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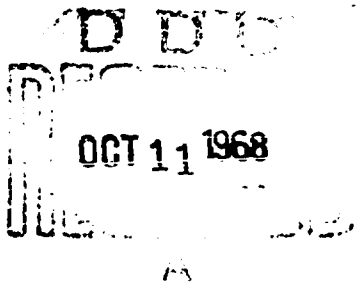


DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Human and Nutritional Aspects
Toxic Fungal Metabolites

/Following is a translation of an article by K. J. van der Merwe, Dr. Rer Nat. Göttingen, Nasionale Chemiese Navorsingslaboratorium, Pretoria, in the South African-language periodical S. A. Tydskrif vir Geneeskunde (South African Medical Journal), Vol. 52, 1965, pages 762-765./

Although it has already been known for more than 70 years that fungi can produce toxic metabolites, the relationship between these toxic substances and certain symptoms of disease, with the only exception of ergotism, has, in general, not been recognized. At present, it is difficult to explain why, notwithstanding the discovery of a large number of antibiotics from fungi, no systematic research into the effect of fungal metabolites on the health of higher organisms has been undertaken.

The recent discovery of the problem of aflatoxins and the realization that, in all probability, other toxic fungal metabolites of comparable importance may exist, has not only given rise to a world-wide interest in the problem of mycotoxicosis, but also to a large-scale search for new toxic fungal metabolites. In a discussion of the work which in this connection is being carried out at the National Chemical Research Laboratory, a few chemical aspects of mycotoxicosis of importance in such research should be mentioned.

Chemical Structure

The chemical structure of a number of known mycotoxins is shown in diagram 1. The change from relatively simple substances, such as oxalate-ion (according to the literature, responsible, inter alia, for the toxicity of Aspergillus niger) and β-nitropropio acid (a cancer creating toxic substance formed by various fungi) to complicated molecules such as "byssochlamien" acid and the peptide-like Islanditoxin. "Byssochlamien" acid, consisting of a relatively unusual nine-link ring and two anhydride groupings, is formed by the fungus Paecilomyces varioti and is connected with the so-called haemorrhagic chicken disease. Islanditoxin is a cancer creating substance which is formed

by the fungus Penicillium islandicum and is primarily responsible for the toxicity of the so-called Islandia yellow rice in Japan. Three of the building blocks in this molecule are of a very extraordinary nature. Treatment with a very much diluted ammonia solution removes the chlorine atom and deprives the substance of its toxicity.

Further examples are given in diagram 2. Sporidesmin, a complicated organic molecule containing both chlorine and sulphur, is a hepatotoxic metabolite formed by the fungus Pithomyces chartarum and is responsible for the so-called "facial eczema" among sheep in New Zealand. One of the better known examples of mycotoxicosis among people is ergotism, a condition occurring sporadically in Central and Northern Europe and which was reported as an epidemic in France as recently as in 1953. It is due to the use of bread prepared from rye infected by the ergot fungus Claviceps purpurea. The active components are alkaloids, which can be derived from the basic skeleton "lisergien" acid.

These examples show that mycotoxins do not belong to any specific class of organic compounds but chemically can be quite different from one another. This does not only partly explain the dissimilar nature of the symptoms of disease caused by them, but makes it impossible, in the case of an unknown mycotoxin to determine beforehand the most appropriate isolation procedure. Such methods can only be obtained on the basis of systematic research in which each step of separation is followed by means of toxicity tests.

The concentration in which mycotoxins are found in fungus contaminated food is usually of the order of one part per million and less. Although it is nowadays possible by means of modern chromatographic methods of separation to isolate the toxic substance even if greatly diluted, the quantity thus obtained usually is insufficient for an extensive chemical and toxicological analysis. That is why it is tried in practice at an early stage of the research in the laboratory to prepare fungus contaminated material of higher toxicity with the aid of a pure fungus culture. However, it should then be kept in mind that the changed conditions under which the fungus is cultivated in the laboratory in some cases may strongly influence the production of the toxic substance.

Environmental Factors

The influence of environmental factors on the production of toxic substances is clearly illustrated in the case of the so-called "alimentary toxic aleukia". This disease was especially prevalent among people in Russia between 1942 and 1947 and reached its climax in 1944, when in certain parts of the Orenburg district it caused the death of more than 10% of the population. Death was caused by the consumption of bread prepared from grain which had been covered with snow for some time and had been contaminated by the fungi Fusarium sporotrichoides, Cladosporum epiphylum and Cladosporum fagi. The toxic substances causing the disease are shown in diagram 3. It has been found that none of these toxic substances develop at normal room temperature, but do develop at low temperatures, especially around 0°C. These toxic substances are so stable that even after six years they are still present in

grain which has been stored; not even in the process of bread baking are they destroyed.

Still another example is shown in diagram 4. "Dikumarien" is the mycotoxin responsible for the so-called haemorrhagic sweet clover [original text: soetklawer] poisoning among animals. It is caused by Aspergillus formed from "kumarien", a substance found in clover. Because the fungus itself cannot synthesize "kumarien", the production of mycotoxin is completely dependent on the presence of "kumarien" in its environment.

