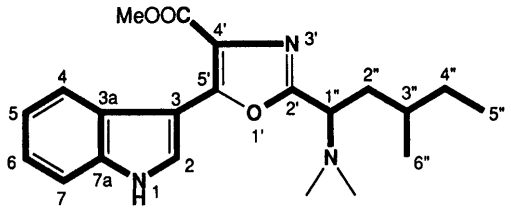

Fig. 3. Chemical Structure of **2**

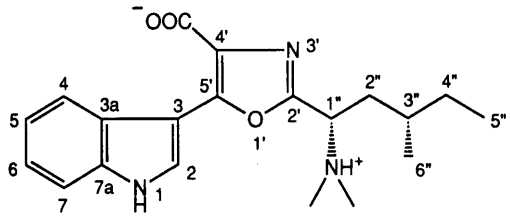

Fig. 4. Chemical Structure of Martefragin A (**1**)

oxazolylindole skeleton are rare. Until now, only a few compounds have been isolated from a species of Actinomycetes.[8—10]

Inhibitory Activity on Lipid Peroxidation We compared the inhibitory activity of martefragin A (**1**) and other antioxidants on NADPH-dependent lipid peroxidation. The activity of **1** was weaker than butylated hydroxytoluene (BHT), a synthetic antioxidant, but 30 times and 70 times stronger than α-tocopherol and ascorbic acid, respectively. Indole and tryptamine, which form the partial structures of **1**, had only a weak inhibitory effect (Table 3). Martefragin A (**1**) displayed no chelation with ferrous ion (data not shown). The mechanism of inhibition of NADPH-dependent lipid peroxidation is thought to be due to radical stabilization by its extended conjugated system. However, further investigation is necessary to elucidate the detailed mechanism.

Experimental

Optical rotation was measured with a JASCO DIP-1000. The IR spectrum was recorded with a PERKIN ELMER JAPAN 1650QS spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM GSX-500 spectrometer. FAB-MS and HR EI-MS were measured with a HITACHI M-80 spectrometer. The UV spectrum was recorded with a HITACHI 557 spectrometer. Column chromatography was carried out on Kieselgel 60 (Art. 9385, 230—400 mesh, Merck), Lobar column (LiChroprep Si 60, size B, Merck) and Sephadex LH-20 (Pharmacia).

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **2** in $CDCl_3$

δ_C (ppm)	δ_H (ppm)	Position
11.1	0.85 (3H, t, J=7.3 Hz)	5″-C
19.0	0.95 (3H, d, J=6.5 Hz)	6″-C
29.6	1.20 (1H, m)	4″-C
	1.40 (1H, m)	
31.3	1.36 (1H, m)	3″-C
37.9	1.74 (1H, ddd, J=13.2, 8.8, 5.9 Hz)	2″-C
	2.20 (1H, ddd, J=13.2, 9.8, 4.9 Hz)	
41.9	2.40 (6H, s)	N($\underline{C}H_3$)$_2$
52.0	3.98 (3H, s)	COO$\underline{C}H_3$
60.6	4.01 (1H, dd, J=9.8, 5.9 Hz)	1″-C
104.2	—	3-C
111.7	7.47 (1H, br d, J=7.3 Hz)	7-C
121.0	8.19 (1H, br d, J=7.3 Hz)	4-C
121.6	7.28 (1H, td, J=7.3, 1.5 Hz)	5-C
122.7	—	4′-C
123.2	7.32 (1H, td, J=7.3, 1.5 Hz)	6-C
125.2	—	3a-C
129.6	8.86 (1H, d, J=2.9 Hz)	2-C
135.9	—	7a-C
154.9	—	5′-C
159.4	—	2′-C
163.5	—	$\underline{C}OOCH_3$
	8.77 (1H, br s)	1-N$\underline{H}$

Isolation of Martefragin A (1) *Martensia fragilis* (26 kg, wet weight) was collected off the coast of Uozu, Toyama Prefecture in July 1994. It was extracted with methanol, chloroform : methanol (3 : 1) and chloroform successively. The extracted solutions were combined, dried, and partitioned with butanol and water. The butanol layer was dried *in vacuo* to give a residue. Methanol (50 ml) was added to this residue and the insoluble part was removed. Acetone (11) was added to this methanol solution and the insoluble part was removed. This solution was dried and partitioned with hexane and methanol. Chloroform and water were added to the methanol layer, and the organic layer concentrated to give the chloroform extract (76 g). This frac-

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for Martefragin A (**1**) in CD_3OD

δ_C (ppm)	δ_H (ppm)	Position
12.2	0.78 (3H, t, J=7.3 Hz)	5″-C
19.9	0.88 (3H, d, J=6.4 Hz)	6″-C
31.7	1.16 (1H, m)	4″-C
	1.31 (1H, m)	
33.7	1.23 (1H, m)	3″-C
38.6	1.74 (1H, ddd, J=13.2, 9.3, 4.4 Hz)	2″-C
	2.28 (1H, ddd, J=13.2, 11.2, 3.9 Hz)	
42.8	2.51 (6H, s)	$N(\underline{C}H_3)_2$
63.1	4.15 (1H, br s)	1″-C
105.4	—	3-C
113.8	7.43 (1H, br d, J=8.0 Hz)	7-C
122.3	8.06 (1H, br d, J=8.0 Hz)	4-C
122.6	7.12 (1H, td, J=8.0, 1.0 Hz)	5-C
124.3	7.16 (1H, td, J=8.0, 1.0 Hz)	6-C
127.4	—	3a-C
128.5	—	4′-C
131.7	8.71 (1H, s)	2-C
138.6	—	7a-C
155.2	—	5′-C
158.8	—	2′-C
168.9	—	$\underline{C}OO^-$

