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Biosynthetic Studies of the Eudistomins in the Tunicate *Eudistoma olivaceum*

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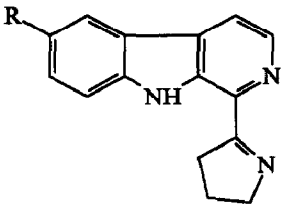
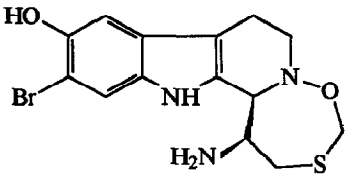
Abstract: The origin of eudistomin I (**2**) in the Floridian tunicate *Eudistoma olivaceum* has been investigated by *in vivo* techniques. Tryptophan and proline are the primary precursors to this antibiotic agent, while trypamine serves as an intermediate.

The tunicate *Eudistoma olivaceum* produces a series of β-carboline derivatives,[1,2] the eudistomins (e.g. **1-3**), which display a variety of pharmacological activities, including potent antiviral (HSV-1, HSV-2, *Vaccinia*)[1c] activity in the oxathiazepine-bearing eudistomins (e.g. **3**), antimicrobial activity in most other *E. olivaceum* eudistomins,[1c] and cytotoxicity in eudistomin K[3] as well as eudistomin analogs known as eudistomidins,[4] the latter from the Pacific *E. glaucus*.



1. Eudistomin H: R = Br
2. Eudistomin I: R = H



3. Eudistomin C

Eudistoma olivaceum is distributed throughout the Caribbean, where it is typically found on mangrove roots. It can be found in Florida both among mangroves and as a member of the fouling community. Many of the eudistomins are produced by the tunicates in exceedingly small quantities[1] leading researchers to undertake synthetic[5] efforts to address supply problems experienced by the biomedical community.

Little biosynthetic investigation has been carried out on tunicate metabolites,[6] which are typically amino acid derived.[7] In one of the few investigations, the tunichromes were shown to derive from phenylalanine.[8] Biosynthesis of β-carboline ring systems, such as that in the eudistomins, is well studied in plants and known to derive from tryptamine via condensation with an aldehyde or α-keto acid.[9] We have undertaken biosynthetic studies of the eudistomins to elucidate the origin of these potential pharmaceuticals and report here our initial efforts.

Eudistoma olivaceum collected on bridge pilings at Ft. Pierce, Florida, can be maintained in aquaria for several months at a time; however, freshly collected animals are best for biosynthetic investigation. Experiments were carried out on small colonies of a dozen or fewer zooids isolated in a separate chamber.

Radiolabeled precursors (Table I) were administered to the tunicates over a one to two day feeding period and metabolism monitored by harvesting several zooids every few days for three weeks. Analysis of the zooids involved treatment with a chloroform solution of cold carrier, reisolation of the carrier, then repeated HPLC purification until constant specific activity was attained. We have investigated both eudistomins H (1) and I (2), since they are the predominant metabolites in our collections of tunicate; eudistomin H (1) is up to ten times more abundant even than eudistomin I (2). Cold carrier (1, 2) was prepared synthetically by the method of Hino[10] and its integrity verified by comparison with the natural product.

Table I. Specific Activity of Eudistomins H (1) and I (2) following *In Vivo* Incorporation of Amino Acids by *Eudistoma olivaceum*

Precursor		Specific Activity (Days After Uptake)				
[*ethyl*-^{3}H]Tryptamine	H:	90 (3)	70 (6)	110 (13)	70 (20)	
	I:	1100 (3)	1500 (6)	2600 (13)	2100 (20)	
L-[3-^{14}C]Tryptophan	H:	430 (0)	560 (3)	1800 (7)	3600 (14)	5000 (21)
	I:	60 (0)	80 (3)	140 (7)	450 (14)	750 (21)
L-[2,3-^{3}H]Proline	H:	160 (2)	1300 (7)	100 (14)	190 (21)	
	I:	190 (2)	3100 (7)	210 (14)	80 (21)	
L-[2,3-^{3}H]Arginine	H:	20 (0)	20 (3)	20 (14)	BG[11](14)	BG (21)
	I:	BG (0)	10 (3)	BG (7)	BG (14)	BG (21)
L-[2,3-^{3}H]Ornithine	H:	50 (0)	130 (3)	250 (7)	60 (14)	20 (21)
	I:	10 (0)	40 (3)	40 (7)	20 (14)	10 (21)

Specific activities (DPM/mg) of product eudistomins (Table I) are a good measure of the success of a precursor; however, since zooids selected for analysis varied in mass between 0.67 g and 2.53 g and added carrier varied between 1.3 mg and 3.1 mg, there is no basis of comparison among precursors. Instead, total recovered counts expressed on a per gram basis of dry zooid (Figure 1 and 2) provide a means of comparison. Proline and tryptophan are clearly the best utilized precursors for the pyrrolinyl-bearing eudistomins, though eudistomins H (1) and I (2) show some differences in specificity (Figure 2).

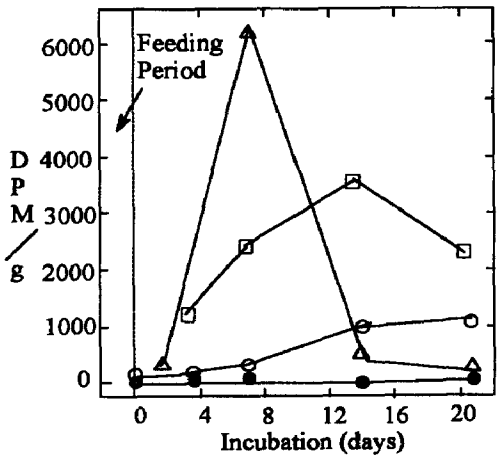


Figure 1. Incorporation (DPM/g zooid) vs. time in eudistomin I (2) for precursors proline (△), tryptophan (○), tryptamine (□), ornithine (●), and arginine (●).

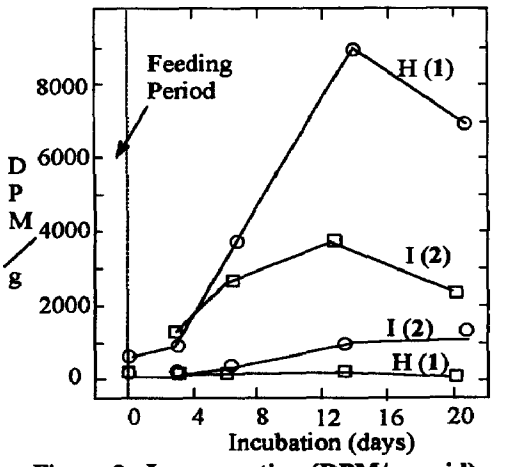


Figure 2. Incorporation (DPM/g zooid) vs. time in eudistomin H (1) and I (2) for precursors tryptophan (○) and tryptamine (□).

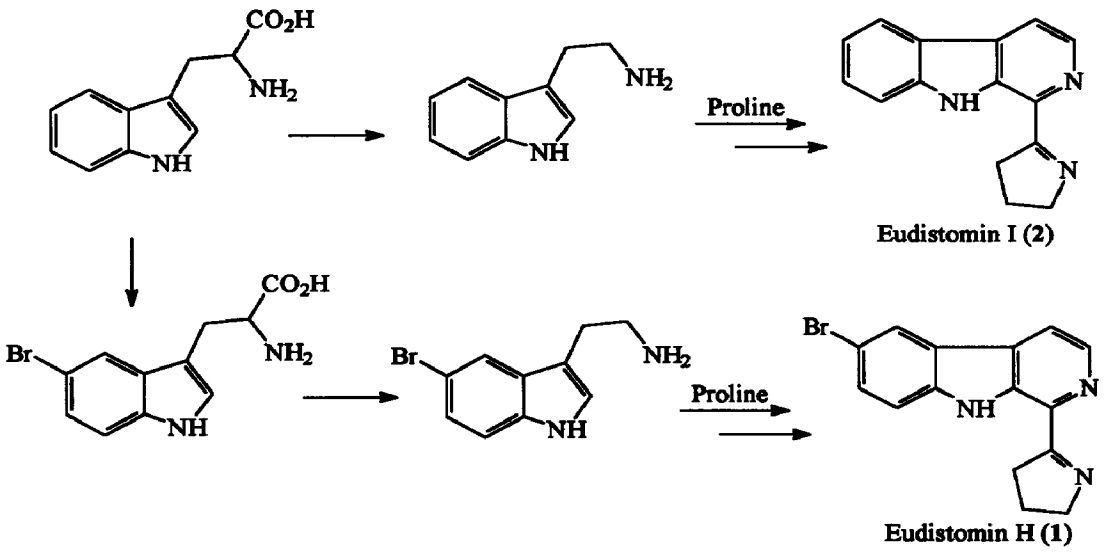
Thus, L-[*side chain* 3-^{14}C]tryptophan and L-[2,3-^{3}H]proline were shown to label both eudistomin H (**1**) and I (**2**) (Table I). Further, tryptophan was demonstrated to be a specific precursor of eudistomin I, using double-label techniques (Table II), while L-[2,3-^{3}H]ornithine and L-[2,3-^{3}H]arginine were not utilized significantly by *E. olivaceum*. Eudistomin I (**2**) is labeled by [*ethyl*-^{3}H]tryptamine, to the exclusion of eudistomin H (**1**).

Table II. Incorporation of L-[5-^{3}H]Tryptophan/L-[*side chain* 3-^{14}C]Tryptophan [^{3}H/^{14}C = 1.0] by *Eudistoma olivaceum* into Eudistomin I (2)

Sample	Incubation (days)	Specific Activity (DPM/mg) ^{3}H	^{14}C	Ratio of Counts (^{3}H/^{14}C)
1	2	819	916	0.9
2	7	947	1077	0.9
3	21	108	120	0.9

These results lead us to postulate the early stages of the biosynthetic route to these pyrrolinyl-bearing eudistomins (Scheme). In the pathway leading to halogenated eudistomins, decarboxylation must follow halogenation of tryptophan, since tryptamine labels only eudistomin I (**2**). Decarboxylation must be the first step of eudistomin I (**2**) biosynthesis since tryptamine would otherwise not be utilized. That tryptamine might show incorporation due to lack of specificity of the enzyme cannot be the case, since eudistomin H (**1**) would also be labeled by tryptamine. Our current efforts to follow up on this postulated pathway are directed at investigating the role of 5-bromotryptophan and its derivatives in an effort to demonstrate their possible role in eudistomin H (**1**) biosynthesis.

Scheme



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11. Specific activity equal to background.

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