Phytochemistry, 1965, Vol. 4, pp. 341 to 343. Pergamon Press Ltd. Printed in England

INFLUENCE OF AUXINS ON THE PRODUCTION OF ERGOT ALKALOIDS IN SAPROPHYTIC CULTURES

E. TEUSCHER

Laboratory of Pharmakognosy, Department of Botany, University of Greifswald, Greifswald, GDR

(*Received* 10 *August* 1964)

NEARLY all strains of ergot (*Claviceps purpurea* (Fries) Tulasne) are capable of producing alkaloids when grown on rye, but only a few biochemical races are able to produce these compounds also under saprophytic conditions. The reasons of this behaviour are still unknown.

The indole character of ergot alkaloids led us to the idea that either auxins of the host plant or similar substances originating from tryptophan metabolism of the fungus may play a role in the biosynthesis of these alkaloids. The fact that β-^{14}C-labelled tryptophan is incorporated in ergot alkaloids is well known,[1] but we have only little information about reaction sequence of biosynthesis. Our observation[2] that indolyl-3-acetic acid (IAA) enhances alkaloid production and the finding of Yamano *et al.*[3] that ergot strains are able to convert tryptophan to IAA and indolyl-3-isopropionic acid should be mentioned in this connexion. To clear up this problem we fed various compounds known to influence growth of higher plants to ergot in submerged culture.

We carried out the experiments utilizing 7-day-old cultures of strain SD 58 of *Claviceps purpurea* which produces clavine-type alkaloids in saprophytic culture, and used the replacement technique. The substances to be tested were dissolved in phosphate buffer, pH 5·9, and after addition, the flasks were placed for 3 days at 25° on a reciprocal shaker. Alkaloids were estimated after extraction from the basified replacement medium with chloroform, and retransfer to tartaric acid solution, by van Urk reaction. IAA, indolyl-3-butyric acid (IBA) and indolyl-3-propionic acid (IPA) were determined after extraction from the medium at pH 3 with peroxide-free ether with Salkowski's reagent according to Gordon and Paleg.[4] Tryptophan in the medium and the actively taken up tryptophan extracted with boiling water from the washed mycelium was estimated enzymatically by the use of tryptophanase as indole.[5] The protein-bound tryptophan was determined after extraction of soluble compounds from the mycelium with 80% ethanol by the method of Spiess and Chambers.[6] For details see previous publications.[2,7,8]

Table 1 shows that addition of IAA, IBA, IPA, 2,4-dichlorphenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA) at levels of 0·60 mM increases the yield of alkaloids significantly. The effect of these additives is smaller under conditions of higher alkaloid

[1] D. GRÖGER, H. J. WENDT, K. MOTHES and F. WEYGAND, *Z. Naturforsch.* **14B**, 355 (1959).
[2] E. TEUSCHER, *Pharmazie* **16**, 570 (1961).
[3] T. YAMANO, K. KISHINO, S. YAMATODANI and M. ABE, *Ann. Rep. Takeda Res. Lab.* **21**, 83 (1962).
[4] S. A. GORDON and L. G. PALEG, *Physiol. Plant.* **10**, 39 (1957).
[5] T. A. SCOTT, *Biochem. J.* **80**, 462 (1961).
[6] J. R. SPIESS and D. C. CHAMBERS, *Analyt. Chem.* **21**, 1249 (1949).
[7] E. TEUSCHER, *Pharmazie* **18**, 561 (1963).
[8] E. TEUSCHER, *Flora* (In press).

production in the control. In the presence of IBA, IPA and NAA in higher concentrations (3 mM) no alkaloid is produced whereas with IAA an enhanced effect is found. The effect of low IAA concentrations is enhanced by the addition of Co^{++}-ions. (In higher plants Co^{++} increases the growth-promoting effect of IAA.[9,10]) Coumarin, a plant growth inhibitor, supresses IAA effect.

