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First Total Synthesis of Martefragin A, a Potent Inhibitor of Lipid Peroxidation Isolated from Sea Alga

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Abstract

The first total synthesis of martefragin A, a potent inhibitor of lipid peroxidation isolated from sea alga, has been accomplished and the absolute configurations of two stereogenic centers were determined. Synthetic martefragin A, its stereo isomers, and some analogs showed strong inhibitory activity against lipid peroxidation using rat liver microsome.

Keywords: *Alkaloids; Asymmetric Synthesis; Oxazoles;*

Martefragin A (**1**) was isolated from a sea alga *Martensia fragilis* Harvey, collected at Toyama Bay in Japan, by Saito and co-workers, and has been reported to be a strong inhibitor of lipid peroxidation using rat liver microsome [1, 2]. Using NMR techniques, the same group showed that the structure of **1** is a 5-(3'-indolyl)oxazole skeleton with a 1"-dimethylamino-3"-methylpentyl side chain, as shown in Scheme 1. However, the stereochemistry of **1** has not been established. Due to its strong activity (30-fold more potent inhibitor than α-tocopherol)[1] and considering its relatively simple structure, we became interested in the mechanism of its inhibitory activity. Therefore, we started a synthetic study of **1** and related compounds. We report here the determination of the absolute configuration of **1** by the first enantioselective syntheses of **1**, three other stereoisomers of **1** and some analogs, including preliminary results on their biological activity.

Since the configurations of the two stereogenic centers in the side chain of **1** had not been determined, we planned to synthesize all four possible stereoisomers. We expected that the characteristic 5-(3'-indolyl)oxazole skeleton of **1** could be constructed by the oxidative cyclization of dipeptide **2** (Scheme 1) [3], which consists of protected tryptophan and an amino acid; homoisoleucine **3**. Although, the preparation of an isomer of homoisoleucine in an optically active form, by enzymatic hydrolysis of **4** using α-chymotrypsin, has been reported previously [4], an asymmetric synthesis of **3** was used because of the need for all four stereoisomers of homoisoleucine. Evans' asymmetric azidation [5] of **5** which could be

[1] Indole and tryptamine did not show such activity. See ref. 2.

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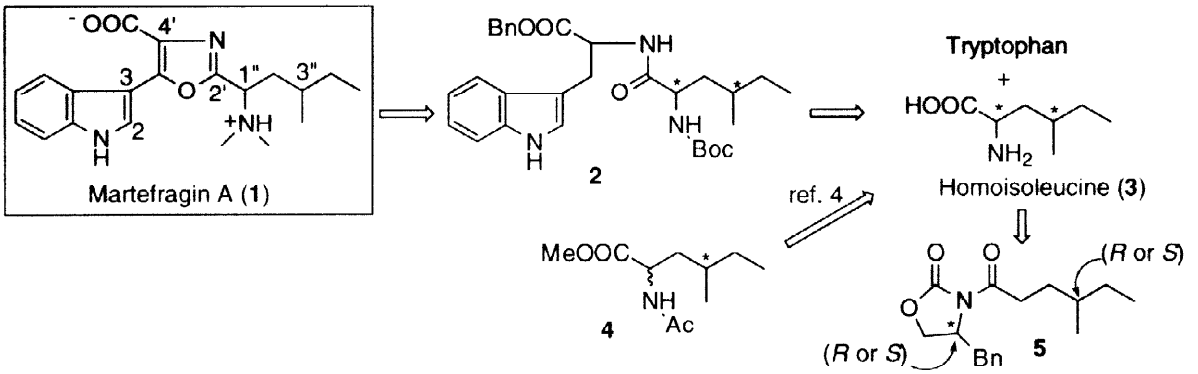
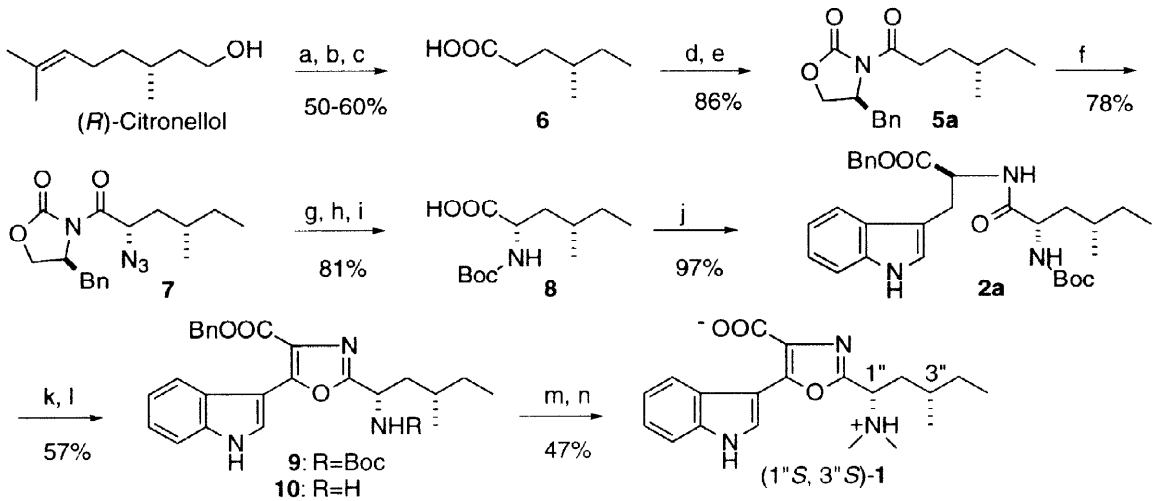


