PHYSIOLOGICAL STUDIES ON ERGOT. THE INDUCTION OF ERGOT ALKALOID BIOSYNTHESIS BY THE TRYPTOPHAN BIOISOSTERES, β-1- AND β-2-NAPHTHYLALANINE

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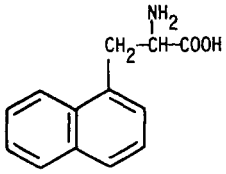
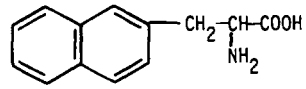
ABSTRACT.—β-Naphthylalanines induced the formation of ergoline alkaloids in submerged cultures of *Claviceps* species, strain SD 58. Comparisons of the induction effect were made between DL-β-1, DL-β-2, L-β-1-naphthylalanine (**1**, **2**) and tryptophan. Of these it was demonstrated that DL-β-2-naphthylalanine produced the greatest effect in stimulating alkaloid biosynthesis, and the L isomer of β-1-naphthylalanine was as effective as the DL-mixture at one-half the concentration.

A replacement in the imino radical of the indole ring of tryptophan by the vinylene group results in the formation of the bioisosteric β-1-naphthylalanine (**1**) also known as α-amino-1-naphthalenepropanoic acid. A related tryptophan bioisostere is the isomeric β-2-naphthylalanine (**2**), α-amino-2-naphthalenepropanoic acid. Bioisosterism is a term coined to designate compounds which possess groups of atoms imparting similar physical, chemical, and biological properties to these compounds due to similarities in size, electronegativity, or stereochemistry (1). In comparing the indole ring system to the naphthalene system, there is



(1)



(2)

an equivalence because both systems contain 10 pi electrons. For this reason one might speculate that the naphthylalanines may have biological activity similar to that of tryptophan. In fact Fornal *et al.* (2) have demonstrated in rats that β-1-naphthylalanine, as did tryptophan, reduced slow-wave sleep latency, increased slow-wave sleep, and produced an attenuation of catecholamine activity in the brain. An analog of luteinizing hormone-releasing hormone (LH–RH) in which β-2-naphthylalanine was substituted for tryptophan was prepared and tested by Prasad *et al.* (3). They found that this analog of LH–RH upon subcutaneous injection into immature male rats caused a release of 1.2 times as much LH and 0.8 times as much follicle stimulating hormone as did synthetic LH–RH.

Work in our laboratory (4) has demonstrated quite convincingly that tryptophan not only serves as a biosynthetic precursor to the ergoline ring system but also, at least in the case of *Claviceps* species, strain SD 58, can induce alkaloid synthesis. Working with tryptophan as well as the tryptophan analogs, 5-methyltryptophan and thiotryptophan, we demonstrated a clear parallel between activity levels of the first pathway-specific enzyme for alkaloid synthesis, namely dimethylallyltryptophan synthetase, and alkaloid production leading us to believe that the induction effect involves *de novo* synthesis of this enzyme.

It was of interest to determine the effect of the naphthylalanines on alkaloid biosynthesis in the ergot fungus since the fungus and the induction phenomenon provide a system for testing the biological equivalency of these compounds with tryptophan. We report the results of work which demonstrates that the naphthylalanines are effective inducers of alkaloid biosynthesis in *Claviceps* species, strain SD 58.

MATERIALS AND METHODS

ORGANISM AND CULTURING PROCEDURES.—The strain of ergot used for this study was *Claviceps* species, strain SD 58 (ATCC 26019). It was cultivated by a method which has been previously described (4).

QUANTITATION OF ALKALOIDS.—The amount of alkaloid was determined by a previously described method (5) in micrograms per milligram of mycelial dry weight.

INDUCTION OF ALKALOID PRODUCTION.—Two sets of experiments were performed: one in which 100 ml of culture medium was used per 500-ml culture flask and the other where 30 ml of medium was placed in 125-ml flasks. Two-milliliter volumes of resuspended mycelium were used to inoculate experimental flasks when they contained 100 ml of medium; whereas, For flasks containing 30 ml of medium, 0.5 ml of inoculum was used. In all cases the medium used was NL-406 medium with the yeast extract omitted. This medium was employed in the control cultures, and a 4 mM concentration of the various potential inducers was added to the experimental cultures. The compounds which were tested were DL-tryptophan, DL-5-methyltryptophan, DL-β-1-, and DL-β-2-naphthylalanines as well as L-β-1-naphthylalanine, which was used in a 2 mM concentration. Multiple flasks were prepared and tested for each data point.

RESULTS AND DISCUSSION

As seen from fig. 1, DL-β-1-naphthylalanine is an effective inducer of alkaloid biosynthesis in *Claviceps* species, strain SD 58. In fact it shows, on the average,

