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Influence of tryptophan and related compounds on ergot alkaloid formation in *Claviceps purpurea* (Fr.) Tul.

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L-Tryptophan did not exert any influence on peptide alkaloid formation in an ergotamine and in an ergosine-accumulating *C. purpurea* strain. A different picture was observed in a series of related *C. purpurea* strains. Tryptophan showed a slight stimulatory effect on the ergotoxine producer Pepty 695/S. A blocked mutant of it, designated as Pepty 695/ch which was able to accumulate secoclavines gave similar results. In a high-yielding elymoclavine strain Pepty 695/e, the progeny of the former one, tryptophan up to a concentration of 25 mM stimulated remarkably clavine biosynthesis. Furthermore, tryptophan could overcome the block of synthesis by inorganic phosphate. Increased specific activities of chanoclavine cyclase but not DMAT synthetase were observed in cultures of strain Pepty 695/e supplemented with tryptophan. 5-Methyltryptophan and bioisosteres of tryptophan were ineffective in alkaloid stimulation. These results are compared with those obtained with the grass ergot strain SD 58 and discussed with the relation to other induction phenomena.

Ergot alkaloids are mainly found in various species of the parasitic fungus *Claviceps* (Ascomycetes). These secondary metabolites are also synthesized under saprophytic conditions (GRÖGER 1972, ARCAMONE 1977).

Tryptophan and an isoprenoid unit serve as essential building blocks for the ergoline nucleus (FLOSS 1976). Addition of D,L-tryptophan at the beginning of the fermentation triggered a dramatic stimulation of lysergic acid derivatives production in *Claviceps paspali* (ARCAMONE *et al.* 1961). Unfortunately, it has not yet been clarified if this phenomenon is merely a precursor effect or not. In the case of clavine alkaloids-forming grass-ergot strains it was well established during the last 20 years that tryptophan has a dual role in ergoline biosynthesis: it serves as precursor but also acts as inducer of alkaloid formation. The inducer function of tryptophan were first corroborated by FLOSS and MOTHES (1964) and later also proposed by others (BU'LOCK and BARR 1968, VINING 1970). Most convincing results were obtained by H. G. FLOSS' group (For review: ROBBERS and FLOSS 1976, FLOSS *et al.* 1979).

Tryptophan and its analogues 5-methyltryptophan and thiotryptophan β -(1-benzothien-3-yl)-alanine at concentrations of 4 mM induce alkaloid synthesis in strain SD 58 — *Claviceps fusiformis*. The latter compound which is not effective as feedback regulator of tryptophan biosynthesis proved to be in many cases superior to tryptophan as inducer (KRUPINSKI *et al.* 1976). High concentrations of inorganic phosphate (ten times the normal level) inhibited alkaloid synthesis in *Claviceps* strain SD 58 practically completely (ROBBERS *et al.* 1972). Tryptophan and thiotryptophan but not 5-methyltryptophan can overcome the block of alkaloid formation by inorganic phosphate (KRUPINSKI *et al.* 1976). Some evidence was obtained that in "high-phosphate" media alkaloid degradative enzymes may be operative (ROBBERS *et al.* 1978).

Apparently the induction of alkaloid biosynthesis by tryptophan and its analogues is mainly due to enhanced levels of DMAT-synthetase in supplemented cultures compared with noninduced controls. Induction involves *de novo* synthesis of the first ergoline-pathway specific enzyme (KRUPINSKI *et al.* 1976, FLOSS *et al.* 1979). Recently the

induction of ergoline biosynthesis by so-called tryptophan bioisosteres, naphthylalaminines, were described (ROBBERS *et al.* 1982).

In this paper we present observations on the influence of tryptophan and related compounds on alkaloid formation in various strains of *Claviceps purpurea*. The mutants used are the progeny of an ergotoxine-accumulating *Claviceps purpurea* strain. Also some other peptide-type alkaloids producing strains were investigated.

