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Isolation and structure of new toxins from plants

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Abstract - Livestock poisoning is a serious problem in many countries of the world whereever cattle is raised on grassland. A figure of 500.000 animals per year in South America only is estimated as loss. Beyond these cases of acute poisoning, the cases of chronic poisoning seem to be even more important, as from the food chain such substances or metabolites thereof may become harmful to man. Under these aspects we have investigated a number of poisonous plants during the past years. Thiloa glaucocarpa contains four tannins, vescalagin, castalagin,stachyurin, and casuarinin as toxic principles. Investigation of five Senecio species from Brazil showed the pyrrolizidine alkaloids integerrimin, senecionin, neosenkirkin retrorsin, and florosenin to be responsible for the symptoms of poisoning. The toxicity of Baccharis coridifolia is well documented since the beginning of this century . Eight macrocyclic trichothecenes as well as O-acetyl-verrucarol and a metabolite thereof have been isolated. Two of the macrocyclic trichothecenes , roridin A and roridin E were already known, five of the toxic compounds were novel; they were named miotoxins A, B, C, D, and iso-D. Two more of the trichothecenes , miophytocenes A and B, were non-toxic. As could be shown, the trichothecenes are not original products of Baccharis coridifolia but are produced by a soil fungus, Myrothecium verrucaria which could be isolated from the roots of the plant. This is the first case of a symbiosis of a soil fungus and a higher plant.

INTRODUCTION

Toxic plants have been known for centuries. They have been extensively used by all primitive cultures for medicinal purposes as well as arrow poisons. Thus, many toxic plants are well known . Despite this, unintentional poisoning still occurs in both, humans and animals as a result of the accidental ingestion of poisonous plants and the misuse of plants of medicinal value.
The isolation of the toxic factors from poisonous plants became a serious occupation around the turn of the century, and interest in this field has continued to grow. It has become clear that poisoning by plants is not always as straighforward as is commonly supposed and recent investigations into a number of plants responsible for the large-scale poisoning of livestock have produced a number of unexpected results.
Cattle poisoning must be considered from two standpoints. Firstly, the acute envenomations have to be mentioned as they may be of considerable economic relevance due to the loss of animals. The second aspect, however, the chronic envenomations are even more important, as it is well known that plant toxins are either accumulated in the animal or in certain organs or they are metabolized and excreted with the milk. By this food chain , toxins or metabolites thereof may become harmful to humans.

THILOA GLAUCOCARPA

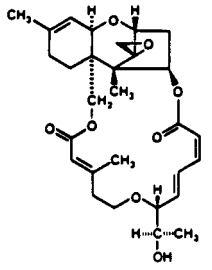
In the beginning of every rainy season in Brazil, numerous cattle are lost by a disease known as "popa-inchada", or "venta seca", or "mal-da-rama". All these cases are one and the same disease, caused by ingestion of leaves of Thiloa glaucocarpa (ref. 1). Usual work-up and structure elucidation of the pure toxins showed that tannins were responsible for this disease. At this point we observed that the same symptoms were caused by another disease common in Germany, the so-called "Oak disease" in cattle (ref. 2). In both cases the same substances are responsible for the symptoms, and we were able to identify four compounds: vescalagin, castalagin, stachyurin, and casuarinin (ref. 3).

SENECIO spp.

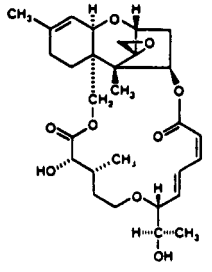
A worldwide problem is poisoning by plants of the genus Senecio. Seneciosis is described under names like "Winton disease" ,"Pictou disease", "Moltena disease", and "Schweinsberger disease". They all are caused by one or more Senecio species which contain pyrrolizidine alkaloids responsible for disease and death (ref. 4).
Acute poisoning is observed already after ingestion of a single portion of plant material exceeding 2o g/kg body weight, while chronic poisoning may be observed after ingestion of smaller amounts over a longer period of time. Depending on the uptake of plant material, chronic poisoning may occur without showing any symptoms for quite a while. As the alkaloids are excreted with the milk, humans may also be involved in "chronic seneciosis".
The following species were investigated: S. brasiliensis, S. heterotrichus, S. leptolobus, S. selloi, S. cisplatinus. From the methanol extract the alkaloids could be isolated by a sequela of chromatographic separations. The structures were determined by 1H-, 13C-, and 2D-NMR spectroscopy. The alkaloids responsible for the disease are: neosenkirkine, retrorsine, florosenine, integerrimine, and senecionine.

