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The Pharmaceutical Utilization of Phytopathogenic Microorganisms

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Abstract

A large number of microorganisms which belong to nearly all the taxonomic groups of bacteria and fungi are the etiological agents of plant diseases. A certain number of these microorganisms produces compounds with some demonstrated or potential pharmaceutical applications. *Claviceps paspali* and *purpurea* and *Gibberella zeae* are by far the most important examples producing respectively the pharmaceutically important ergot alkaloids and zearalenone.

Other phytopathogenic microorganisms produce compounds with different kinds of antibiotic activity. While there are evidences that in *Claviceps* and in *Gibberella*, the overproduction of the respective metabolites can somehow increase their virulence, in all the other cases a relationship between virulence and overproduction does not seem to exist.

Keywords: pharmaceutical, *Claviceps*, *Gibberella*, Zearalenone, ergot alkaloids, microorganisms

1. Introduction

A large number of compounds produced by microorganisms find useful applications in human or animal health care. They belong to many chemical classes and show various activities: antibiotics, enzymes, vitamins, alkaloids, amino acids, etc. The microorganisms producing these substances are spread through almost all the taxonomic groups of bacteria and fungi. The phytopathogenic microorganisms, although numerous and present in many different taxa, have nevertheless a unique feature which characterize them: the ability to parasitize in some way the plant.

* Invited lecture

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Generally speaking, the ability of a microorganism to overproduce metabolites increases its competitivity against other organisms and therefore its survival (Demain, 1974). It is not known, however, if a mechanism of this kind does somehow operate in phytopathogenic microorganism, that is, if a relationship exists between pathogenicity, or virulence, and overproduction of a metabolite.

In this note a certain number of phytopathogenic microorganisms producing compounds of demonstrated or potential pharmaceutical interest are described and the possible implications of this production ability in the pathogenicity or virulence are discussed.

2. Ergot Alkaloids

The ergot alkaloids are the biologically active substances of the ergot drug which consists of dried sclerotia of *Claviceps paspali*, the well known parasite of rye. The structures of some typical representatives of these compounds are shown in Fig. 1. Besides the sclerotia of *Claviceps*, other fungi, phytopathogenic or not, and several higher plants can contain small amounts of ergot alkaloids (Table 1). The genus *Claviceps* however, is the only source of commercial ergot alkaloids.

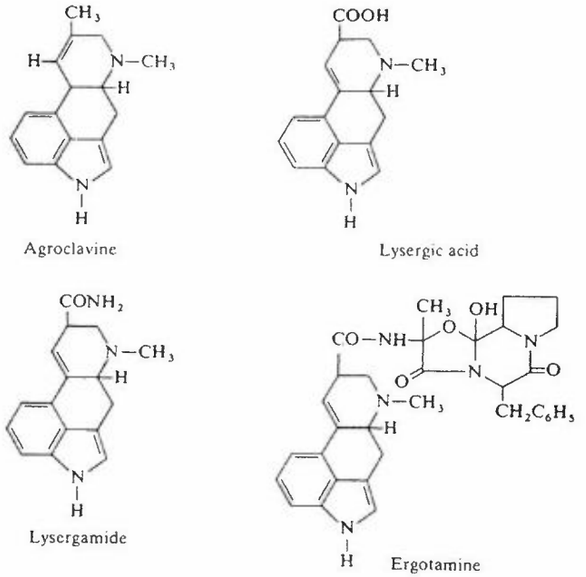


Figure 1. Structure of some typical ergot alkaloids. (from Spalla, 1980).

Table 1. Alkaloids found in plants and in fungi not belonging to the genus *Claviceps* (from Spalla, 1980).

Alkaloids	Species	References
Festuclavine	*Aspergillus fumigatus*	Spilsbury and Wilkinson (1961)
Fumigaclavine A and B	*Aspergillus fumigatus*	Spilsbury and Wilkinson (1961)
Fumigaclavine B	*Rhizopus arrhizus*	Spilsbury and Wilkinson (1961)
Clavine alkaloid	*Penicillium roqueforti*	Taber and Vining (1958)
Costaclavine	*Penicillium chermesinum*	Agurell (1964)
Lysergamide	*Rivea corymbosa*	Hofmann and Tscherter (1960)
Lysergamide	*Ipomoea tricolor*	Hofmann and Tscherter (1960)
Chanoclavine	*Ipomoea tricolor*	Hofmann and Tscherter (1960)
Ergosine	*Ipomoea argyrophylla*	Stauffacher *et al.* (1965)
Penniclavine	*Sclerotium delphinii*	El-Refai *et al.* (1977)
Ergocornine	*Sclerotium delphinii*	El-Refai *et al.* (1977)
Clavines	*Sclerotium cepivorum*	El-Refai *et al.* (1977)