The toxicity of mycotoxin is not necessarily restricted to people and animals only but sometimes also affects the growth of microorganisms and plants. This is reflected in the fact that certain antibiotic-active substances, discovered in the search for antibiotics and found to be too toxic, are nowadays known to us as mycotoxins. One example is "patulien" (diagram 4), a known antibiotic-active metabolite from Penicillium urticae, which is responsible for malt fodder poisoning among animals.

However, it should be kept in mind that toxic antibiotic-active metabolites and "toxic" phytotoxins have been isolated in another context and thus need not always be of great importance with regard to mycotoxicosis. For some fungi are capable of synthesizing more than one toxic substance, as can clearly be illustrated in the case of Aspergillus flavus (diagram 5). The importance of each of these substances with regard to mycotoxicosis depends on the relative quantities in which they are present, as well as on the relative toxicity.

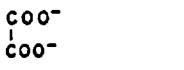
Aspergillus ochraceus is a fungus which occurs on a large scale in nature, and the toxicity of which has recently been proved by the Microbiological Research Group of the WNNR. Aspergillus ochraceus forms part of the microflora of "katsuo bushi" and other fermented fish preparations used in the Far East, while its capacity to cause the desired change in flavor during the fermentation process of coffee is patented. A chemical research of the toxic fungal metabolites of this fungus has been started at the National Chemical Research Laboratory of WNNR at the end of 1963.

This toxic fungus was cultivated on a large scale in the laboratory on the basis of sterilized "mielie" flour, and the procedure of extraction and the fractionating methods used to isolate the pure toxic substance are listed in diagram 6. Toxic analysis, both qualitative and quantitative in nature, was employed after each step to follow the extraction of the toxic substance and to ensure that the active component did not disintegrate in the process. It could finally be shown that only one metabolite, which has been given the name of ochratoxin A, is responsible for the toxic character of this fungus.

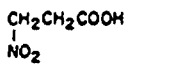
Diagram 7 shows the chemical structure of ochratoxin A and two allied components, ochratoxin B and C, which have recently become apparent, especially through the aid of physical chemical methods such as mass spectrometry, nuclear magnetic resonance, infrared and ultraviolet spectroscopy. Only ochratoxin A

is toxic, and its toxicological aspects have already been dealt with by Dr. Theron. The structure of ochratoxin B and C shows that removal of chlorine or the esterification of the carboxyl group removes the toxic nature of ochratoxin A.

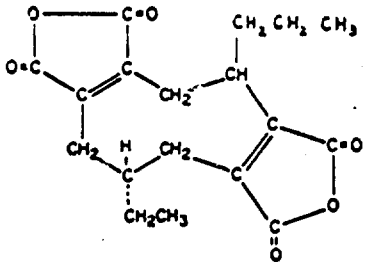
Diagrams



Oksalaat
(Aspergillus niger)



β-nitro-propioonsuur

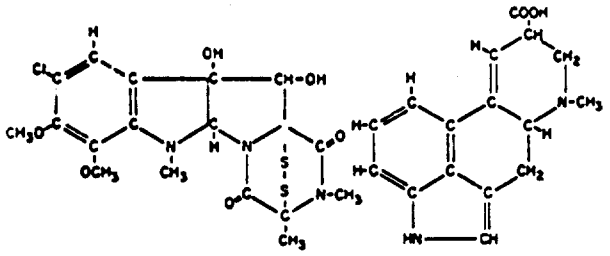


L-seriel-L-seriel-L-dichloroprotiel-β-feniel-β-aminopropioniel-L-α-aminobottersuur anhidried.

Byssochlamiensuur

Islanditoksien
(Penicillium islandicum)

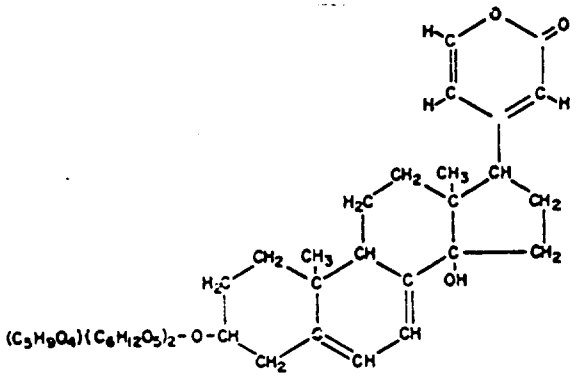
Afb. 1



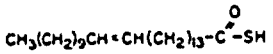
Sporidesmin
(Pithomyces chartarum)

Lisergiensuur

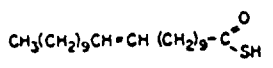
Afb. 2



Fusariogenien (Fusarium sporotrichoides)

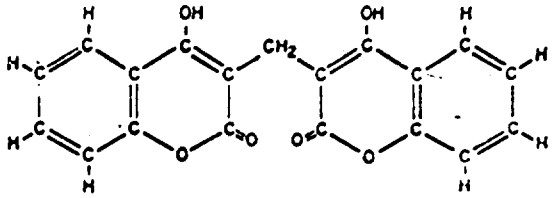


Epicladosporiensuur (Cladosporum epiphylum)