BACCHARIS CORIDIFOLIA

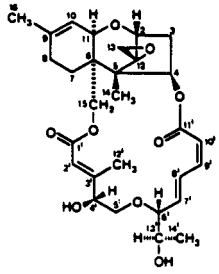
One of the most important plants responsible for the poisoning of cattle in South America is Baccharis coridifolia. This herbaceous shrub is a characteristic representative of the savannah vegetation of Southern Brazil, Uruguay, Paraguay, and Argentine. Although the plant has been known to be toxic since the beginning of this century, no experimental work was formally published before 1975 (ref. 5). All parts of the plant are toxic, but the flowers, leaves and seeds are particularly dangerous. Death occurs between 4 h and 34 h following the ingestion of a lethal amount of the plant (o.35 g/kg body weight). More than 5o.ooo animals die in Brazil per year from this plant.



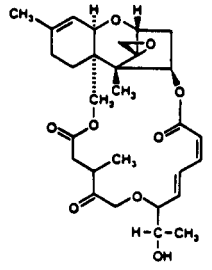
RORIDIN E (1)



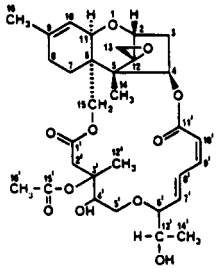
RORIDIN A (2)



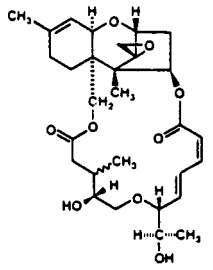
MIOTOXIN A (3)



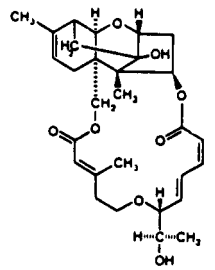
MIOTOXIN B (4)



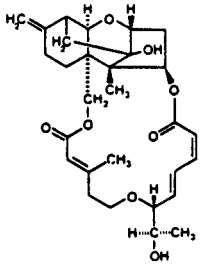
MIOTOXIN C (5)



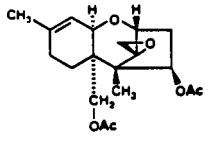
MIOTOXIN D (6)
ISO-MIOTOXIN D (7)
(epimeric at C-3')



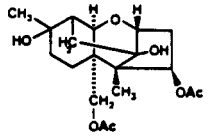
MIOPHYTOCEN A (8)



MIOPHYTOCEN B (9)



DI-O-ACETYL-
VERRUCAROL (10)



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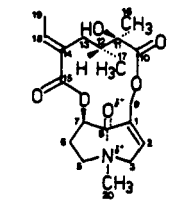
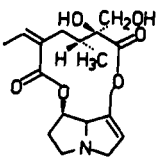
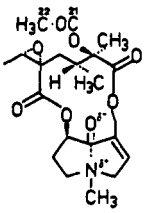


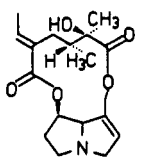
(1) Neosenkirkine



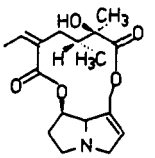
(2) Retrorsine



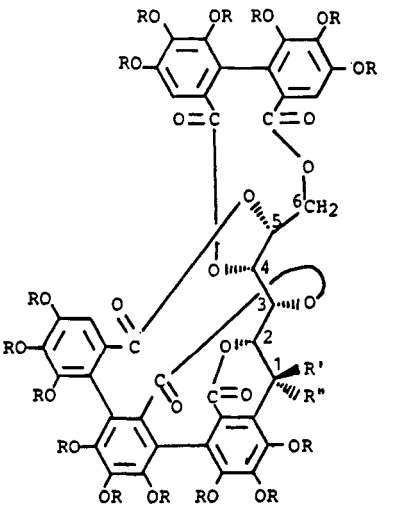
(3) Florosenine



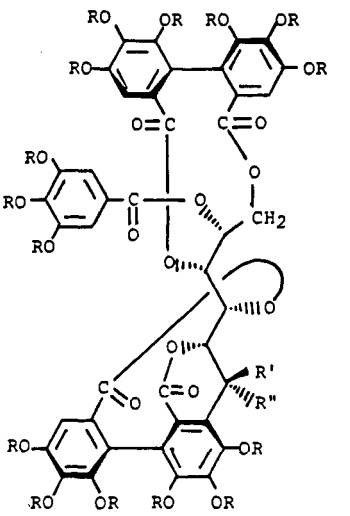
(4) Integerrimine



(5) Senecionine



	R	R'	R"
1	H	H	H
1m	Me	H	OH
1ma	Me	H	OAc
1a	Ac	H	OAc
2	H	OH	H
2m	Me	OH	H
2ma	Me	OAc	H
2a	Ac	OAc	H



	R	R'	R"
3	H	H	OH
3m	Me	H	OH
3ma	Me	H	OAc
3a	Ac	H	OAc
4	H	OH	H
4m	Me	OH	H
4ma	Me	OAc	H
4a	Ac	OAc	H

Extraction of the plant with chloroform, followed by chromatographic separation, yielded eight macrocyclic trichothecenes, as well as di-O-acetyl-verrucarol and a metabolite thereof. All these compounds were isolated in pure form, and the structures elucidated by means of mass spectrometry and two-dimensional n.m.r. spectroscopy. Two of the macrocyclic trichothecenes, roridin E and roridin A , were well known as metabolites of Fungi imperfecti. Five of the compounds were novel (although closely related to the roridins) and they were named miotoxins A, B, C, D, and iso-D, after the native name of the plant, mio-mio. Two more of the trichothecenes, miophytocenes A and B, were non-toxic.

The toxic compounds possess an intact oxirane system , while in the non-toxic miophytocenes this oxirane ring has been opened. Since it is well known that oxiranes are highly reactive chemical groups, it seems likely that an intact oxirane ring is essential and directly responsible for the toxicity of the trichothecenes.

The fact that the roridins A and E as well as acetyl-verrucarol have been known for a long time as metabolites of Fungi imperfecti , and that such compounds are well known to be phytotoxins, led to the assumption that the compounds isolated from the plant are not original products of Baccharis coridifolia. Infestation of the plant material, however, could be excluded. Further studies led to the finding that the roridins isolated are in fact produced by a soil fungus, Myrothecium verrucaria, which could be isolated from the surface of the roots of the plants. Obviously the trichothecenes produced by Myrothecium verrucaria are taken up from the roots and then metabolized within the plant, in part to the non-toxic miophytocenes (ref. 6) . A similar observation was made by Kupchan et al. (ref. 7) who isolated trichothecenes from Baccharis megapotamica.

Another plant of the same genus, Baccharis dracunculifolia has been considered toxic in some cases, but not in others. We have checked this plant without finding any evidence of toxicity, and no trichothecenes could be isolated. Reports of the toxicity therefore may be due to improper classification of the plant and confusing it for B. coridifolia.

Two questions still remain open. What is the reason for this kind of symbiosis between a higher plant and a fungus ? And what is the reason that B. coridifolia is able to store and metabolize large amounts of trichothecenes which are otherwise able to kill plants in amounts of a few p.p.m.

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