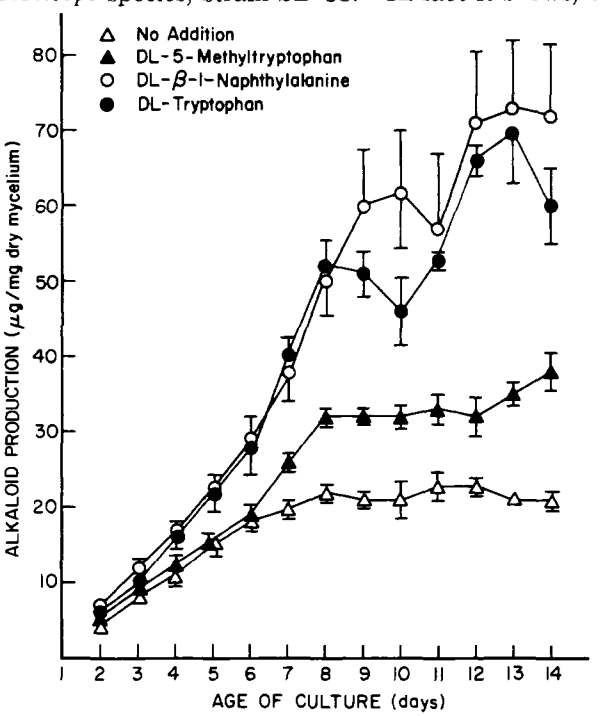


FIG. 1. Time course of alkaloid production in cultures of *Claviceps* species, strain SD-58, showing the induction effect of tryptophan and tryptophan bioiososteres on alkaloid production. The organism was cultivated in NL-406 medium minus yeast extract plus a 4 mM concentration of the various compounds to be tested. Each point plotted represents the average value obtained from four separate cultures with the standard error indicated by vertical bars. Standard errors are within the symbols, if not shown.

induction activity in the same order of magnitude as tryptophan with a 3.5 fold increase in alkaloid production over the control at day 13 of the fermentation as compared to a 3.3 fold increase for DL-tryptophan. At the same time, naphthylalanine is twice as effective as 5-methyltryptophan in causing induction. A somewhat similar result has been obtained in previous work (4) when the induction effect of thiotryptophan was compared to that of tryptophan and 5-methyltryptophan. In that prior study it was determined from measuring the activity of certain key enzymes of the tryptophan biosynthetic pathway that thiotryptophan was ineffective as a substitute for tryptophan in the end-product regulation of tryptophan biosynthesis; whereas, 5-methyltryptophan approached the ability of tryptophan to control tryptophan biosynthesis. As a consequence, even though thiotryptophan and 5-methyltryptophan both stimulated the production of alkaloid synthesizing enzyme, in this case dimethylallyltryptophan (DMAT) synthetase, there was a decreased amount of endogenously synthesized tryptophan available for alkaloid biosynthesis in the cultures containing 5-methyltryptophan resulting in a lower alkaloid titer in those cultures. In the work reported here, the results obtained with β-1-naphthylalanine strongly suggest a parallel situation with that of thiotryptophan. Namely, β-1-naphthylalanine probably stimulates the production of alkaloid synthesizing enzyme(s), most likely DMAT synthetase, but it may be an inefficient substitute for tryptophan in the end-product regulation of tryptophan biosynthesis. Both thiotryptophan and the naphthylalanines possess ring systems with 10 pi electrons, as does tryptophan. Consequently, one might speculate that this isoelectronic feature is required in order for these compounds to elicit the induction effect. Apparently, different and/or additional structural features are required before these bioisosteres will exhibit end-product inhibition of tryptophan biosynthesis.

Additional information on structural features of the naphthylalanines required to elicit the induction effect was obtained by comparing the magnitude of the induction effect obtained from various naphthylalanine isomers with that obtained with tryptophan. Table 1 illustrates that DL-β-2-naphthylalanine produces,

TABLE 1. A comparison to tryptophan of the induction effect produced by naphthylalanines on alkaloid production in submerged cultures of *Claviceps* species, strain SD 58.

Group	Age of culture in days											
	2	3	4	5	6	7	8	9	10	11	12	13
4 mM DL-Tryptophan.....	21±5[a]	25±5	35±6	57±9	66±10	61±8	61±3	64±3	65±4	64±2	75±10	92±17
4 mM DL-β-1-Naphthylalanine..	3±0.4	9±2	21±3	34±2	43± 1	49±1	57±3	63±5	67±7	68±8	71±11	82±11
2 mM L-β-1-Naphthylalanine..	3±0.7[b]	9±6	24±3	42±4	54± 3	56±3	57±4	66	72±7	72±3	83±2	99±6
4 mM DL-β-2-Naphthylalanine..	3±0.6	6±3	25±1	48±3	56± 3	56±2	71±5	89±9	89±4	87±0.6	109± 7	134±12

[a]Average values (±S.E.) of alkaloid production in μg/mg mycelial dry weight for 3 separate cultures.
[b]Average values (±S.E.) for 2 separate cultures.

on the average, an approximately 45% greater stimulation of alkaloid production at day 13 of the fermentation than does DL-tryptophan; whereas, the induction produced by DL-β-1-naphthylalanine is slightly less than that produced by tryptophan in this set of experiments.

The alkaloid titers in the tryptophan-containing cultures shown in table 1 are somewhat inflated early in the fermentation period because tryptophan which has not yet been taken up by the mycelium contributes to the colorimetric reaction

used to quantitate the alkaloids. The β-naphthylalanines do not react with this color reagent.

With regard to the enantiomers of β-1-naphthylalanine, a 2 mM concentration of L-β-1-naphthylalanine stimulates alkaloid production to the same degree, in fact slightly higher, than does a 4 mM concentration of the DL mixture. These results indicate that the L isomer causes the induction of alkaloid biosynthesis, while the D isomer may have a slightly inhibitory effect on alkaloid formation. Unfortunately, D-β-1-naphthylalanine was not available for testing.

ACKNOWLEDGMENTS

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