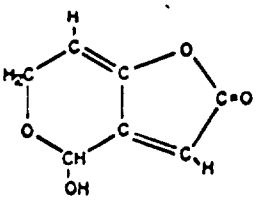


Fogicladosporiensuur (Cladosporum fagi)

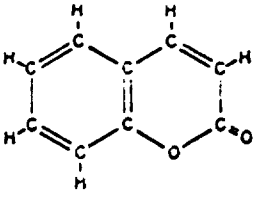
Afb. 3



Dikumarien



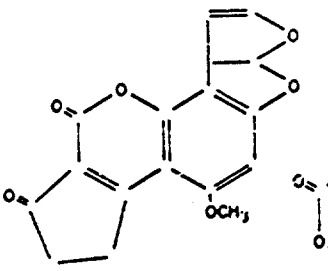
Patulien
(Penicillium urticae)



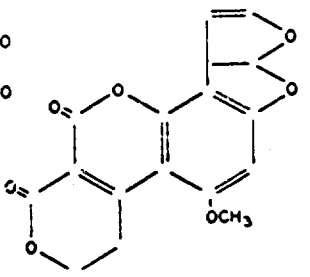
Kumarien

Afb. 4

Die Aflatoksiene

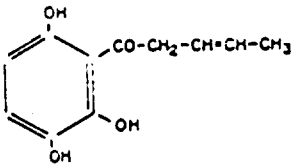


B_1



G_1

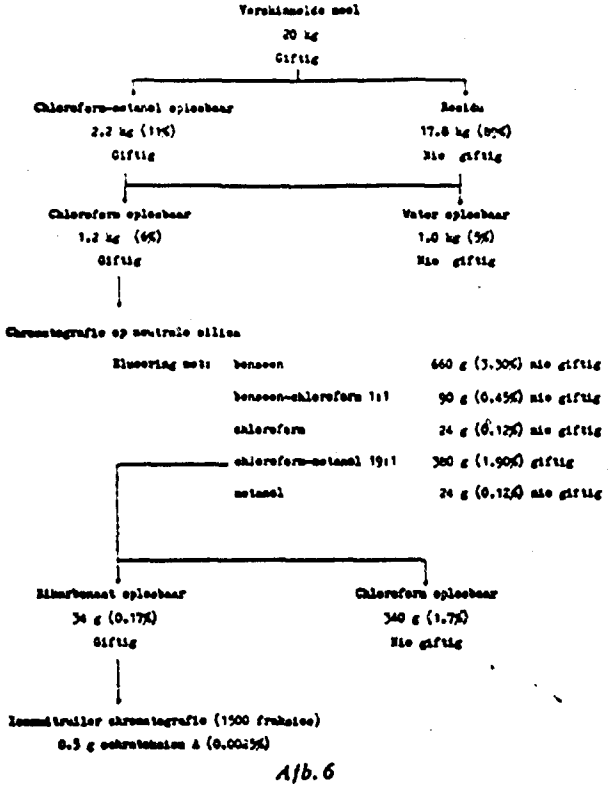
Asperçiliensuur
Flavusien
Oksaalsuur
Kojiensuur



Maltoryzien
(Aspergillus oryzae var. microsporus)

Afb. 5

Diagrams (continued)



Afb. 6

Translator's note to Diagram 6

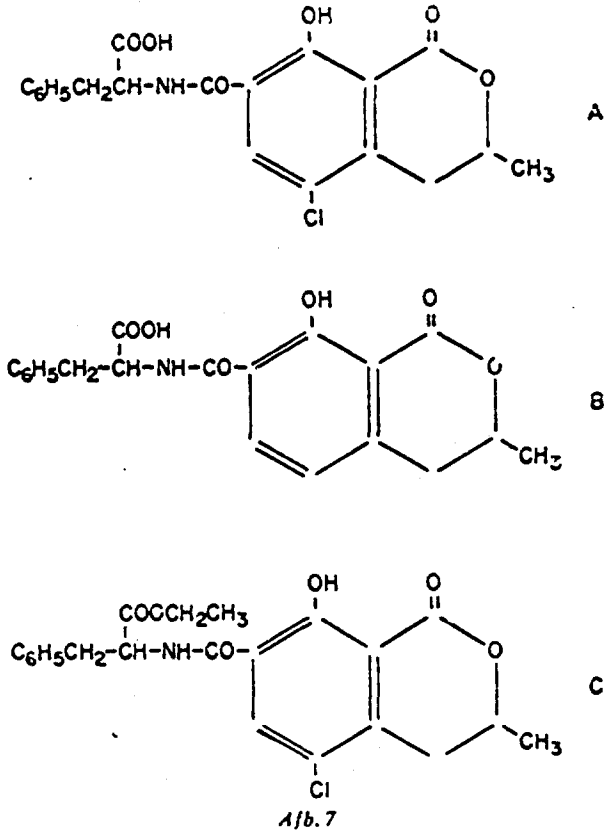
Verskimmelde meel: fungus contaminated flour;

Oplosbaar: soluble;

Giftig: toxic;

Nie Giftig: non-toxic.

Die Ochratoksiene



Afb. 7