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BIOLOGICALLY ACTIVE COMPOUNDS FROM HIGHER FUNGI

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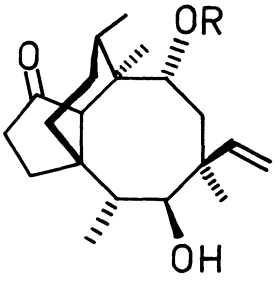
Abstract - From mycelial cultures and fruiting bodies of Higher Fungi several metabolites with antibiotic and cytotoxic activity have been isolated. Some recent progress in this field is discussed including the chemistry of sesquiterpene antibiotics, striatals, strobilurins and hemiterpenoids. Myxomycetes appear to be a source of unusual pigments which often exhibit antibiotic properties.

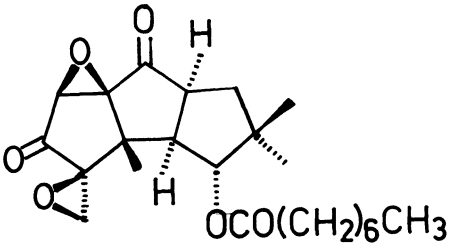
Fruiting bodies of Higher Fungi show many different colours, colour reactions, odors and tastes which are useful characters for their identification. The chemistry behind these phenomena opens a wide field of research, and in recent years several new types of pigments, terpenes, amino acids, alkaloids and polysaccharides have been isolated from mushrooms and toadstools.

The screening of mycelial cultures from Basidiomycetes for antibiotics has led to the discovery of pleuromutilin (1) (Ref. 1) from which tiamulin (2) has been prepared, an antibiotic used in veterinary medicine for treatment of infections caused by gram-positive bacteria and mycoplasmas (Ref. 2). Diketocoriolin B (3), an oxidation product of coriolin B from Coriolus consors, has received great attention recently because of its ability to increase the number of antibody-forming cells at very low concentrations (Ref. 3).



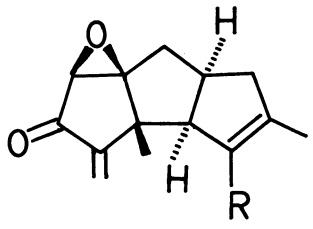
1, R= $COCH_2OH$

2, R= $COCH_2SCH_2CH_2NEt_2$



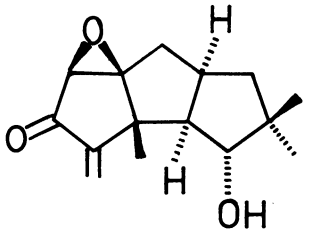
3

During a systematic screening carried out by Dr. T. Anke and coworkers at Tübingen, three new antibiotics have been isolated from cultures of *Pleurotellus hypnophilus* which are closely related to the coriolins (Ref. 4). Pleurotellol (4) and pleurotellic acid (5) possess a rearranged hirsutane skeleton. The location of the hydroxy group in the third compound, hypnophilin (6), points to its possible role as precursor in the biosynthesis of the rearranged compounds. All three metabolites inhibit the growth of several bacteria, 4 and 6 show pronounced cytotoxic activity.



4, R= CH_2OH

5, R= CO_2H



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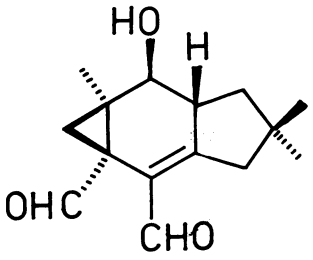
Another highly active antibiotic, merulidial (7), is produced by cultures of *Merulius tremellosus* (Ref. 5). It has the same carbon skeleton as isolactaro= rufin from fruiting bodies of *Lactarius rufus* (Ref. 6). On oxidation with pyridinium chlorochromate 7 is transformed into the homo-p-quinone derivative 8 with retention of the antibiotic activity. Merulidial gives a positive Ames test for mutagenic activity which is of interest in respect to the occurence of similiar dialdehydes like isovelleral (9) in the fruiting bodies of edible *Lactarius* species (Ref. 7). The presence of sesquiterpene dialdehydes causes the peppery taste of these mushrooms which enjoy great popularity as food in Eastern and Northeastern Europe.



