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Letters to the Editor

From: Dr. Ian R. Tebbett, Director Forensic Toxicology, Dept of Pharmacodynamics, The University of Chicago, Box 6998, Chicago, Illinois 60680, USA

18 November 1991

Sir,

I refer to the article "Cortinarins in *Cortinarius speciosissimus*? A critical revision", by L. Matthies and H. Laatsch that appeared in the June 1991 edition of Experientia [Vol. 47/No. 6/pp. 634–640], and a very similar paper by the same authors which appeared in October's Mycologia under the title "Fluorescent compounds in *Cortinarius speciosissimus*: investigation for the presence of Cortinarins". I feel that the authors' criticism of the work which Dr. Caddy and myself described in 1984 is over zealous, misleading and unfair.

From methanolic extracts of *Cortinarius* species, we isolated three compounds (the cortinarins) two of which produced toxic responses in mice consistent with reports of toxicity caused by the ingestion of *Cortinarius* mushrooms. On chemical examination, these toxic compounds showed NONE of the properties of orellanin. I think it was reasonable to infer from this observation that *Cortinarius* species contain toxic compounds in addition to orellanin. Nonetheless, this seemingly innocuous statement which we first made in 1982, created an uproar from certain members of the European mycological community and the work has been the subject of ridicule ever since. For some reason, which escapes me, research grants are still being used almost 10 years later, for the purpose of disproving the possibility that toxic polypeptides may be present in these fungi.

Matthies and Laatsch state that flourescence in extracts of *C. speciosissimus* MUST be attributed to decomposition products of orellanin and to steroids, and could not possibly be associated with peptides. Their reasoning is based on so-called decisive errors in the structure elucidation of the peptides. I agree entirely that the cleavage of the cyclic peptide is difficult to explain, but this is what happened and we reported our findings.

The NMR spectrum, which was not published but was represented in my thesis, is criticized at length being used as proof that the structure elucidation of the cortinarins was incorrect. In fact, my thesis clearly states that the 90 MHz NMR spectrum was poorly resolved, and merely indicated the presence of a methoxy group, aromatic region and N-H. Unfortunately, I did not have the benefit of a Fourier Transform instrument.

The fact that I reported the isolation of 3 mg of indole from a hydrolysate is criticized because this exceeds the theoretical yield.This isolation requires several steps including preparative TLC and separation on a Sephadex column. It is little wonder that the final weight did not match up to the theoretical yield, but again this is what I found.

Based on the analytical findings of myself and others in the research group we published the most PROBABLE structure of the cortinarins as cyclic polypeptides. I fully accept that minor revisions to the structure may be appropriate. However the points outlined by Matthies and Laatsch are hardly decisive!

In fact the articles by these workers have taken isolated points from our work and presented them out of context. Indeed they identified steroids in ether extracts of the fungi. This is very interesting but in the work in which we described the presence of polypeptides, the ether extracts were simply used as a clean-up stage and were discarded. The methanolic extraction is more interesting; the authors isolated fractions which gave reactions with either cinnamaldehyde/HCl or ninhydrin. Analysis of these fractions failed to show the presence of cortinarins. This does not surprise me since the cortinarins do not react with either compound.

I have some further criticisms of this paper: in the results and discussion section it is stated that, based on Rf values and reaction with acidic ninhydrin, grzymalin and benzonin were found and these were presumably identical to Tebbett's Cortinarins A and C respectively. This is an

incredible presumption based on a single TLC analysis with no standards. Indeed the authors make great importance out of Rf values, even quoting Rf values from my thesis of seven years ago. In fact Rf values are notoriously variable as a result of minor changes in solvent composition, temperature, humidity, and even different batches of TLC plates.

The comment that authentic samples were not available, in spite of substantial efforts is irrelevant to the scientific value of this article and is meant to infer that we have something to hide. It refers to two letters to the University of Strathclyde requesting samples of cortinarins. There is no mysterious reason why these samples are not available. Our work with these compounds ended in 1984, and I emigrated to the United States in 1988.

The authors conclude that they were unable to find any substance relating to cortinarins and based on the criticisms outlined above, the structural determination of the cortinarins must be incorrect. Matthies and Laatsch conveniently omit to mention that the cortinarins showed chemical and spectral properties consistent with the presence of an indole, that amino acid analysis and peptide sequencing was performed both by us and by an independant laboratory, that the amino acid sequence was confirmed by GC-MS and that the cortinarins were shown to cause renal necropsis in mice. In addition, they were unable to find any substance relating to cortinarins. How can they therefore conclude that the structural identification MUST be incorrect?

Finally, Matthies and Laatsch as well as some eminent mycologists would have us believe that orellanin is the sole cause of *Cortinarius* toxicity, probably because this fits into their chemotaxonomic divisions of this genus. However, despite considerable chemical investigation, orellanin has only been found to be present in a few species within the genus *Cortinarius*, notably *C. orellanus* and *C. speciosissimus*. This does not explain the reported toxicity of *C. splendens*, *C. limonius*, *C. sanguineus*, *C. phoenicius* and other species which are not closely related to the orellanus group. Incidentally, all of these species were found to contain toxic peptides. *C. splendens* in particular, which contains fairly high concentrations of cortinarin A, has been the cause of several severe poisonings in France and Switzerland.