The genus Claviceps

In nature the ergot fungus has a rather complex life cycle. The sclerotia show normally the approximate form and shape of the seeds of the parasitized plant. When fully developed they fall on to the ground and remain dormant until the following spring. At that time they germinate producing the spheridia in which ascocarps and then ascospores are formed (sexual reproduction). Ascospores are ejected from the ascocarps and are borne by air currents to host plants where the infection of the ovary takes place. During the next stages of the infection, numerous conidia are formed (asexual reproduction). A sticky liquid, with a very high content of sugars and of conidia, and called honeydew, transports the conidia to neighboring plants thus effecting a secondary infection. When the production of honeydew ceases, the mycelium continues to grow in the place of the developing seed forming the sclerotium which is the "ergot" of commerce.

All the species of the genus *Claviceps* can be grown easily on the common nutrient media such as potato agar or malt agar. On these media they grow as a filamentous mycelium which reproduces by means of conidia but does not form sclerotia or ascospores.

Production of alkaloids

The only known practical source of the ergot alkaloids until 1960 was the sclerotia of *C. paspali* grown parasitically on rye. The sclerotia were obtained through artificial infection of the host plants, normally rye, with a suspension of conidia of selected strains of *C. paspali* grown saprophytically on a nutrient medium. This technique shows a number of disadvantages, first of all, the large dependence on the seasonal variations which can strongly influence the final yield. Another disadvantage is that sclerotia normally contain a mixture of alkaloids, some of which are presently considered of poor pharmaceutical interest, and this makes the extraction and the purification of the wanted alkaloids very difficult and expensive. This last problem has been at least in part solved by utilizing for the infection selected strains able to produce mainly one alkaloid (Bekesy, 1973).

The described drawbacks prompted researches to find a process for the production of alkaloids by cultivating selected strain of *Claviceps* in saprophytic and submerged conditions, with the techniques largely utilized for the production of antibiotics. The production of biologically active ergot alkaloids in saprophytic conditions had been attempted in many laboratories: the first paper on this subject goes back to 1922 (Bonns, 1922). No attempt, however, had been successful in obtaining more than trace amounts until 1960 when a group of academic and industrial scientists at the Instituto Superiore di Sanità in Rome described the production in submerged culture with a strain of *Claviceps paspali*, a parasite of *Paspalum distichum*, of reasonable yields (about 1000μg/ml) of lysergic acid α-hydroxyethylamide, a new simple lysergic acid derivative (Arcamone et al., 1961). The first announcements concerning the production in mg/ml amounts of the pharmacologically important peptide alkaloids, mainly consisting of ergotamine, by cultivation of *C. paspali* in submerged conditions, appeared in the scientific literature in 1966 (Tonolo, 1966; Amici et al., 1966). Some years later Amici et al. (1969) described three new strains of *C. paspali* able to produce, under submerged conditions, high yields of ergokryptine and ergotamine, ergocornine and ergosine, and ergocristine, respectively. The main alkaloids which can be produced today in commercial amounts in submerged culture and the producing strains are reported in Table 2.

The genetic problems related to the production of ergot alkaloids have been thoroughly reviewed and discussed in detail (Spalla, 1973). An important characteristic of the species of *Claviceps* studied so far in different laboratories is their variability, which is however remarkably decreased in highly

Table 2. Species of the genus *Claviceps* and alkaloids produced under submerged culture (from Spalla, 1980).

Species	Produced alkaloid	References
C. fusiformis	Clavines	Tonolo and Udvardy-Nagy (1968)
	Clavines	Banks *et al.* (1974)
C. paspali	α-hydroxyethylamide of lysergic acid $\Delta^{8,9}$	Arcamone *et al.* (1961)
	lysergic acid	Kobel *et al.* (1964)
C. purpurea	Ergotamine	Amici *et al.* (1966)
	Ergotamine	Tonolo (1966)
	Ergosine	Amici *et al.* (1969)
	Ergocryptine	Amici *et al.* (1969)
	Ergocornine	Amici *et al.* (1969)
	Ergocristine	Amici *et al.* (1969)
	β-ergocryptine	Bianchi *et al.* (1976)

productive strains obtained through selection. The variability involves both the morphology and the amount of alkaloid produced and has been related to the heterokaryotic nature of the corresponding strains.

