

Synthesis of Entadamide A and Entadamide B Isolated from *Entada phaseoloides* and Their Inhibitory Effects on 5-Lipoxygenase¹⁾

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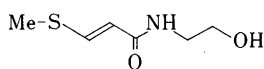
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Two new sulfur-containing amides, entadamide A (1) and entadamide B (2), isolated from the seeds of *Entada phaseoloides*, were synthesized by the addition reaction of methanethiol to propiolic acid (5) followed by condensation with ethanolamine by the use of dicyclohexylcarbodiimide. 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.

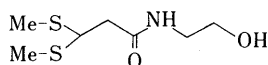
Keywords synthesis; sulfur-containing amide; lipoxygenase inhibitor; *trans*-*N*-(2-hydroxyethyl)-3-methylthiopropenamide; *N*-(2-hydroxyethyl)-3,3-bis(methylthio)propanamide; entadamide A; entadamide B; *Entada phaseoloides*; Leguminosae

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.



entadamide A (1)



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

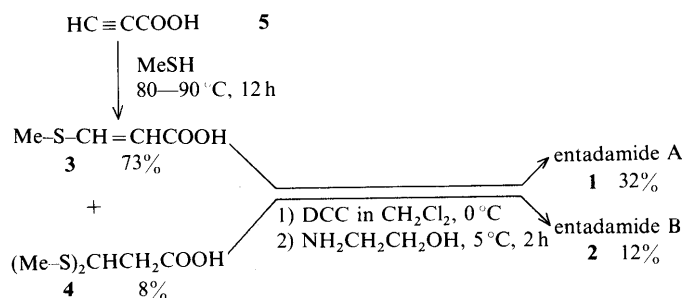


Chart 1. Synthesis of Entadamide A (1) and Entadamide B (2)

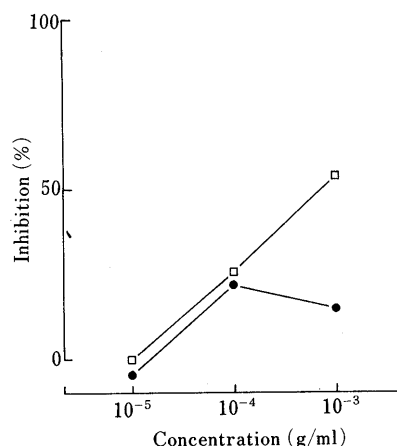


Fig. 1. Effect of Entadamide A (1) and Entadamide B (2) on 5-Lipoxygenase Activities

●, 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

60F₂₅₄ pre-coated plates (0.25 mm, Merck) using CH₂Cl₂-MeOH (8:1, v/v) as a solvent, unless otherwise indicated; spots were visualized by exposing the plate to UV light (254 nm) or I₂ vapor and by spraying with iodoplatinate reagent. [1-¹⁴C]Arachidonic acid (59.6 mCi/mmol) and [5,6,8,9,11,12,15-³H] 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 I₂ (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 CCl₄ 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 CH₂Cl₂. The mixture was stirred under ice-cooling for 15 min, and then ethanolamine (0.63 g) in 15 ml of CH₂Cl₂ 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 $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 270. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3200–3500, 1640, 1580. EIMS m/z (rel. int.): 161 [M^+] (9), 114 (10), 101 (100), 73 (31), 45 (30). The ¹H- and ¹³C-NMR spectral data (in CDCl₃) 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 Lee⁸⁾ 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 [1-¹⁴C]arachidonic acid (0.1 μ Ci, 16.8 nmol), 1 mM CaCl₂ 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 CHCl₃-MeOH (1:2, v/v). The substrate and metabolites were extracted by the method of Bligh and Dyer¹⁰⁾ to avoid lactone formation. Lipid extract was then chromatographed on Si gel 60F₂₅₄ aluminum plates along with standards using petroleum ether-Et₂O-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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