The scientific community has a responsibility to advise the general public of the potential effects of exposure to toxic materials. We found toxic polypeptides to be present to some extent in all but one of over 100 species of *Cortinarius* which we examined. Because of the possibility of cumulative effects of these compounds on the kidney with continued exposure, we advised the public that ingestion of *Cortinarius* fungi should be avoided. How many more case reports of *Cortinarius* mushroom poisoning must appear before scientists involved in this field accept that toxins other than orellanin are present in this genus? Perhaps then they will adopt a more responsible attitude.

Ian R. Tebbett

Response: Prof. Dr. H. Laatsch and Dr. L. Matthies, Institut für Organische Chemie, Universität Göttingen, Tammannstrasse 2, D-W3400 Göttingen

31 January 1992

Sir,

Even recently, several investigations have attributed the nephrotoxicity of *Cortinarius orellanus* and *C. speciosissimus* exclusively to their content of orellanin; in contrast to Tebbett's findings, orellanin apparently displays the same toxic activity as the whole mushroom [1]. No other potent toxin except orellanin was found [2], and none of the numerous investigations on toxic *Cortinarius* mushrooms revealed nephrotoxic peptides.

It is however well-known, that plants and mushrooms of different geographic origin often develop significant chemical modifications. We therefore admit that Tebbett may have isolated (from Scottish mushrooms) fluorescent toxic compounds other than orellanin. According to his paper, the peptidic structure of these constituents seems clear as their mass fragmentation pattern is of extraordinary quality and allows an unusual precision of structure elucidation for Cortinarin A.

However, the chromatographic behavior of Tebbett's cyclopeptides, cyclohexane/ethylacetate (3:1) being the eluent, stands in complete contradiction to practical experience with peptides in general, and especially with the structurally related *Amanita* toxins. Even the well-known variability of chromatographic results cannot explain such a deviation.

We are also surprised that the cortinarins are not stainable with cinnamaldehyde/HCl, because the structurally similar *Amanita* toxins display a purple (amanitin) or blue (phalloidin) color. In general cinnamaldehyde/HCl is an excellent reagent for the detection of indole derivatives.

Following the procedures in Tebbett's cortinarin publications, we found only orellanin (resp. its decomposition products) and a minor amount of steroids being responsible for the fluorescence of the methanol extract of *C.*

speciosissimus. On chromatograms a color reaction with acids (or acidified ninhydrin) can be observed. This has perhaps led to Testa's erroneous assignment of these steroids as peptides[3]. We did not find any substances referable to cortinarins in methanol extracts of mushrooms from different locations, collected over a period of several years.

Summarizing our results we can state: a) The trifluoroacetic acid hydrolysis of Cortinarin C is inexplicable and cannot be corroborated with a model peptide, b) Our NMR spectra of synthetic 4-methoxyindole are distinctly different from data given by Tebbett (according to the original papers measured at 250 MHz, not 90 MHz). Despite the rapid H/D exchange in deuterated methanol, a sharp indole-NH signal was found by Tebbett. We only detected this signal by measuring in chloroform. c) The instability of hydroxylated tryptophanes and their ethers in acids has been extensively documented by Wieland and others. Nevertheless, 4-methoxytryptophane was isolated in excellent yield after hydrolysis of Cortinarin C. In polar *C. speciosissimus* extracts, not even traces of 4-hydroxy- and 4-methoxytryptophane were found by us after hydrogenation and hydrolysis, though suitable protection procedures were used and the synthetic acids served as internal standards.

Meanwhile orellanin has also been found in *Cortinarius brunneofulvus, C. fluorescens, C. henrici, C. orelannoides* and *C. rainieresis*[4]. Therefore we agree that the ingestion of *Cortinarius* mushrooms should be strictly avoided. Other *Cortinarius* mushrooms may contain further toxic components, but we question the involvence of peptides with structures of the cortinarins; all details leading to this result have been published.

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H. Laatsch
L. Matthies

Announcements

Pfizer Research Prize 1992

2 Awards of Swiss Fr. 30.000.– each.
Applicants should be scientists working in Switzerland, age limit is 45 years.
Subject: Basic or clinical research on the cardiovascular system. Published or ready-to-publish work originating mainly from Switzerland.
Jury: Independent scientific committee of the Pfizer Foundation presided by Proffs. C. Perret (Lausanne) and F. Follath (Zurich).
Deadline for application: 15 July 1992.
The Prizes will be awarded in January 1993.
Details for applications can be obtained from:
Stiftung Pfizer-Forschungspreis,
Postfach, CH-8048 Zurich.

Endowment of the Sandoz Prize for Therapeutically Relevant Pharmacological Research

Basic research in pharmacology and the clinical application of medicaments belong together. Nevertheless, constant effort is necessary to prevent the two disciplines growing apart from each other, and to make sure that the ultimate aim – progress in therapeutic applications – remains central. Sandoz AG of Nürnberg, together with the German Society for Pharmacology and Toxicology, wants to emphasize this point with its new prize of 20.000 DM.

The prize will be awarded for work bridging the gap between pharmacological and clinical research.

Applications for the prize can be made by pharmacologists and by interdisciplinary teams working in Germany. Nominations can also be made by third parties.

The members of the jury will be neutral experts, independent of the firm. The prize will be awarded at the Annual Congress of the German Society for Pharmacology and Toxicology. It will be awarded for the first time in March 1993. The final date for applications and nominations (with all the necessary documentation) is August 1st 1992.

Instructions for applicants, and further information, can be obtained from the secretariat of the Sandoz Prize for Therapeutically Relevant Pharmacological Research, Sandoz AG Nürnberg, Deutschherrnstr. 15, D-W 8500 Nürnberg 80, Fed. Rep. of Germany.