Scheme 1.



Key: ***a*** MsCl, Et_3N, ***b*** $LiAlH_4$, Et_2O, ***c*** $KMnO_4$, $NaIO_4$, aq. acetone, ***d*** $SOCl_2$, benzene, ***e*** lithium (*S*)-4-benzyl-2-oxo-1,3-oxazolidine, ***f*** 1) $KN(TMS)_2$, THF, -78 °C, 2) 2,4,6-triisopropylbenzenesulfonyl azide, -78 °C, 3) HOAc, -78 °C-rt, ***g*** LiOH, aq. THF, ***h*** H_2, 10% Pd-C, EtOH, ***i*** Boc_2O, NaOH, aq. dioxane, ***j*** benzyl L-tryptophan•HCl, diethyl phosphonocyanidate, Et_3N, THF, 0 °C-rt, ***k*** DDQ, THF, reflux, 2h, ***l*** CF_3COOH, CH_2Cl_2, ***m*** formaline, AcOH, dioxane, H_2, 10% Pd-C, ***n*** H_2, 10% Pd-C, AcOEt.

Scheme 2.

obtained from chiral 4-methylhexanoic acid, appeared to be a suitable choice for our purpose.

Both (*S*)- and (*R*)-4-methylhexanoic acids were prepared from (*R*)- and (*S*)-citronellol by known procedures with a slight modification (Scheme 2) [6-8]. Thus, a hydroxy group of (*R*)-citronellol was removed to give chiral (*S*)-(*E*)-2,6-dimethyl-2-octene in two steps (methylsulfonylation and reduction with lithium aluminum hydride). Oxidative cleavage of the double bond by the successive addition of sodium periodate and potassium permanganate gave the chiral 4-methylhexanoic acid **6** in good yield. Compound **6** was then converted to acid chloride and coupled with lithium salt of (*S*)-4-benzyl-2-oxo-1,3-oxazolidine. Asymmetric azidation was carried out according to the procedures developed by Evans. Thus, oxazolidinone **5a** was treated with KHMDS (1.5 equiv.) at -78 °C and then reacted with trisylazide. After adding acetic acid, the reaction was warmed to room temperature. Usual workup gave the desired azide **7** in 78% yield in a diastereomerically pure form.[2] In this reaction, when the amount of KHMDS was insufficient (1.2 equiv.), the yield of the azide was

[2] The absolute configuration of a new stereogenic center in 7 was confirmed by single crystal X-ray analysis.

considerably lowered (10%) and the diazo compound was obtained (20%) as a by-product. The azide **7** was converted to the protected (2*S*, 4*S*)-homoisoleucine **8** in three steps, and **8** was condensed with *O*-benzyl-L-tryptophan hydrochloride to give the dipeptide **2a**.