Materials and methods

Organisms: The work was carried out with submerged cultivated mycelia of various strains of *Claviceps purpurea* (Fr.) Tul. The fungi are deposited in the culture collection of the Institute of Plant Biochemistry, Academy of Sciences of the GDR, Halle (S.): Strain Pepty 695/S, producing up to 1 mg/ml alkaloids, mainly ergotoxines (50–60%). Strain Pepty 695/ch: A blocked mutant from the ergotoxine strain. Pepty 695/ch, accumulating the secoergolines chanoclavine-I and chanoclavine-I-aldehyde in the proportion (3:1), on the average 300–350 µg/ml. Strain Pepty 695/c: A mutant derived from Pepty 695/ch accumulating clavine alkaloids, mainly elymoclavine (90%). Total alkaloid in general 1500–1800 µg/ml. Strain JAP 471 produces a mixture of ergotamine (70%) and clavines (30%) up to the range of 800 µg/ml. Strain MUT 170 accumulates preferentially ergosine besides clavine alkaloids up to a range of 700 µg/ml.

Media and cultivation: The stock cultures were maintained on asparagine/sucrose agar slants or petri dishes.

Fermentation media: NL 720 (g/l): Sucrose 200; ammonium citrate 15; KH_2PO_4 0.25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.03; pH adjusted to 5.2–5.4 with NH_4OH .

NL 833 (g/l): Sucrose 300; ammonium citrate 20; $\text{Ca}(\text{NO}_3)_2$ 1.0; KH_2PO_4 0.25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.03; pH adjusted to 5.2–5.4.

NL 614 (g/l): Mannitol 50; sucrose 50; succinic acid 5.4; yeast extract (DIFCO) 3.0; KH_2PO_4 0.1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.004; pH adjusted with ammonium hydroxide solution to 5.2–5.4.

The strains were grown in submerged cultures at 24 °C at 240 rpm on a VEB FANAL rotary shaker. For preparing the inoculum mycelium of 4 weeks old agar slants were used. The 7 day old preculture mycelium was transferred either into 500 ml flat bottom flasks containing 100 ml broth or 100 ml ERLÉNMEYER flasks containing 25 ml medium. The inoculation rate amounted to 1:10. Alkaloid production was achieved with strains Pepty 695/S, Pepty 695/ch, Pepty 695/e in medium NL 720 and using strains JAP 471 and MUT 170 with medium NL 833. Each experiment was performed in duplicate.

Analytical methods: For dry-weight determination the mycelium was removed from the culture filtrate by vacuum filtration intensively washed with water and finally dried in an oven at 80 °C for 24h.

For total alkaloid determination 1 ml culture filtrate was made alkaline by adding 0.2 ml of 10% ammonia solution and was extracted with an appropriate amount of CHCl_3 . This was repeated twice and the combined extracts were evaporated to dryness. The residue was taken up with 2% tartaric acid solution and subsequently VAN URK's reagent was added in the ratio 1:2. The optical density was determined at 580 nm. The quantity of alkaloids were calculated according to a standard curve using elymoclavine for clavine-producing strains or the appropriate peptide alkaloids for ergopeptine producers.

The quantitative determinations of the various compounds of the alkaloid mixture of strain Pepty 695/e were done after TLC separation according to MAIER *et al.* (1980). The alkaloids were chromatographed on silica PF₂₅₄ "MERCK" plates using as solvent system chloroform:methanol (8:2).

Protein was determined according to BRADFORD (1976).

Chanoclavine-I-cyclase (ERGE *et al.* 1973) was assayed in the following manner: Mycelia grown for 4 days in NL 720, if not otherwise stated, were recovered from the fermentation broth by filtration, washed with water and immediately lyophilized. Lyophilized mycelium was ruptured by grinding with dry ice in a mortar and suspended in 0.1 M Tris-HCl buffer (pH 7.8) containing 10% glycerol, 2 mM EDTA and 2 mM mercaptoethanol. The suspension was centrifuged at 20 000 xg for 30 min. The supernatant was used as crude enzyme extract. The reaction mixture in a total volume of 2 ml contained: 0.250 mg chanoclavine-I; 1.25 µmol NADPH; 10 µmol ATP; 20 µmol Mg^{2+} ; 200 µl 1 M Tris-HCl buffer (pH 7.8) and 0.5 ml enzyme solution. The mixture was incubated for 3 h at 32 °C. The extraction and purification of the reaction product (agroclavine) and the substrate (chanoclavine-I) was essentially done as described (ERGE *et al.* 1973). Determination of di-

methylallyltryptophan (DMAT) synthetase was performed according to MAIER and GRÖGER (1976). Lyophilized mycelia were used to prepare the crude enzyme extract. Mevalonic acid-2-¹⁴C served as substrate.

