# Synthesis of Entadamide A and Entadamide B Isolated from *Entada phaseoloides* and Their Inhibitory Effects on 5-Lipoxygenase[1]

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Two new sulfur-containing amides, entadamide A (1) and entadamide B (2), isolated from the seeds of *Entada phaseoloides*, were synthesized by the addition reaction of methanethiol to propiolic acid (5) followed by condensation with ethanolamine by the use of dicyclohexylcarbadiimide. These compounds inhibited the 5-lipoxygenase activity of RBL-1 cells at $10^{-4}$ g/ml. This finding suggests that entadamides A and B may be examples of a new type of anti-inflammatory drug.

**Keywords**   synthesis; sulfur-containing amide; lipoxygenase inhibitor; *trans*-N-(2-hydroxyethyl)-3-methylthiopropenamide; N-(2-hydroxyethyl)-3,3-bis(methylthio)propanamide; entadamide A; entadamide B; *Entada phaseoloides*; Leguminosae

In our recent papers,[2,3] we reported the isolation and structural elucidation of two new sulfur-containing amides, named entadamide A (1) and entadamide B (2), from the dry seed kernels of *Entada phaseoloides* MERR. (Leguminosae).

In order to confirm that entadamide A (1) and entadamide B (2) are *trans*-N-(2-hydroxyethyl)-3-methylthiopropenamide and N-(2-hydroxyethyl)-3,3-bis(methylthio)propanamide, respectively, we have chemically prepared these new compounds. We now present the synthesis of these new sulfur-containing amides (1, 2) and describe their inhibitory effects on 5-lipoxygenase activity.

Me-S\/\/NH\/\OH
          ‖
          O

entadamide A (1)

Me-S\/\/NH\/\OH
Me-S/      ‖
          O
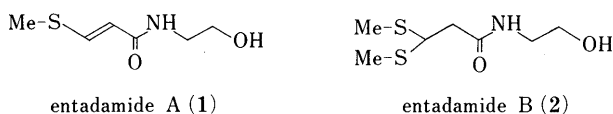
entadamide B (2)

## Results and Discussion

**Synthesis of Entadamides A (1) and B(2)**   Entadamide A (1) and entadamide B (2) were synthesized by condensation of *trans*-3-methylthioacrylic acid (3b) and 3,3-bis(methylthio)propionic acid (4), respectively, with ethanolamine according to the method of Beck *et al.*[4] as shown in Chart 1. Pure *trans*-3-methylthioacrylic acid (3b) was obtained by heating a mixture of *cis*- (3a) and *trans*-isomers (3b) of 3-methylthioacrylic acid (3) and then by repeated recrystallization from n-hexane and ethyl acetate, while 3 was obtained as a mixture of *cis*- and *trans*-isomers (2 : 1) in 73% yield together with 4 by the addition reaction of methanethiol to propiolic acid (5) according to a modification of the method of Mueller.[5] This thermal isomerization was accelerated by the addition of a small amount of I₂. The
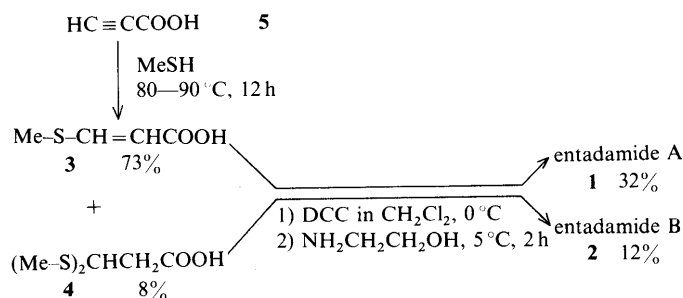
HC≡CCOOH     5

      │ MeSH
      │ 80—90 °C, 12 h
      ▼

Me–S–CH=CHCOOH \
3     73%                    \
                              \→ entadamide A
      +                            1   32%
                      / 1) DCC in CH₂Cl₂, 0 °C \
                     / 2) NH₂CH₂CH₂OH, 5 °C, 2 h \→ entadamide B
(Me–S)₂CHCH₂COOH /                               2   12%
4      8%

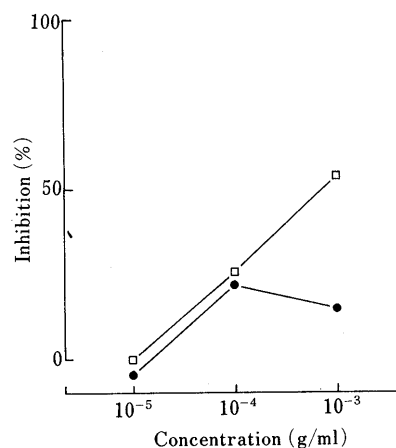Chart 1.   Synthesis of Entadamide A (1) and Entadamide B (2)



Fig. 1.   Effect of Entadamide A (1) and Entadamide B (2) on 5-Lipoxygenase Activities

●, entadamide A; □, entadamide B.

photochemical isomerization was also effective to obtain the *trans*-isomer (3b). The resulting 3b and 4 were then individually allowed to condense with ethanolamine to give entadamide A (1) and entadamide B (2) in yields of 32% and 12%, respectively. The synthetic compounds were found to be identical with natural 1 and 2 by comparison of spectral data.[2,3]

**Biological Activity**   Entadamide A (1) and entadamide B (2) showed marked inhibitory effects on the arachidonate 5-lipoxygenase of rat basophilic leukemia (RBL-1) cells, as shown in Fig. 1. The inhibition (%) values were 25.1 and 53.5% at $10^{-4}$ and $10^{-3}$ g/ml of 2 and 21.5 and 14.5% at $10^{-4}$ and $10^{-3}$ g/ml of 1, respectively. These results indicated that 2 was more effective than 1 as an inhibitor of 5-lipoxygenase activity, though the inhibitory effects of these compounds were less than that of a microbial metabolite, KF8940.[6] The present findings suggest that entadamide A (1) or entadamide B (2) may be useful to treat inflammatory diseases such as bronchial asthma.