When **2a** was heated in THF at reflux for 1 h in the presence of 2.5 equiv. of dichlorodicyanobenzoquinone (DDQ), oxidative cyclization proceeded to give the oxazole derivative **9** in 63% yield. The structure of the oxazole **9** was supported by spectral data and finally confirmed by X-ray analysis after conversion to **10** (Figure 1). *N*, *N*-Dimethylation followed by debenzylation gave (1"*S*, 3"*S*)-**1**. Using the same protocol, three other isomers of homoisoleucine, (2*R*, 4*S*)-, (2*S*, 4*R*)-, and (2*R*, 4*R*)-**3**, were synthesized in an optically pure form, and were converted to **1**.

^{1}H and ^{13}C-NMR spectra of both (1"*S*, 3"*S*)- and (1"*R*, 3"*S*)-**1** were compared to those reported for natural martefragin A [2]. Clear differences were observed between the ^{1}H-NMR spectra of natural and (1"*R*, 3"*S*)-**1**, especially in the region of the side-chain proton (C2"-H, C3"-H, and C4"-H). Although the ^{1}H-NMR spectra of (1"*S*, 3"*S*)-**1** were almost the same as those reported for natural **1**, subtle differences were also observed. Thus, methyl esters of (1"*S*, 3"*S*)- and (1"*R*, 3"*S*)-**1** were prepared as described above. ^{1}H and ^{13}C-NMR spectra obtained from methyl ester of (1"*S*, 3"*S*)-**1** showed good agreement with those reported for methyl ester of natural **1** (Table 1). Therefore, the absolute configurations of natural **1** should be (1"*S*, 3"*S*) or (1"*R*, 3"*R*).

Table 1. Selected ^{1}H and ^{13}C-NMR Data of Natural, (1"*S*, 3"*S*)-, and (1"*R*, 3"*S*)-**1** Methyl Ester

number	Natural-**1** Methyl Ester [ref. 2]		(1"*S*, 3"*S*)-**1** Methyl Ester		(1"*R*, 3"*S*)-**1** Methyl Ester	
	^{1}H δ (ppm, $CDCl_3$)	^{13}C	^{1}H δ (ppm, $CDCl_3$)	^{13}C	^{1}H δ (ppm, $CDCl_3$)	^{13}C
2	8.86 (d, *J*=2.9 Hz)	129.6	8.86 (d, *J*=2.9 Hz)	129.5	8.86 (d, *J*=2.9 Hz)	129.5
3		125.2		125.2		125.2
2'		159.4		159.3		159.8
4'		122.7		122.8		122.9
5'		154.9		154.9		154.7
1"	4.01 (dd, *J*=9.8, 5.9 Hz)	60.6	4.00 (dd, *J*=10.0, 5.6 Hz)	60.6	4.05 (t, *J*=7.6 Hz)	60.2
2"	2.20 (ddd, *J*=13.2, 9.8, 4.9 Hz)	37.9	2.20 (ddd, *J*=13.6, 9.6, 4.7 Hz)	37.9	2.07 (ddd, *J*=13.8, 7.8, 5.9 Hz)	37.6
	1.74 (ddd, *J*=13.2, 8.8, 5.9 Hz)		1.74 (ddd, *J*=13.6, 8.3, 5.5 Hz)		1.81 (dt, *J*=13.9, 7.7 Hz)	
3"	1.36 (m)	31.3	1.34 (m)	31.3	1.54 (m)	31.3
4"	1.40 (m)	29.6	1.40 (m)	29.6	1.44 (m)	29.4
	1.20 (m)		1.20 (dq, *J*=13.2, 7.2 Hz)		1.24 (dq, *J*=13.6, 7.0 Hz)	
5"	0.85 (t, *J*=7.3 Hz)	11.1	0.85 (t, *J*=7.2 Hz)	11.1	0.91 (t, *J*=7.3 Hz)	11.2
6"	0.95 (d, *J*=6.5 Hz)	19.0	0.95 (d, *J*=6.6 Hz)	19.1	0.90 (d, *J*=6.6 Hz)	19.0

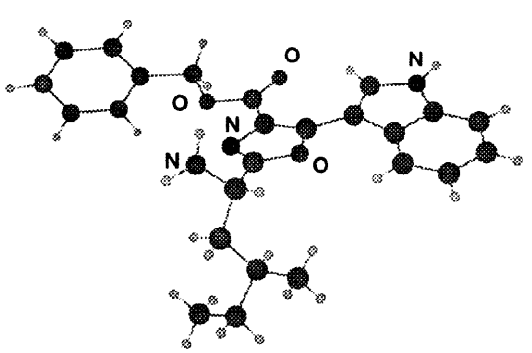


Figure 1. X-Ray Structure of **10**

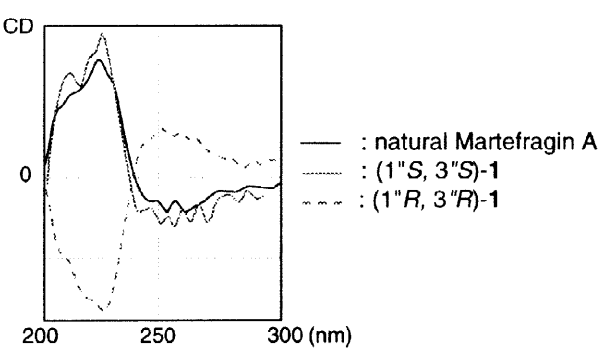


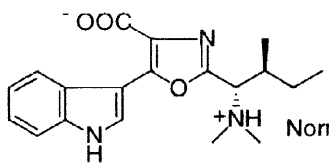
Figure 2. CD Spectra of Natural Martefragin A, (1"*R*, 3"*R*) and (1"*S*, 3"*S*)-**1**

Since only a few milligrams of natural **1** were available, the CD spectra of (1"*S*, 3"*S*)- and (1"*R*, 3"*R*)-**1** were compared with that of natural **1**. The CD spectra of (1"*S*, 3"*S*)-**1** showed a strong positive Cotton effect peak at 225 nm and a negative Cotton effect peak at 250 nm, which was superimposed on that of natural **1**, whereas the CD spectra of (1"*R*, 3"*R*)-**1** showed a mirror image (Figure 2). Therefore, we concluded that the absolute configurations of martefragin A are 1"*S* and 3"*S*.

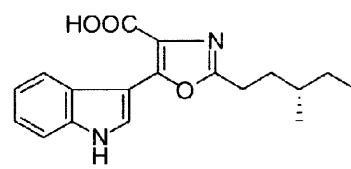
The inhibitory activities of synthetic martefragin isomers were measured using rat liver microsome and compared with that of natural martefragin A. Synthetic (1"*S*, 3"*S*)-**1** showed strong inhibitory activity against lipid peroxidation, similar to that of natural **1** [IC_{50} for natural and synthetic (1"*S*, 3"*S*)-**1**: 1.00 and 1.07 μg/mL, respectively]. Interestingly, other stereoisomers also exhibited the same level of activity against lipid peroxidation. These results are shown in Table 2 relative to the activity of (1"*S*, 3"*S*)-**1**. Normartefragin A (**11**), which was synthesized from a protected dipeptide Ile-Trp by the procedure described above, also showed nearly the same activity. Among the analogs tested, deaminomartefragin A (**12**), synthesized from tryptophan and **6**, exhibited the strongest activity. Further studies on the construction of related molecule libraries, the structure-activity relationships of martefragin A, and the mechanism of the inhibition of lipid peroxidation are now underway.

Table 2. Relative Activities for Antiperoxidation of Lipid

	(1"*S*, 3"*S*)-**1**	(1"*S*, 3"*R*)-**1**	(1"*R*, 3"*S*)-**1**	(1"*R*, 3"*R*)-**1**	**11**	**12**
relative activity	1.00	0.98	1.09	0.86	0.95	1.74



Normartefragin A
(**11**)



Deaminomartefragin A
(**12**)

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