Results

Recently we obtained two mutants of *Claviceps purpurea* which showed a metabolic block in different positions of the alkaloid pathway (MAIER *et al.* 1980, SCHUMANN *et al.* 1982). The parent strain Pepty 695/S accumulates as main products ergotamine alkaloids. The strain Pepty 695/ch derived from it was not able to form tetracyclic clavines. Both strains do not differ in morphology and pigmentation. The second mutant Pepty 695/e which was obtained among others from Pepty 695/ch accumulates as endproducts clavines predominantly elymoclavine (Fig. 1). Surprisingly the

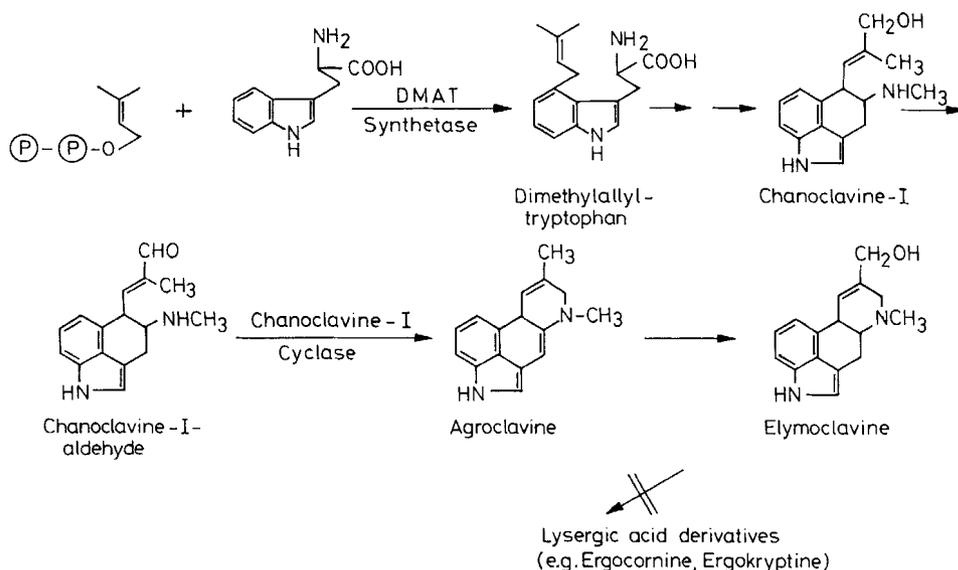


Fig. 1.

Biosynthetic pathway of ergoline formation. Bars indicate position of block in strain Pepty 695/e

total alkaloid yield was about 5 times higher in Pepty 695/e compared to its progenitor. It seemed now worthwhile to investigate the influence of tryptophan on alkaloid formation in *C. purpurea* strains in order to check if there are any induction processes like those observed with strains derived from *Pennisetum ergot*. We used mainly strain Pepty 695/e which closely resembles the *Pennisetum* strains (e.g. SD 58) regarding the alkaloid pattern. This strain was cultivated in a chemically defined medium NL 720 in the presence of increasing amounts of L-tryptophan (Table 1). Up to a concentration of 25 mM L-Try we observed a stimulation of alkaloid synthesis. The maximum total alkaloid yield was reached after 14 days of cultivation. It is interesting to note that the amount of elymoclavine increased steadily of the incubation time was prolonged after day 14 up to 21 days. This means the agroclavine hydroxylase is also still operative in older mycelium. In the next set of experiments we compared the alkaloid production of strain Pepty 695/e in two different media: medium NL 720, which is generally used for ergopeptide formation and medium NL 614, containing

Table 1

Stimulatory effect produced by varying amounts of L-tryptophan on alkaloid production in shake cultures of strain Pepty 695/e, 100 ml NL 720 per flask

Addition of L-Try	Alkaloid yield $\mu\text{g/ml}$ after			Composition of alkaloid fraction % after								
	14 d	16 d	21 d	14 d			16 d			21 d		
				Ch	A	E	Ch	A	E	Ch	A	E
control	1490	1410	1410	14	9	77	14	—	86	15	—	85
2.5 mM	1720	1650	1660	13	13	74	12	6	82	13	—	87
5.0 mM	1805	1780	1780	11	21	68	12	12	76	11	—	89
12.5 mM	1960	1950	1990	11	27	62	11	18	71	10	—	90
25 mM	2330	2230	2390	10	36	54	10	27	63	9	11	80

Abbreviations: Ch = chanoclavine-I; A = agroclavine; E = elymoclavine

mannitol/sucrose/succinate and yeast extract. The latter one is widely employed for clavine alkaloid production with grass ergot strains (ROBBERS *et al.* 1972, 1978). A stimulatory effect of L-tryptophan was observed in both media. Furthermore, the tryptophan analogue DL-5-methyltryptophan caused an inhibition of alkaloid formation, roughly 1/3 of the alkaloid yield was obtained compared with the unsupplemented control flasks. This is in contrast to previously reported results with the *Pennisetum* ergot strain SD 58, indicating an induction of alkaloid synthesis by 5-methyltryptophan (ROBBERS and FLOSS 1970, KRUPINSKI *et al.* 1976). The effect on alkaloid production of a one-time addition of 25 mM tryptophan at different times during the course of fermentation is shown in Fig. 2. Four days after supplementation the cultures were harvested. It is seen that the stimulatory effect of L-tryptophan is most pronounced in young mycelium up to the age of 3 days.

High levels of inorganic phosphate (1.1 g/l) suppress alkaloid formation in *C. fusiformis* strain SD 58 practically completely. Addition of tryptophan could partially overcome the phosphate inhibition of alkaloid biosynthesis (ROBBERS *et al.* 1972,

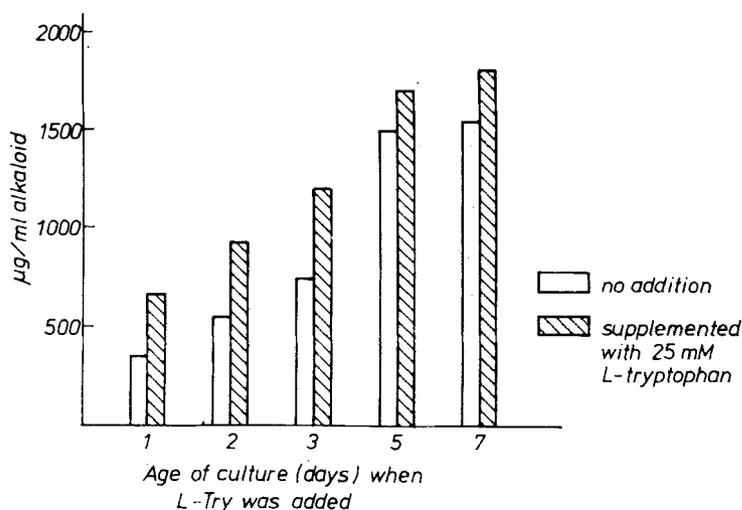


Fig. 2.

Influence of L-tryptophan on alkaloid formation in strain Pepty 695/e added at various stages of fermentation. Cultures were harvested four days after supplementation

Table 2
Influence of potential inducers on alkaloid production of *C. purpurea* strain Pepty 695/e cultivated in different media

Age of culture	Alkaloid yield			Dry weight			Composition of alkaloid fraction %									
	$\mu\text{g/ml}$			mg/ml			Chanoel.			Agrocl.			Elymoel.			
	a ¹⁾	b	c	a	b	c	a	b	c	a	b	c	a	b	c	
				NL 614												
3	140	190	50	10	10	9	5	5	10	10	10	5	85	85	85	
7	1150	1360	140	12	12	12	11	7	5	36	41	10	53	52	85	
14	1425	2470	380	19	16	18	8	8	—	20	33	22	72	59	78	
16	1425	2400	360	17	14	16	10	7	5	13	29	15	77	64	85	
21	1392	2450	450	20	16	16	8	7	—	10	18	15	82	75	85	
				NL 720												
3	220	420	90	7	5	8	Sp	10	20	35	36	20	65	54	60	
7	850	1590	331	11	10	13	14	6	21	31	65	25	55	35	54	
14	1540	2560	625	14	12	14	7	8	7	12	46	8	81	46	85	
16	1505	2450	655	12	12	13	7	7	Sp	7	39	20	86	54	80	
21	1540	2475	640	13	13	13	11	6	Sp	Sp	16	Sp	89	78	100	

¹⁾ a = 100 ml medium/flask; b = 100 ml medium plus 25 mM L-tryptophan; c = 100 ml medium plus 25 mM D,L-5-Me-tryptophan

Table 3

Effect of excess inorganic phosphate and L-tryptophan addition in medium NL 720 on alkaloid production in *Claviceps* strain Pepty 695/e. Harvested after 14 days of cultivation

Medium	Addition	Alkaloid production µg/ml	Composition of alkaloid fraction %		
			Chanoel.	Agrocl.	Elymoel.
100 ml NL 720	—	1650	10	18	82
100 ml NL 720	1 g KH ₂ PO ₄ /l	1000	5	5	90
100 ml NL 720	1 g KH ₂ PO ₄ /l 5 mM L-Try	1200	n. d.	n. d.	n. d.
100 ml NL 720	1 g KH ₂ PO ₄ /l 25 mM L-Try	2800	7	13	80
100 ml NL 720	1 g KH ₂ PO ₄ — 50 mM L-Try	3100	6	24	70

Table 4

Effect of excess inorganic phosphate and L-tryptophan addition on alkaloid production in *Claviceps* strain Pepty 695/ch

Medium	Addition	Alkaloid yield µg/ml	dry weight mg/ml
NL 720	control	350	20
NL 720	2.5 mM L-Try	415	
NL 720	5.0 mM L-Try	480	
NL 720	12.5 mM L-Try	450	
NL 720	25.0 mM L-Try	410	
NL 720	50.0 mM L-Try	440	
NL 720	1 g KH ₂ PO ₄ /l	50	20
NL 720	1 g KH ₂ PO ₄ /l plus 5 mM L-Try	70	24
NL 720	1 g KH ₂ PO ₄ /l plus 25 mM L-Try	80	21

Table 5

Effect of different compounds on alkaloid formation in submerged culture of *Claviceps* strain Pepty 695/e; 25 ml NL 720; Harvested after 14 days of cultivation

Group	Alkaloid yield	Composition of alkaloid fraction %		
		Chanoel.	Agrocl.	Elymoel.
No addition	2540	8	9	83
12.5 mM L-Tryptophan	3000	7	19	74
25 mM L-Tryptophan	3100	6	23	72
4 mM 5-Me-Tryptophan	400	5	5	90
12.5 mM 5-Me-Tryptophan	460	5	5	90
4 mM D,L-β-1-Naphthylalanine	2500	8	16	76
12.5 mM D,L-β-1-Naphthylalanine	2550	8	16	76
4 mM D,L-β-1-Naphthylalanine	2700	8	14	78
12.5 mM D,L-β-1-Naphthylalanine	2600	7	14	79

KRUPINSKI *et al.* 1976). Excess inorganic phosphate in medium NL 720 hampered alkaloid formation also in our elymoclavine-producing *C. purpurea* strain Pepty 695/e but caused a far less pronounced inhibition amounting to 40%. An addition of 5 mM L-tryptophan did only slightly influence the phosphate inhibition. However, a high dose of L-Try (25 mM) could completely restore the full capacity of alkaloid formation

comparable with that observed in NL 720 medium containing the "normal" level of phosphate (Table 3).

The progenitor of strain Pepty 695/e is strain Pepty 695/ch accumulating about 350–450 $\mu\text{g/ml}$ secoclavines. Tryptophan applied in a wide range of concentrations stimulated also here alkaloid formation but was much less effective than in strain Pepty 695/e. High levels of phosphate could also block in strain Pepty 695/ch alkaloid biosynthesis. In contrast to other strains tryptophan was not able to overcome inhibition of alkaloid formation (Table 4).

ROBBERS *et al.* (1982) found that naphthylalanines are effective inducers of alkaloid biosynthesis in strain SD 58. These compounds may be regarded as bioisosteres of tryptophan. D,L- β -2-naphthylalanine produced the greatest effect. With regard to the enantiomeres of β -1-naphthylalanine a 2 mM concentration of L- β -1-naphthylalanine stimulated alkaloid production to the same degree than does a 4 mM concentration of the D,L-mixture.

In various sets of experiments we compared the effect of naphthylalanines on elymoclavine formation in strain Pepty 695/e (Tab. 5 and 6). Unsupplemented cultures gave in these experiments total alkaloid yields above the average values. Nevertheless, the stimulatory effect of tryptophan and the inhibition of alkaloid biosynthesis caused by 5-methyltryptophan could be clearly demonstrated. But there was no indication that naphthylalanines even in rather high concentrations did influence growth of mycelium or alkaloid production.

Table 6

Influence of various bioisosteres of tryptophan on alkaloid formation in submerged culture of *Claviceps* strain Pepty 695/e; 100 ml NL 720; Harvested after 14 d

Group	Alkaloid yield $\mu\text{g/ml}$	Dry weight mg/ml
No addition	1900	11
10 mM L-Try	2600	12
10 mM D,L- β -1-Naphthylalanine*)	2000	12
10 mM D,L- β -2-Naphthylalanine*)	1950	12
10 mM L- β -1-Naphthylalanine*)	2100	10
10 mM L- β -2-Naphthylalanine*)	2000	12

*) not entirely solved at the beginning of the fermentation

Table 7

Influence of varying amounts of L-tryptophan on alkaloid production in shake cultures of different peptide alkaloid-producing strains. Cultivation time 14 d

Addition of L-Try	Strain: JAP 471 Alkaloid yield $\mu\text{g/ml}$	Strain: MUT 170 Alkaloid yield $\mu\text{g/ml}$	Strain: Pepty 695/S Alkaloid yield $\mu\text{g/ml}$
control	840	650	1000
5 mM	800	700	1200
12.5 mM	800	750	1390
25.0 mM	750	760	1230
50.0 mM	720	720	1400

Table 7 shows that tryptophan employed in increasing concentrations from 5 to 50 mM did not stimulate alkaloid formation in the ergotamine-strain JAP 471 and the ergosine-strain MUT 170. Somewhat different results were obtained with the ergotamine producer Pepty 695/S which is the progenitor of both clavine alkaloids-accumu-

lating strains Pepty 695/ch and Pepty 695/e. An increase of alkaloid production in tryptophan-supplemented cultures was detectable about in the same order of magnitude as observed with the secoclavines-producing strain Pepty 695/ch. High levels of inorganic phosphate diminished remarkably alkaloid formation in Pepty strain 695/S which could only be partially overcome by a high dose of L-tryptophan (Table 8).

The first pathway-specific enzyme in ergoline biosynthesis is the dimethylallyl-tryptophan (DMAT) synthetase. Evidence has been presented that in *Claviceps* strain SD 58 the induction effect caused by tryptophan and some analogues of it is related to higher levels of DMAT synthetase in those cultures compared with the non-induced controls. Apparently induction involves *de novo* synthesis of this key enzyme (KRUPINSKI *et al.* 1976, FLOSS *et al.* 1979).

Table 8

Effect of high levels of inorganic phosphate and L-tryptophan on alkaloid production in *C. purpurea* strain Pepty 695/S

Medium	Addition	Alkaloid yield $\mu\text{g/ml}$	Dry weight mg/ml
NL 720	control	1290	38
NL 720	1 g $\text{KH}_2\text{PO}_4/\text{l}$	240	32
NL 720	1 g $\text{KH}_2\text{PO}_4/\text{l}$ plus 25 mM L-Try	520	29
NL 720	2 g $\text{KH}_2\text{PO}_4/\text{l}$	200	26
NL 720	2 g $\text{KH}_2\text{PO}_4/\text{l}$ plus 25 mM L-Try	380	24

Table 9

Alkaloid formation in strain Pepty 695/e and activity of some enzymes. Cultivation period 4 d

Condition	Alkaloid yield $\mu\text{g/ml}$	DMAT-Synthetase dpm in DMAT*)	Chanoclavin-I cyclase**) agroclavine %
No addition	475	2.2×10^5	20
25 mM L-Tryptophan	675	2.0×10^5	30

*) Assay mixture of dimethylallyltryptophan (DMAT) synthetase reaction contained mevalonic acid: 2.2×10^6 dpm

**) Assay mixture contained 500 μg chanoclavin-I

We observed in strain Pepty 695/e no differences in DMAT synthetase activity in tryptophan supplemented cultures and in non-supplemented controls (Table 9). Also a high phosphate level in the medium did not exert a negative influence on DMAT synthetase activity (data not shown). Another key enzyme in ergoline formation is the chanoclavin-I-cyclase catalyzing the conversion of secoclavines to agroclavine. This particular enzyme is lacking in strain Pepty 695/ch but present in the mutant strain Pepty 695/e which is derived from the former one and is able to accumulate tetracyclic clavines. As shown in Fig. 3 the enhancement of alkaloid formation by tryptophan in both normal and high-phosphate (2 g/l) cultures of strain Pepty 695/e is inter alia apparently related to increased levels of chanoclavin-I-cyclase activity. The same phenomenon has been earlier observed in strain SD 58 (ERGE *et al.* 1973). The same levels of chanoclavin cyclase activity were detectable in with tryptophan-supplemented and non-supplemented cultures of peptide alkaloid strain Pepty 695/S

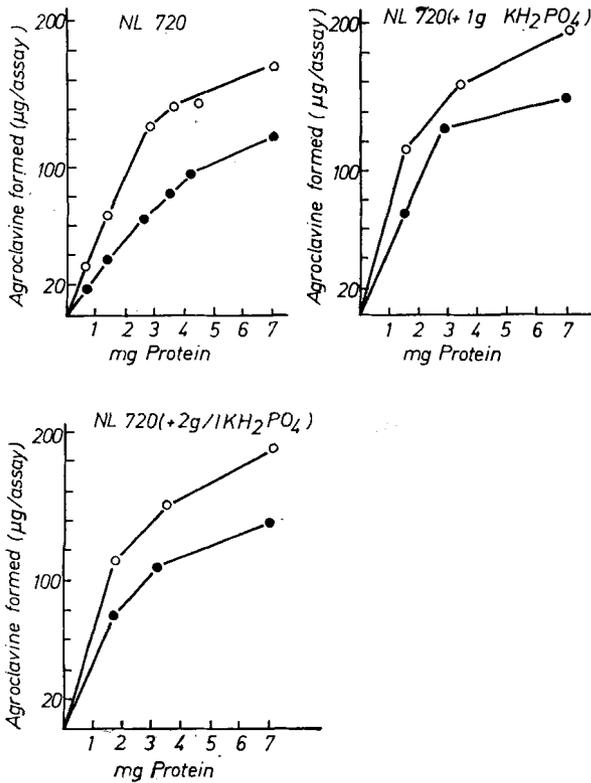


Fig. 3. Chanoclavine-I cyclase activity at the 4th day of cultivation of mycelia of strain Pepty 695/e grown in medium NL 720 and in high-phosphate medium. Dependence of the agroclavine formation on protein concentration. The assay mixture contained 0.250 mg chanoclavine-I
 ● No addition; ○ 25 mM L-Tryptophan

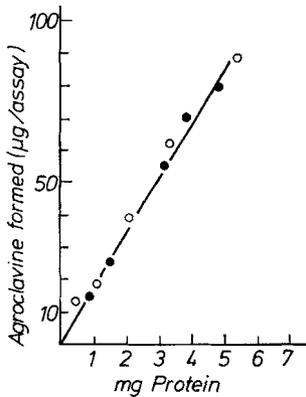


Fig. 4. Chanoclavine-I-cyclase activity at the 4th day of cultivation. Mycelia of strain Pepty 695/S in NL 720. Dependence of the agroclavine formation on the protein concentration.
 ● No addition; ○ 25 mM L-Tryptophan

which shows a far less pronounced stimulatory effect on alkaloid formation by tryptophan (Fig. 4).

Discussion

There are only few examples regarding the regulatory role of primary metabolites as inducers of secondary metabolite formation. In *Streptomyces fradiae* fosfomycin biosynthesis is stimulated by methionine (ROGERS and BIRNBAUM 1974). More data are available on the methionine stimulation of cephalosporin C biosynthesis in *Cephalosporium acremonium* (DREW and DEMAIN 1973 and 1977). The methionine effect is not a function of its ability to donate sulfur for antibiotic formation nor did methionine repress or inhibit the metabolism of cysteine, a known precursor to the antibiotic. Methionine gave greatest stimulation when added during the trophophase. Furthermore, methionine supplementation caused drastical changes in the morphology of the producer organism. Interestingly also a methionine analog, norleucine, induced both a stimulation of cephalosporin C formation and fragmentation of mycelia into arthrospores. Recently it was shown that both DL-methionine and D,L-norleucine in a concentration of 3.1 g/l in the medium increased in cells of *Cephalosporium acremonium* the level of antibiotic synthetases (SAWADA *et al.* 1980). There are quite striking similarities in the inductive effect of methionine on Cephalosporin C formation and the induction of alkaloid biosynthesis in *Claviceps* species, strain SD 58 caused by tryptophan.

As it was demonstrated in this study tryptophan acts on alkaloid formation in various *Claviceps* strains in a different manner. No influence on alkaloid production was seen in an ergotamine and an ergosine accumulating strain. It is of special interest to compare the tryptophan effect on alkaloid formation in the series of related *C. purpurea* strains designated Pepty. A slight stimulation of ergotoxine production (Table 7) by high doses of tryptophan was observed with strain Pepty 695/S. A blocked mutant, Pepty 695/ch, which is the progeny of *C. purpurea* strain Pepty 695/S gave similar results. High phosphate levels in the medium impaired considerably alkaloid biosynthesis. In this case tryptophan could not overcome the block of alkaloid formation. In contrast tryptophan in increasing concentrations up to 25 mM stimulates remarkably alkaloid formation in the high-yielding elymoclavine strain Pepty 695/e (Tables 1 and 2). High levels of inorganic phosphate inhibit alkaloid formation much less as in strain Pepty 695/ch and this inhibitory effect could be easily overcome by L-tryptophan (Table 3). It is still an open question why such high concentrations of L-tryptophan for optimal stimulation are necessary. A similar situation is found in the directed biosynthesis of ergopeptines (BEACCO *et al.* 1978) where amino acids or their analogues are administered in the range of 4–5 g/l. Obviously the relative concentrations of amino acids in the internal pool partially control the biosynthesis of the peptide moiety. The tryptophan analogue 5-methyltryptophan diminishes alkaloid production and the tryptophan bioisosteres β -1- and β -2-naphthylalanine had no effect on the alkaloid yield. There was no enhancement of DMAT synthetase activity in tryptophan supplemented cultures in *Claviceps* strain Pepty 695/e compared with the unsupplemented ones. But higher activities of chanoclavine synthetase, a key enzyme in the formation of tetracyclic ergolines, were found both in normal and high phosphate cultures in the presence of tryptophan. *C. fusiformis* strain SD 58 and *C. purpurea* strain Pepty 695/e accumulate the same alkaloidal endproduct elymoclavine, but show remarkable differences regarding the stimulatory effect of tryptophan and its analogues. In the grass ergot strain it is well documented that these particular compounds may act as true inducers of alkaloid formation involving the *de novo* synthesis of DMAT synthetase. Such a clear-cut evidence is lacking in strain Pepty 695/e. The results of this study tends to argue that the stimulatory effect of tryptophan is a rather complex process and it seems that L-Try acts here mainly as precursor. On the

other hand it should not be overlooked that tryptophan stimulates clearly chanoclavine synthetase activity and is also able to reverse the inhibition of alkaloid synthesis caused by high levels of inorganic phosphate.

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