Pergamon

Tetrahedron Letters, Vol. 35, No. 28, pp. 4923-4926, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)00970-8

Biosynthetic Studies of Eudistomin H in the Tunicate *Eudistoma olivaceum*

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Key Words: tunicate, β-carboline, biosynthesis, antibiotic, bromination, decarboxylation

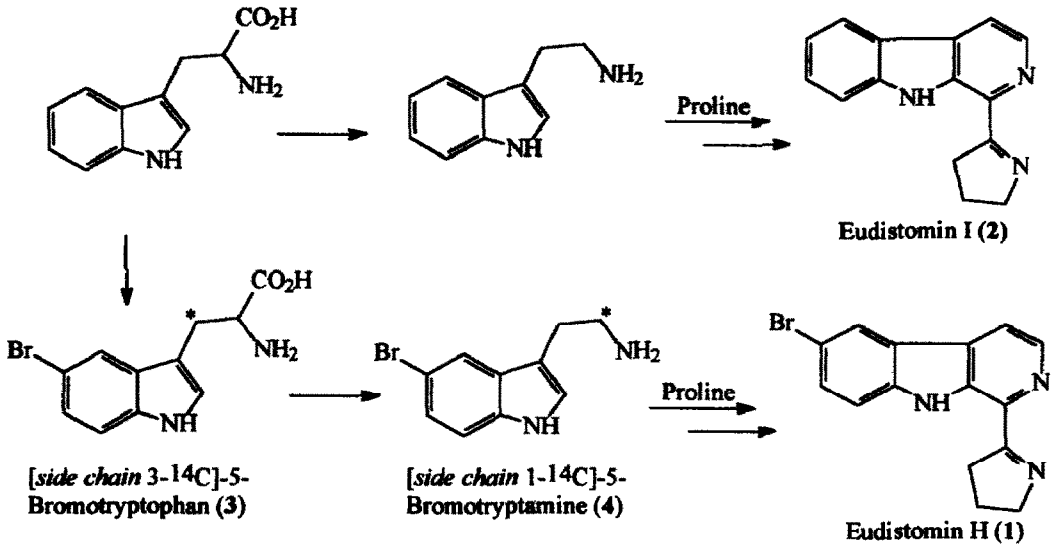
Abstract: Eudistomin H (1) is an antibiotic alkaloid isolated from the tunicate *Eudistoma olivaceum*. In our continuing investigation of the biosynthetic origin of β-carboline derivatives in this marine invertebrate, we have evaluated 5-bromotryptamine and 5-bromotryptophan as intermediates. Both of these amino acid derivatives served efficiently as precursors to eudistomin H.

The eudistomins are a series of amino acid derived[1] β-carboline antibiotics produced by the tunicate *Eudistoma olivaceum*.[2] Tunicate metabolites have held considerable attention because of their pharmacological activities, especially the antiviral oxathiazepine-bearing eudistomins[3] and the didemnins,[4] and ecteinascidins,[5] which are potent and selective cytotoxins in various stages of clinical or preclinical development.[6] Given the low level of production of many tunicate metabolites, a knowledge of their metabolic origin may lead to development of biotechnological techniques for production of those of interest to the biomedical community.[7,8] Only recently have biosynthetic investigations of tunicate metabolites been undertaken.[1,9,10]

The pyrrolinyl-bearing eudistomins display, as do the other classes of eudistomins, a variety of substitution on the indole ring, including bromination and hydroxylation. These substitution patterns apparently influence bioactivity, with bromine containing derivatives generally more potent.[11] Brominated analogues of natural products are often more bioactive than their non-halogenated counterparts.[12] Based on our previous observation that tryptamine was not utilized in eudistomin H (1) biosynthesis but was a precursor to eudistomin I (2), we proposed that bromination and decarboxylation occur early in eudistomin biosynthesis (Scheme).[1] In our continuing investigation of β-carboline biosynthesis in *Eudistoma olivaceum*, we have examined the role of brominated tryptophan (3) and tryptamine (4) as precursors to eudistomin H.

The tunicates were collected and acclimated to a small aquarium as previously reported.[1] Despite the considerably reduced size and darker color of the animals compared to our earlier collections, due apparently to the winter season, these zooids proved capable of producing eudistomins. Our precursors,

Scheme. Biosynthetic Origin of Eudistomins H (1) and I (2) in *Eudistoma olivaceum*



[*side chain* 3-^{14}C]-5-bromo-DL-tryptophan (**3**) and [*side chain* 1-^{14}C]- 5-bromotryptamine (**4**), and cold-carrier eudistomins H (**1**) and I (**2**), were prepared by standard methods.[13,14] Colonies of *E. olivaceum* were administered 20 μCi aliquots of potential precursor and incubated for up to two weeks. During the incubation period, several zooids were removed roughly every four days, freeze-dried, then treated with a chloroform solution containing 2-3 mg of both cold carriers. Recovered eudistomins were repeatedly purified by HPLC until constant specific activity was attained (Table).

The results of these incorporation experiments clearly demonstrate that 5-bromotryptophan (**3**) and 5-bromotryptamine (**4**) are utilized by *Eudistoma olivaceum* in the biosynthesis of eudistomin H (**1**). Corrected[1]

Table. Specific Activity of Eudistomins H (1) and I (2) Following *In vivo* Incorporation of Radiolabeled 5-Bromotryptamine and 5-Bromotryptophan

Precursor		Specific Activity, DPM/mg (days after uptake)			
[*side chain* 3-^{14}C]-DL-5-bromotryptophan (**3**)	H:	9600 (4)	13700 (8)	11800 (13)	13000 (17)
	I:	50 (4)	60 (8)	20 (13)	19 (17)
[*side chain* 1-^{14}C]-5-bromotryptamine (**4**)	H:	2300 (4)	2700 (8)	4200 (11)	5300 (16)
	I:	200 (4)	150 (8)	200 (11)	90 (16)

for animal and carrier masses (Figures 1 and 2), it is apparent that the amino acid serves as the more efficient precursor. Bromotryptophan and bromotryptamine are both incorporated significantly better than their non-halogenated precursors, tryptophan and tryptamine.[1]

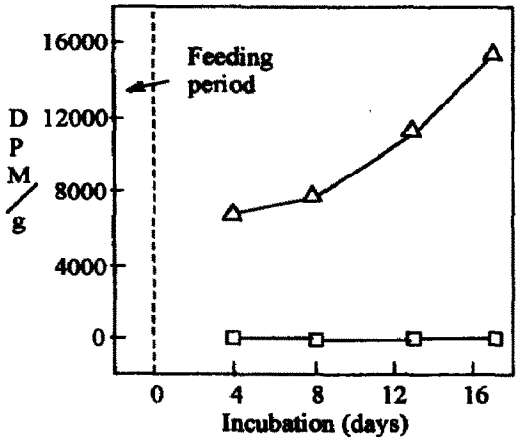


Figure 1. Incorporation (DPM/g dry animal) vs. time in eudistomin H (△) and I (□) from [*side chain* 3-^{14}C]-DL-5-bromotryptophan

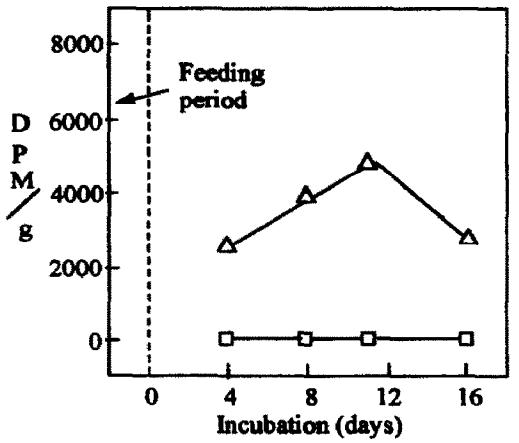


Figure 2. Incorporation (DPM/g of dry animal) vs. time in eudistomin H (△) and I (□) from [*side chain* 1-^{14}C]-5-bromotryptamine

These results substantiate our proposed biosynthetic pathway to the pyrrolinyl-bearing eudistomins in *Eudistoma olivaceum* (Scheme). To summarize the evidence for the proposed scheme, we previously demonstrated[1] that tryptophan and proline are the amino acid precursors to eudistomins H (1) and I (2) and that tryptamine is selectively incorporated into eudistomin I; our current results further support this scheme by demonstrating that bromotryptophan (3) and bromotryptamine (4) are selectively incorporated into eudistomin H. Bromination of tryptophan is clearly the preferential secondary metabolic pathway in our collections of *Eudistoma olivaceum*, based on the efficiency of bromotryptophan utilization and the predominance of eudistomin H in our collections of the tunicate.[1] The preferential utilization of bromotryptophan over bromotryptamine may indicate that decarboxylation and subsequent condensation with proline occur in a multienzyme complex which restricts the efficient utilization of exogenous bromotryptamine. Significantly better incorporation of the brominated amino acid derivatives is to be expected since non-halogenated amino acids have other destinations in both primary and secondary metabolism whereas, once halogenated, the amino acid is committed to secondary metabolism.

Acknowledgments. Support for this work was provided by the National Institutes of Health (AI311256-01) and the National Science Foundation, which provided funds for the purchase of an NMR spectrometer (CHE 9013145). Eli Lilly and Company is gratefully acknowledged for a generous equipment gift, and we thank Florida Tech Professors John H. Trefry, Alan C. Leonard, and Julia Grimwade for use of their equipment.

References:

1. Shen, G. Q.; Baker, B. J. *Tetrahedron Lett.* **1994**, *35*, 1141-1144.
2. Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Mascal, M.; Holt, T. G.; Shield, L. S.; Lafargue, F. *J. Am. Chem. Soc.* **1987**, *109*, 3378- 3387, and references cited therein.
3. Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Hughes, R. G., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1984**, *106*, 1524-1526.
4. Rinehart, K. L.; Kishore, V.; Nagarajan, S.; Lake, R.J.; Gloer, J. B.; Bozich, F. A.; Li, K.-M.; Maleczka, Jr., R. E.; Todsen, W. L.; Munro, M. H. G.; Sullins, D. W.; Sakai, R. *J. Am. Chem. Soc.* **1987**, 109, 6846-6848, and cited references.
5. a) Wright, A. E.; Forleo, D. A.; Gunawardana, G. P.; Gunasekera, S. P.; Koehn, F. E.; McConnell, O. J. *J. Org. Chem.* **1990**, *55*, 4508-4512. b) Rinehart, K. L., Jr.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512-4515.
6. Ireland, C. M.; Copp, B. R.; Foster, M. P.; McDonald, L. A.; Radisky, D. C.; Swersey, J. C. In *Marine Biotechnology*; Attaway, D. H. and Zaborsky, O. R., Eds.; Plenum: New York, 1993; Vol. 1, pp 1-76.
7. Garson, M. J. *Chem. Rev.* **1993**, *93*, 1699-1733.
8. Scott, A. I. *Tetrahedron* **1992**, *48*, 2559-2578.
9. Steffan, B.; Brix, K.; Putz, W. *Tetrahedron* **1993**, *49*, 6223-6228.
10. He, X.; Kustin, K.; Parry, D. L.; Robinson, W. E.; Ruberto, G.; Nakanishi, K. *Experientia* **1992**, *48*, 367-371.
11. Gribble, G. W. *J. Nat. Prod.* **1992**, *55*, 1353-1395.
12. Rinehart, K. L.; Shield, L. S.; Cohen-Parsons, M. In *Marine Biotechnology*; Attaway, D. H. and Zaborsky, O. R., Eds.; Plenum: New York, 1993; Vol. 1, pp. 309-342.
13. Radiolabeled bromotryptophan and bromotryptamine were made by appropriate modifications to Snyder, H. R.; Smith, C. W. *J. Am. Chem. Soc.* **1944**, *66*, 348-351 and Snyder, H. R.; Smith, C. W.; Stewart, J. M. *J. Am. Chem. Soc.* **1944**, *66*, 201-203, respectively. The details will be published elsewhere.
14. Hino, T.; Lai, Z.; Seki, H.; Hara, R.; Kuramochi, T.; Nakagawa, M. *Chem. Pharm. Bull.* **1989**, *37*, 2596-2600.

(*Received in USA* 21 *March* 1994; *revised* 6 *May* 1994; *accepted* 18 *May* 1994)