Table 3. Effect of Martefragin A (**1**) on Lipid Peroxidation in Rat Liver Microsomes

Compounds	IC_{50} (μM)
Martefragin A (**1**)	2.8
BHT	0.55
α-Tocopherol	87
Ascorbic acid	200
Indole	330
Tryptamine	260

tion was subjected to silica gel chromatography (700 g) using EtOAc, EtOAc : MeOH=96 : 4, 92 : 8, 8 : 2, and EtOAc : MeOH : H_2O=8 : 2 : 1. Fractions 45—55 (18.4 g) eluted with EtOAc : MeOH=8 : 2 and EtOAc : MeOH : H_2O=8 : 2 : 1 had inhibitory activity against lipid peroxidation. A part of this fraction (4 g) was further separated by silica gel chromatography (200 g), Lobar column ($CHCl_3$: MeOH : NH_4OH=7 : 3 : 0.3), Sephadex LH-20 (MeOH) and a further Lobar column using the same conditions, to give martefragin A (**1**, 347 mg).

Martefragin A (1): White powder, mp 147—148 °C. FAB-MS (positive) *m/z*: 356 ($[M+H]^+$). FAB-MS (negative) *m/z*: 354 ($[M-H]^-$), 310 ($[M-H-44]^-$). UV λ_{max} (MeOH) nm (ε): 224 (20200), 248 (sh, 8600), 295 (sh, 8800), 323 (14200). IR (KBr) cm^{-1}: 3180, 1593, 1396, 1126, 952, 808, 745. $[\alpha]_D^{26}=-20.3°$ (c=0.760, MeOH). ^{1}H- and ^{13}C-NMR spectral data are shown in Table 2.

Methylation of 1 Martefragin A (**1**) (*ca.* 50 mg) in MeOH (5 ml) was treated with an etheral solution of diazomethane (excess) for 2 h. After concentration, the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH=19 : 1) to yield a methyl ester (**2**, 32 mg).

Martefragin A Methyl Ester (2): Pale yellow gum. HR-EIMS *m/z*: 369.2058 (Calcd for $C_{21}H_{27}N_3O_3$: 369.2025). ^{1}H- and ^{13}C-NMR spectral data are shown in Table 1.

X-Ray Crystal Analysis The hydrochloric acid salt of martefragin A was crystallized from MeOH–EtOAc, to form pale yellow prisms with the tetragonal crystal system. Crystal data were as follows: space group $P4_12_12$, a=10.2082 (8), c=44.25 (1) Å, V=4612 Å^3, Z=8. Reflection data were collected on a Rigaku AFC5R diffractometer using graphite monochromated Cu K_α radiation in the 2θ-ω scan technique to a maximum 2θ value of 120.2° at a speed of 32°/min. The structure was solved by a direct method using 2155 unique reflections. Non-hydrogen atoms were refined either anisotropically or isotropically by a full-matrix least-squares method to give the conventional R value of 0.178.[11] The ORTEP drawing of the result is shown in Fig. 5.

Preparation of Rat Liver Microsomes[12] Male rats (Wistar-ST, 200—250 g) were used. Livers were removed after washing out the blood by infusing physiological salt solution into the portal vein under ether anesthesia and were homogenized by a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 8000 ***g*** for 20 min and to the supernatant was added one tenth volume of 88 mM calcium chloride. The microsome fraction precipitated and was collected by centrifugation and suspended in 0.1 M Tris–HCl buffer (pH 7.5). The microsomes were stored at −80 °C until use.

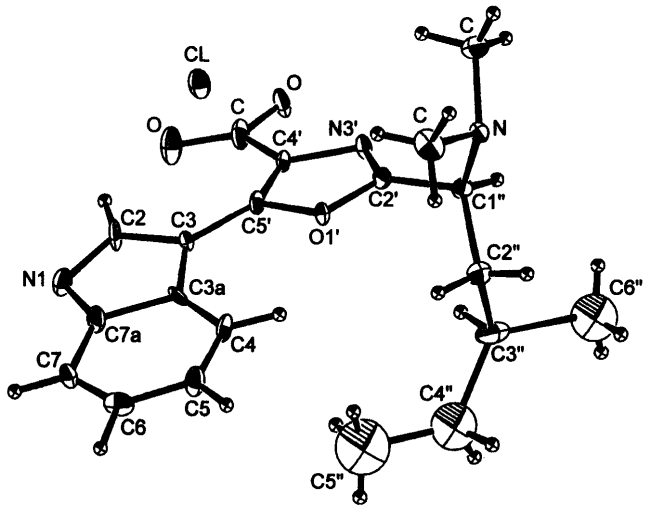

Fig. 5. ORTEP Drawing of Martefragin A (**1**) Hydrochloride

NADPH P-450 Reductase-Dependent Lipid Peroxidation in Liver Microsomes[13] Rat liver microsomes (0.2—1 mg protein) were preincubated with an extract or a compound at 37 °C for 5 min in 50 mM Tris–HCl buffer (pH 7.5) containing 7 mM magnesium chloride and further incubated at 37 °C for 10 min with NADPH-regenerating system (0.1 mM NADPH, 4 mM glucose-6-phosphate and 0.5 unit glucose-6-phosphate dehydrogenase), 0.12 mM ferrous chloride and 2 mM adenosine diphosphate (1 ml of final volume). After terminating the reaction by adding 0.375% 2-thiobarbiturate solution (2 ml) containing 0.25 N HCl and 15% trichloroacetic acid, the solution was heated at 100 °C for 15 min. The solution was centrifuged at 1700 ***g*** for 5 min after cooling and the absorbance of the adduct at 535 nm was measured.

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