TABLE 1. EFFECT OF PLANT GROWTH-PROMOTING SUBSTANCES ON THE YIELD OF ALKALOIDS BY ERGOT

	Concentrations (mM)	IAA, IBA, IPA metabolized (mg/100 mg mycelial dry weight)	Alkaloids produced (μg/100 mg mycelial dry weight)	Increase %
Control	–	–	30	-
IAA	0·60	1·39 (100%)	164	446
Co^{++}	0·16	–	70	133
IAA + Co^{++}	0·60 + 0·16	1·39 (100%)	249	730
IPA	0·60	0·075 (9%)	226	653
IBA	0·60	0·96 (57%)	62	107
2,4-D	0·60		36	20
NAA	0·60	-	96	220
IAA	3·0	6·36 (87%)	502	1573
IAA + Co	3·0 + 0·16	5·00 (68%)	445	1383
IAA + NAA	3·0 + 0·60	5·06 (69%)	451	1403
IAA + coumarin	3·0 + 0·60	5·53 (75%)	62	107
2,4-D	3·0		370	1133

is within the limits of error. Addition of NAA, coumarin or Co^{++}-ions decreases IAA destruction.

The respiration of the mycelium is stimulated by the lower concentrations (0·60 mM) of auxins, but inhibited by the higher (3·0 mM). IAA is an exception, it enhances oxygen uptake in both concentrations, much more in the higher.

It has been found that substances influencing tryptophan metabolism have a great effect on the production of ergot alkaloids. Most compounds inhibiting active uptake of tryptophan decrease alkaloid biosynthesis, substances promoting active uptake enhance yield of alkaloids. The uptake of tryptophan was measured by the tryptophan extractable from the mycelium at the end of the experiment.

Table 2 shows the influence of auxins on some steps of tryptophan metabolism. It is evident that IAA, IBA, IPA and NAA diminish the amount of tryptophan extractable from the mycelium and the amount subsequently metabolized. It is therefore improbable that they act as promotors of active uptake of tryptophan. 2,4-D, however, seems to be an exception.

The degradation of tryptophan evidently follows two pathways: one via anthranilic acid, and the second via IAA. The formation of IAA does not involve tryptamine as an intermediate, since feeding tryptamine did not yield any trace of IAA. It should be mentioned that in control experiments no IAA was formed also without feeding tryptophan.

[9] K. V. THIMAN, *Amer. J. Bot.* 53, 241 (1956).
[10] H. BORRISS and G. HEMPEL, In preparation.

TABLE 2. EFFECT OF PLANT GROWTH-PROMOTING SUBSTANCES ON TRYPTOPHAN METABOLISM IN ERGOT

Additives*	Concentrations (mM)	μg/100 mg mycelial dry weight: Substances produced: Alkaloids	Substances produced: Anthranilic acid	Substances produced: IAA	Tryptophan: Stored	Tryptophan: Metabolized
Control	—	392	113	458	1810	6100
+IAA	0·60	490	106	566	1680	5860
+IAA	3·0	807	72	2360	1240	5300
+IBA	0·60	498	106	—	1040	4830
+IPA	0·60	488	102	—	1125	4740
+NAA	0·60	478	137	342	1140	4000
+2,4-D	0·60	253	117	567	1920	6280

* L-Tryptophan (3mM) was present in each case.

Some other strains which were not able to produce alkaloids in submerged culture have little or no ability to take up tryptophan actively or to metabolize it.[7,8] In these cases after feeding tryptophan no IAA was found, neither was given IAA metabolized.

Though no organic compounds besides the substances being tested were added to the samples, protein-bound tryptophan could not be the only source of indole skeleton of the alkaloids, because when both IAA and 2,4-D were fed the amounts of alkaloids produced during the experiment were greater than the loss of bound tryptophan (see Table 3).

TABLE 3. CHANGES OF THE AMOUNTS OF PROTEIN-BOUND TRYPTOPHAN IN ERGOT DURING 3 DAYS

Additive	Concentrations (mM)	μmoles/100 mg mycelial dry weight: Alkaloids	Tryptophan*: At the beginning	Tryptophan*: Loss after 3 days
Control	—	0·12	2·87	0·62
IAA	0·60	0·66	2·87	0·92
IAA	3·0	2·01	2·87	0·85
2,4-D	3·0	1·48	2·87	0·47

* Stored = 0 in each case.

The interpretation of the foregoing results is difficult. It seems possible that IAA serves as a precursor of the ergot alkaloids. Incorporation in the ergolin-ring system via indolyl-3-acetamid is not improbable. The other substances may act synergistic, preventing loss of IAA via oxidation. But it is likewise possible that the effect of the auxins is an indirect one, resulting from stimulation or inhibition of metabolic processes connected with alkaloid biosynthesis. It may be that the promoting effect of tryptophan on alkaloid production is, in reality, an effect of IAA.

Acknowledgements—I wish to thank Prof. H. Borriss and Prof. K. Mothes for valuable advice.