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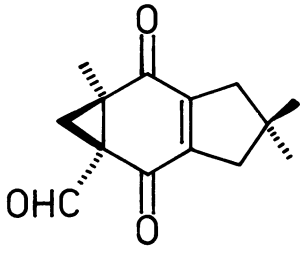
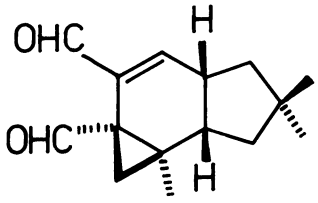


8

Isovelleral is structurally closely related to the well known marasmic acid (10) from cultures of *Marasmius conigenus* (Ref. 8). 10 has strong antibiotic and cytotoxic activity and proved to be mutagenic as indicated by the Ames test (Ref. 9). Reduction of the aldehyde function leads to complete loss of biological activity.



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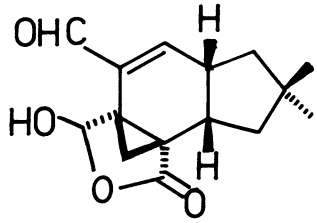
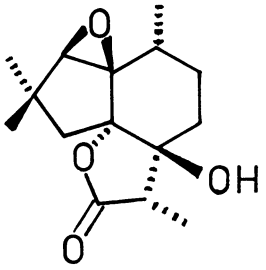


10

Most of the sesquiterpenes from Basidiomycetes are biosynthetically derived from humulene (Ref. 10). This may also be the case for a series of unique lactones formed by *Marasmius alliaceus* which was first studied by Thaller and coworkers (Ref. 11). In addition to alliacolide (11) and alliacol B (13) Anke isolated the primary alcohol alliacol A (12). On acetylation with Ac_2O/pyridine 12 undergoes rearrangement to yield the tertiary acetate 14, indicating the high strain of the endocyclic double bond. In contrast to 11 the two α,ß-unsaturated lactones 12 and 13 show weak antibiotic activity against bacteria and high cytotoxic activity against cells of the ascitic form of Ehrlich carcinoma (Ref. 12).



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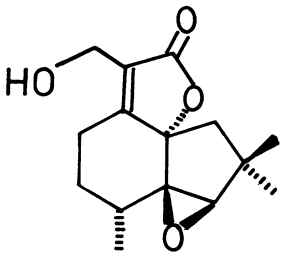

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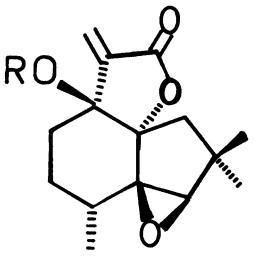
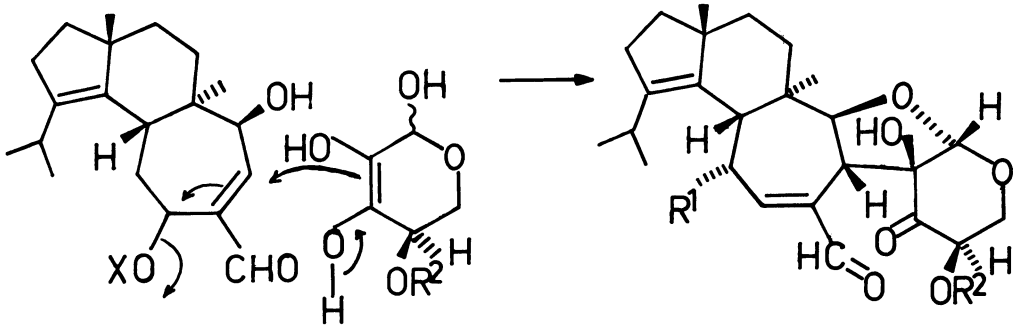