## Experimental

**General**   High- and low-resolution electron impact mass spectra (EIMS) were measured at 70 eV using a direct inlet system. Ultraviolet (UV) spectra in EtOH were taken on a Hitachi 340 recording spectrophotometer. ¹H- and ¹³C-nuclear magnetic resonance (NMR) spectra were recorded at 100 MHz in CDCl₃ with tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was carried out on Si gel

60F$_{254}$ pre-coated plates (0.25 mm, Merck) using CH$_2$Cl$_2$–MeOH (8:1, v/v) as a solvent, unless otherwise indicated; spots were visualized by exposing the plate to UV light (254 nm) or I$_2$ vapor and by spraying with iodoplatinate reagent. [1-$^{14}$C]Arachidonic acid (59.6 mCi/mmol) and [5,6,8,9,11,12,15-$^3$H] 5-hydroxyeicosatetraenoic acid (172 mCi/mmol) were obtained from Amersham. All other chemicals used were of the highest commercial grade available.

**Synthesis of 1 and 2** Methanethiol (4.8 g, 0.1 mol) was added to a mixture of propiolic acid (**5**, 3.5 g, 0.05 mol) and triethylenediamine (56 mg) in a Pyrex tube. The tube was sealed, and the reactant mixture was heated at 80—90 °C for 12 h to yield 3-methylthioacrylic acid (**3**, 4.3 g, 73%) and 3,3-dimethylthiopropionic acid (**4**, 0.7 g, 8%). Since **3** was obtained as the mixture of cis- (**3a**, δ 7.20 ppm, d, $J$ = 10 Hz, cis-CH = CH-COOH) and trans-isomers (**3b**, δ 7.88 ppm, d, $J$ = 15 Hz, trans-CH = CH-COOH) in a ratio of nearly 2:1, the mixture of **3a** and **3b** was refluxed in xylene for 24 h in the presence of I$_2$ (1—5%) to give a mixture of **3a** and **3b** in a ratio of 6:94. A small amount of **3a** coexisting with **3b** was then removed by repeated recrystallization from n-hexane and ethyl acetate to give pure trans-3-methylthioacrylic acid (**3b**), which gave spectral data identical to those described previously.[7] Exposure of the mixture of **3a** and **3b** in CCl$_4$ to sunlight for 6 d was also effective for this isomerization, giving a mixture of **3a** and **3b** (2:98). Pure **3b** was then allowed to condense with ethanolamine to give **1** in a 32% yield as follows. Dicyclohexylcarbodiimide (DCC, 1.75 g) was slowly added to a magnetically stirred, cold solution of **3b** (1.0 g) in 30 ml of CH$_2$Cl$_2$. The mixture was stirred under ice-cooling for 15 min, and then ethanolamine (0.63 g) in 15 ml of CH$_2$Cl$_2$ was added dropwise. After being stirred for 2 h, the separated solid was filtered off and the filtrate was evaporated in vacuo to dryness. The residue was finally subjected to silica gel column chromatography eluted with ethyl acetate to give entadamide A (**1**) as a colorless sirup (0.43 g), which is soluble in organic solvents but insoluble in water. UV $\lambda_{max}^{EtOH}$ nm: 270. IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3200—3500, 1640, 1580. EIMS $m/z$ (rel. int.): 161 [M$^+$] (9), 114 (10), 101 (100), 73 (31), 45 (30). The $^1$H- and $^{13}$C-NMR spectral data (in CDCl$_3$) were identical with those of the natural product.[2] Entadamide B (**2**) was also synthesized from **4** (0.16 g) as a colorless sirup (25 mg) in a 12% yield by the same procedure as described for **1**.[3]

**Biological Activity Assays** The assays were carried out based on the methods of Jakschik and Lee[8] and Steinhoff et al.[9] RBL-1 cells were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum and antibiotic–antimycotic mixture. The harvested cells were washed once with phosphate-buffered saline, suspended in 50 mM Tris–HCl buffer, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid, and sonicated for 4 s four times at 5 s intervals. The resulting homogenate was centrifuged at 105000 $g$ for 1 h to yield the supernatant fraction (cytosol) for assaying arachidonate 5-lipoxygenase. The assay mixtures contained [1-$^{14}$C]arachidonic acid (0.1 $\mu$Ci, 16.8 nmol), 1 mM CaCl$_2$ and cytosolic fraction in 0.5 ml of 50 mM Tris–HCl buffer, pH 7.4, with or without test compound **1** or **2** (EtOH concentration: 2%). The mixtures were incubated at 37 °C for 10 min with shaking and then the reaction was terminated by the addition of 1.9 ml of CHCl$_3$–MeOH (1:2, v/v). The substrate and metabolites were extracted by the method of Bligh and Dyer[10] to avoid lactone formation. Lipid extract was then chromatographed on Si gel 60F$_{254}$ aluminum plates along with standards using petroleum ether–Et$_2$O–HOAc (45:55:1, v/v) as a solvent. Labelled products and substrate were localized by an Aloka radiochromatoscanner (TLC-101). Radioactive peaks were scraped off and finally counted in a Beckman LS 5800 liquid scintillation counter.

**References and Notes**
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