Virulence and alkaloid production

First mention must be made of the important observation by Tonolo (1959) that when rye embryos are infected *in vitro* with different strains of *Claviceps*, some of the strains tested are non-infective, some cause infection but grow in the form of vegetative mycelium without formation of sclerotia, whereas others give rise to both infection and sclerotia formation. Among the last group, a strain from a sclerotium of *Paspalum distichum* L. identified as *C. paspali* able to produce lysergic derivatives was isolated and studied. The results obtained on this strain, as well as other observations, allowed to establish the existence of a direct, positive relationship between virulence, sclerotia formation and alkaloid production.

An explanation could be found in the fact that since the derivatives of lysergic acid have an indole structure, they can display an auxinic activity of their own which also influences the virulence. A similar hypothesis has been used for example to try to explain the disease of rice known as "seedling bligh" caused by *Fusarium fujikuroi* where the severity of the infection and the virulence of the various strains are proportional to the quantity of the growth factor gibberellin they synthesize (Ming et al., 1966).

With the aim of confirming this last hypothesis some derivatives of lysergic acid have been tested in comparison with indolylacetic acid for their ability to stimulate the elongation of rye coleoptiles (Spalla, 1973). The obtained results demonstrated that lysergic acid, ergotamine, ergokryptine and ergocornine, though less active than indolylacetic acid, show auxinic activity. It would be therefore reasonable to suppose that the production of alkaloid could in some extent increase the virulence of *Claviceps*.

Toxopharmacology of ergot alkaloids

Several extensive reviews on the subject have been made available during the last 30 years and since no major knowledge has been gained recently, just a summary of the status of the art will be presented here.

First of all it must be mentioned that even slight modifications of the native structure of these alkaloids induce relevant modifications in their pharmacology. They can be defined as simpathicolytic agents and serotonine antagonists. It is therefore not surprising that they have been investigated in a large variety of clinical indications including: (a) peripheral and cerebral vascular insufficiencies like claudication, T.I.A., etc. (hydergine); (b) uterotonics (methylergometrine); (c) hallucinogens (LSD); (d) mammary hypertrophy, antinidation.

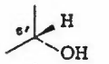
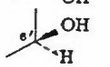
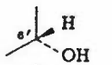
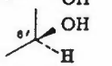
The evaluation of their safety indicated that for dihydroergotoxine, it is strictly limited in man by the production of nausea and vomiting. Chronic ergotism, due to overdosage or to individual unusual susceptibility, such as hepatic insufficiency, can cause vascular striking and gangrene of the extremities.

3. Zearalenone and Derivatives

Zearalenone is a natural resorcilic acid lactone endowed with interesting pharmacological properties (Hidy et al., 1977), produced by *Gibberella zeae* (the perithecial or perfect stage of *Fusarium roseum* f. sp. "*graminearum*"). This fungus is a weak pathogen of various plants, including corn. Once the corn is harvested and stored, if there is humidity enough, it can be invaded by the fungus which produces zearalenone imparting estrogenic properties to the feed grain. Other species or subspecies able to produce zearalenone are *F. tricinctum, F. roseum, F. gibbosum, F. roseum* f. sp. *equiseti, F. roseum* f. sp. *culmorum, F. roseum* f. sp. *graminearum*.

Table 3 summarizes some data on the main derivatives of zearalenone and Fig. 2 indicates their structural relationships (Hidy et al., 1977). Natural

Table 3. Related resorcylic acid lactones from (Hidy et al., 1977).

	Compound	Melting point (°C)	Configuration at C-6′	Trademarks
(I)	Zearalenone	164-165		
(II)	Zearalenol	168-169		
(III)		174-176		
(IV)	Zearalanone	192-193		
(V)	Zearalanol	145-147		RALONE®
(VI)		182-183		RALONE®, RALGRO®

metabolites similar in structure to zearalenone but not in biological activity are curvularin, produced by the fungi *Curvularia sp., Penicillium steckii* and *P. expansum*; radicicol and monorden, produced, respectively, by *Nectria radicicola* (see Mirocha et al., 1971 for a review) and *Monosporium* sp.

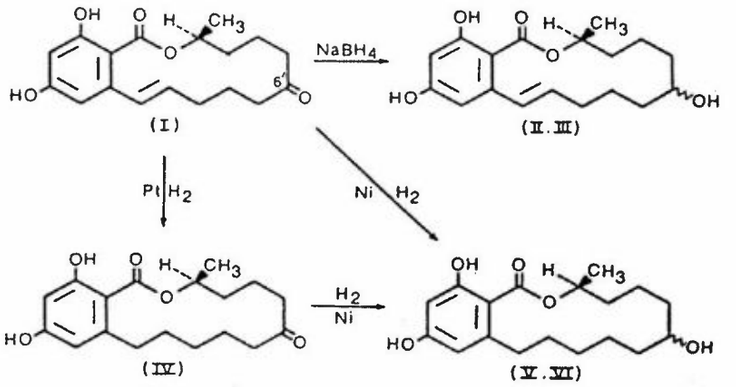


Figure 2. Structures and relationships of zearalenone and the derivatives. (from Hidy et al., 1977)

Production of zearalenone

Initial culture isolation studies were conducted at Purdue University and continued in Commercial Solvents Corporation Laboratories by examining various moldy corn and soil samples. A mutation and selection program was initiated concurrently with media and solid support development. Using the procedure of Eisenstark to treat macroconidia of the Dewar strain with N-methyl-N′-nitro-N-nitrosoguanidine (NTG), a strain (542, ATCC 20273) was selected that not only produced higher titers in surface fermentations, but also produced zearalenone under aerobic submerged conditions (Keith, 1972). Selected strains of *G. zeae* were preserved both by lyophilization and by submersion in liquid nitrogen.

At the beginning the production of zearalenone was done through surface fermentation only, with the inherent disadvantages (low productivity per unit of volume and high costs). The discovery of mutant strains of *G. zeae* capable of producing zearalenone by submerged culture was a major breakthrough in efforts to produce zearalenone on an industrial scale since earlier attempts had been unsuccessful (Keith, 1972; Woodings, 1972). The development of a successful submerged fermentation required a reinvestigation of the medium components as well as a study of other fermentation parameters. Early work on the submerged process was conducted in shake flasks and small fermenters. The fermentation was subsequently scaled up to 80 m^3 industrial fermenters.

Effect of zearalenone on fungi

According to Nelson (1971), zearalenone primarily serves as a hormonal regulator in promoting sexual stage development in the fungi. He offers as evidence for this the following: (a) the concentrations of zearalenone (0.01–1.0μg) necessary to stimulate sexual responses is small, (b) zearalenone stimulates only the early stages of sexual reproduction (perithecia formation) and not development of asci or ascospores, which occurs later, (c) stimulatory abilities of zearalenone are influenced quantitatively by time of application, (d) zearalenone stimulates sexual development but usually has no effect on vegetative growth, and (e) higher, but still physiological, concentrations inhibit sexual reproduction. Many fungi within the *Ascomycetes* as well as *Phycomycetes* are affected by zearalenone. it can therefore be supposed that the ability to produce zearalenone can, in some way, influence the survival, and then the virulence, of phytopathogenic fungi.

Toxopharmacology of zearalenone and related compounds

According to Hidy et al. (1977), over 150 compounds have been tested, but just a few in detail. No further information has been found indicating that these chemical series have been further investigated. Pharmacologically speaking, this chemical family is characterized by no clearcut specific biological targets. Minor behavioural cardiovascular and gastrointestinal effects can be detected at relatively high (single and repeated) doses. Toxicological doses are reached on various animal species.

Vice versa the easily detectable anabolic activity of zearalenone and analogs (tested on the basis of endometrium and vaginal mucosa alteration) allows to classify this chemical species among the anabolic agents with, however, a different profile when compared to 17-β estradiol and/or estrone, and with a weaker activity (1 to 20%). This aspect might be linked to the differences in the bioavailability and metabolization rate of the parent compound and its analogs in the various species studied.

The inhibition of the synthesis and excretion of the pituitary hormone associated with the uterotrophic effect of zearalenone and analogs suggested a potential clinical use for the control of the menopausa syndrome (dose investigated 25/50 mg/day); clinical evidence confirmed the biological hypothesis.

Finally, in dyslipaemic rats, zearalenone with doses of 1 to 10 mg significantly lowered blood rate of cholesterol leaving triglycerids and glucose tolerance unaffected. In conclusion this class of non steroidal agents, interesting alternatives to classical estrogenic agents, confirmed its capacity to displace estradiol from its uterine binding sites (according to the tests and the animal species used), the dose/response curves do not parallel (possibly indicating different sites of actions) and finally the intensity of the estrogenic activity conspicuously varies in the different animal models tested; the oral/parenteral ratio varies between 0.2 and 3. Efficiency in the treatment of estrogenic deficiency in human beings has been attained with oral daily doses of 50 to 75 mg.