13, R= H

14, R= Ac

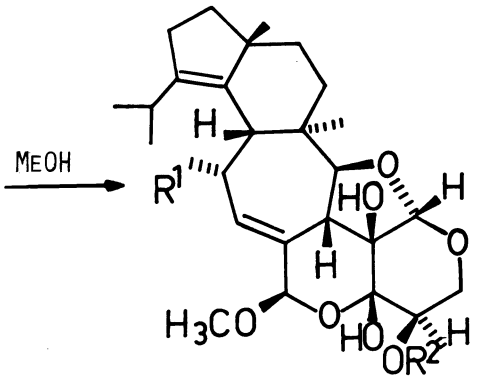
A series of complicated terpenoid compounds which exhibit activity against a range of gram-positive bacteria, some gram-negative bacterial and fungi imperfecti has been isolated from cultures of the bird's nest fungus *Cyathus striatus* (Ref. 13). The striatals A, B and C (15)-(17) may be considered to arise biosynthetically by condensation of a cyathane derivative with a 3-oxopentose unit. Diterpenes with the cyathane skeleton are typical metabolites of *Cyathus* species as has been shown by the elegant work of Ayer and coworkers (Ref. 14). On standing in methanol striatals add one molecule of solvent to give the corresponding striatins 18 - 20 without loss of antibiotic activity.



15, R^1= H, R^2= Ac

16, R^1= OH, R^2= H

17, R^1= OH, R^2= Ac

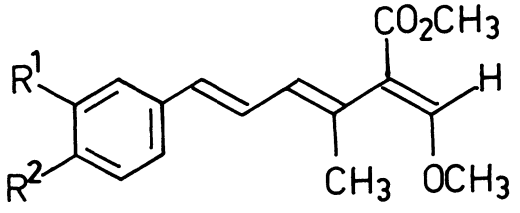


18, R^1= H, R^2= Ac

19, R^1= OH, R^2= H

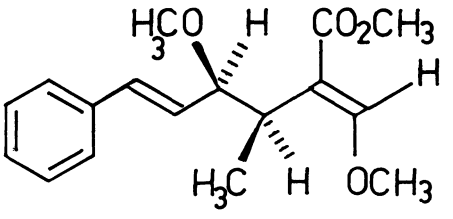
20, R^1= OH, R^2= Ac

In all compounds mentioned so far, the antibiotic and cytotoxic activity is connected with the presence of an α,ß-unsaturated carbonyl system (Ref.15). We have found recently that the strong inhibition of respiration in fungi and Ehrlich carcinoma by strobilurin A (21) and B (22) (Ref. 16) is critically dependent on the presence of the terminal (*E*)-ß-methoxyacrylate function. The synthetic strobilurin A with (*Z*)-geometry of the terminal double bond is devoid of any antibiotic activity.



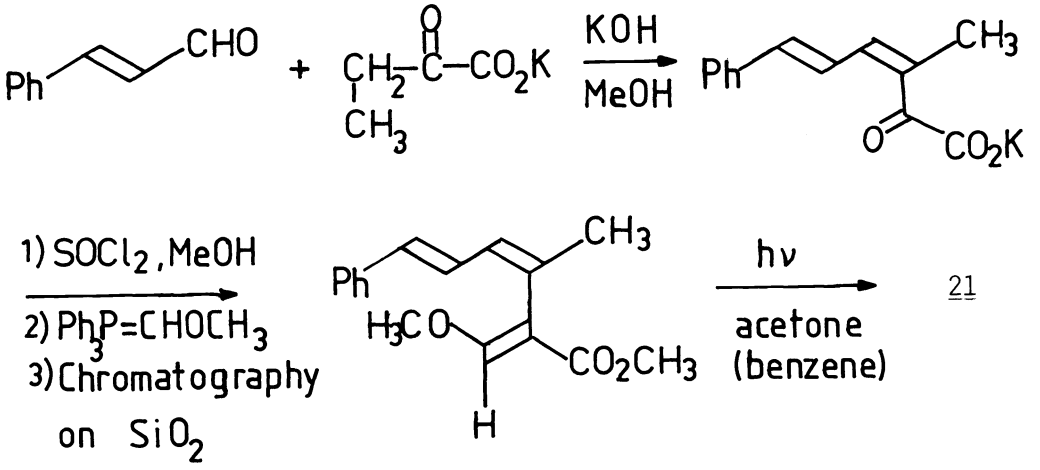
21, $R^1 = R^2 = H$

22, $R^1 = OH$, $R^2 = