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Enzymatic Synthesis of Thia-L-tryptophans

Robert S. Phillips[1]* Louis A. Cohen[2], Ulf Annby[3], David Wensbo[3] and Salo Gronowitz[3]

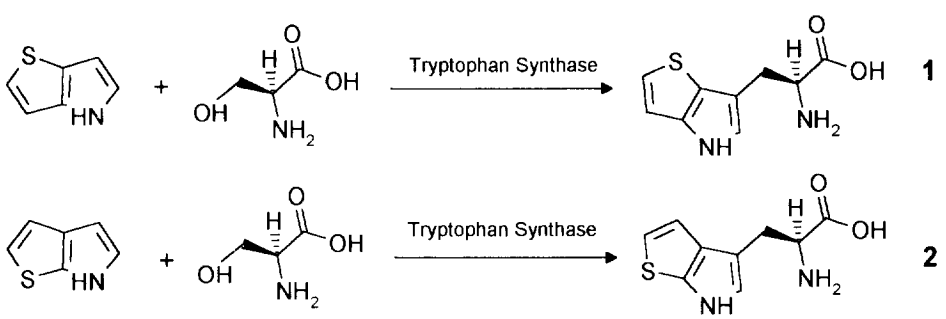
[1]Department of Chemistry, Department of Biochemistry and Molecular Biology, and Center for Metalloenzyme Studies, University of Georgia, Athens, GA 30602-2556 USA

[2]Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892 USA

[3]Department of Organic Chemistry, Chemical Center, Lund University, Box 124, 221 00 Lund, Sweden

Abstract: Thia analogs of L-tryptophan (β-3-thieno[2,3-*b*]pyrrolyl-L-alanine and β-3-thieno[3,2-*b*]pyrrolyl-L-alanine) have been prepared by the reaction of L-serine and thienopyrroles catalysed by *Salmonella typhimurium* tryptophan synthase.

The amino acid L-tryptophan plays essential roles in the structure and function of proteins and peptides and in the biosynthesis of hormones such as serotonin in animals and indole alkaloids in plants. Recently, the metabolism of L-tryptophan in man to produce quinolinic acid after injury, viral or bacterial infection has been implicated in the etiology of neurological disease[1]. Analogs of L-tryptophan are thus of interest as potential drugs and antibiotics. Previously, we demonstrated that *Salmonella typhimurium* trytophan synthase can be used to synthesize ring chlorinated[2] and aza analogues[3] of L-trytophan from the corresponding chloro and azaindoles, respectively. However, the preparation of thia-L-tryptophans (β-3-thienopyrrolyl-L-alanines) has not been reported before. In this communication, we demonstrate that *S. typhimurium* tryptophan synthase can be used to prepare 4,5-thia-L-tryptophan (**1**, β-3-thieno[3,2-*b*]pyrrolyl-L-alanine) and 6,7-thia-L-tryptophan (**2**, β-3-thieno[2,3-*b*]pyrrolyl-L-alanine)[4].



1

2

The thienopyrroles, prepared as described [5], were incubated with L-serine in phosphate buffer containing tryptophan synthase for 18 hours in the dark at 37° C [6]. The reaction mixtures

were then subjected to reverse-phase flash chromatography and lyophilized to obtain the purified thia-L-tryptophans. The rates of reaction of the thienopyrroles with tryptophan synthase is much faster than we observed previously with azaindoles[3], and appear comparable to those of indoles. The ^{1}H and ^{13}C NMR spectra of the isolated products were completely consistent with the structures shown in **1** and **2**[7]. The thiatryptophans are relatively unstable, and **1** decomposed to a black tar when recrystallization from methanol and water was attempted. Even upon storage at -20° in a sealed brown vial, **1** turned brown within 2 weeks; however, **2** was stable in the solid form for several months upon storage at -20°. Studies of the interaction of these novel tryptophan analogues with tryptophan metabolizing enzymes are now in progress.

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References and Notes.

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2. Lee, M., and Phillips, R. S. *Biomed. Chem. Lett.* **1992**, *2*, 1563-1564.
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4. The nomenclature proposed is as follows: In **1**, the 4,5-double bond of tryptophan is replaced by the sulfur atom, so it is designated 4,5-thiatryptophan. Similarly, in **2**, the 6,7-double bond is replaced by the sulfur atom, so it is designated 6,7-thiatryptophan.
5. (a) Thieno[3,2-*b*]pyrrole: Matteson, D. S. and Snyder, H. R. *J. Org. Chem.* **1957**, *22*, 1500; also, Gronowitz, S., Hörnfeldt, A.-B., Gestblom, B., and Hoffman, R. A. *Arkiv. Kemi* **1961**, *18*, 151. (b) Thieno[2,3-*b*]pyrrole: Soth, S., Farnier, M., and Fournari, P. *Bull. Chem. Soc. France* **1975**, 2511.
6. In a typical reaction, 127 mg L-serine, 0.48 mg pyridoxal-5`-phosphate, and 128 mg thieno[2,3-*b*]pyrrole were added to 50 mL of 0.1 M potassium phosphate, pH 7.8. 100 µL of purified *S. typhimurium* tryptophan synthase $\alpha_2\beta_2$ complex (2.2 mg) was added, and the reaction flask was stoppered and incubated in a 37° C water bath with gentle shaking. After 18 hours, the reaction mixture was filtered through Celite, concentrated *in vacuo* to 5 mL, then applied to a flash column (2.5 x 30 cm) of reverse-phase (C18) silica gel (Analtech) and eluted with water. Fractions which were UV and ninhydrin-positive were pooled and lyophilized to give 97.8 mg (47%) of fluffy white solid **2**.
7. **1**: UV (H_2O), λ_{max}=261 nm (log ε = 3.75); 218 (3.81); ^{1}H NMR (H_2O-D_2, 400 Mhz, ppm) 7.07 (d, 1H, J=6 Hz), 6.91(d, 1H, J=6 Hz), 6.88 (s, 1H), 3.8 (dd, 1H, J=5.7, 7.1 Hz, α-H), 3.08 (m, 2H, β-H); ^{13}C NMR (H_2O-D_2, 100 MHz, ppm) 24.5, 50.9, 104.8, 108.5, 118.7, 119.5, 120.4, 135.6, 171.5; MS (ESI), 211.0, MH^+; $[\alpha]^{20}_D$= -5.5 (c=1.096, H_2O).
2: UV (H_2O), λ_{max}=242 nm (shoulder) (log ε = 3.69); 213 (4.20); ^{1}H NMR (H_2O-D_2, 400 Mhz, ppm) 6.94 (d, 1H, J=4.1 Hz), 6.87 (s, 1H) 6.83 (d, 1H, J=4.1 Hz), 3.68 (t, 1H, J=5.9 Hz, α-H), 3.08 (q, 1H, J=4.7 Hz, 16 Hz, β-H), 2.99 (q, 1H, J=7.0 Hz, 14.7 Hz, β-H); ^{13}C NMR (H_2O-D_2, 100 MHz, ppm) 29.1, 55.7, 109.1, 116.9, 118.9, 124.0, 130, 134, 177.4; MS (ESI), 211.0, MH^+; $[\alpha]^{20}_D$= -11.9 (c=1.064, H_2O).

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