Relative large amounts of zearalenone have been made available since 1969 thanks to the improvement of the fermentation processes and the anabolic potential (i.e. growth promoter activity) of the compound has been tested in large animal species. Six mg of a zearalenone analog proved to be superior to an equal dose of diethylstilbestrol in terms of nitrogen retention in the sheep. Implants were commercialized for cattle and sheep when the evidence of the absence of detectable residue in edible tissue was obtained. The evaluation of the safety (Drugs of Today — vol. XII no. 6 p. 243–244 — 1976) of Zeranol

Table 4. Antibiotics produced by some phytopathogenic microorganisms.

Species	Antibiotic	Species	Antibiotic
Fusarium lycopersici[1]	Lycomarasmin	*Ascochyta viciae*	Ascochlorin
Fusarium avenaceum	Lateritin I	*Verticillium cinnabarinus*	Melinacidin
Fusarium fructigenum	Avenacein	*Verticillium sp.*	Verticillin A
Fusarium lateritium	Fructigenin	*Myrothecium verrucaria*	Verrucarin
Fusarium javanicum[1]	Javanicin	*Myrothecium roridum*	Roridin
Fusarium lycopersici[1]	Fusaric acid	*Trichothecium roseum*	Trichotecin
Fusarium fujikuroi	Fusaric acid	*Verticillium sp.*	Cephalochromin
Fusarium vasinfectum[1]	Fusaric acid	*Helminthosporium siccans*	Siccanin
Fusarium bostrycoides[1]	Bostrycoidin	*Helminthosporium sativum*	Helminthosporal
Fusarium moniliforme	Bikaverin	*Ophiobolus miyabeanus*	Ophiobolin
		Alternaria sp.	Alternaric acid

[1] abbreviation of *Fusarium oxysporum* f. sp. *bostrycoides*, f. sp. *javanicum*, f. sp. *lycopersici*, f. sp. *vasinfectum*.

in animal indicated low acute and chronic toxicity. The LD 50 in mice was around 4 g/kg and 10 g/kg in rats by I.P. route. Chronic toxicity was studied for 300 days in rats and dogs with daily dosage of 1,66; 3,32 and 10 mg/kg by oral route. The only observed alterations were slight weight increase and morphologic modifications of adrenal liver and kidneys in the animal at the top dose level.

4. Other Microbial Products or Activities

Many phytopathogenic fungi form products or show activities with potential pharmaceutical interest. A significant part of them are listed in Table 4. At present, however, none of them found practical application. Other phytopathogenic microorganisms showed the ability to transform important pharmaceutical substances like steroid. The best known of them, even if so far not utilized, are *Calonectria decora, Gibberella fujikuroi, Cladosporium herbarum, Helicostilum pyriforme, Fusarium oxysporum* f. sp. *lini, Cephalothecium roseum,* and *Ophiobolus herpotrichus.*

5. Discussion

A comparison between the number of products with proved or potential pharmaceutical interest formed by phytopathogenic microorganisms and that of the same kind of products formed by common microorganisms clearly points out a very scarce importance of the former ones. In fact, out of a total of about 7000 compounds produced by microorganisms, some tens only come from phytopathogenic microorganisms. Also taking well into account

that the phytopathogenic microorganisms studied are by sure in lower number, we can conclude that a positive correlation between active metabolite formation and pathogenicity does not generally exist. On the other hand, the genus *Streptomyces*, which produces about 70% of all the known antibiotics, practically does not include phytopathogenic strains (with the only exception of *Streptomyces scabies*).

The species listed in Table 4 as producers of antibiotics are not specialized parasites but, on the contrary, can also behave as common saprophytes and it is probably that, as such, they can find advantages for their survival in producing antibiotic just as all other saprophytes do.

Completely different seems to be the case of *Claviceps* and of *Gibberella* as producers of alkaloids and of zearalenone respectively. There are in fact evidences that the produced compounds can act by increasing the virulence. In these cases too, however, the mechanism through which the products increase the virulence have nothing in common with the pharmacological activity of the product itself. It can therefore be concluded that no relation exists between phytopathogenicity of a microorganism and its ability to produce compounds of pharmaceutical interest.

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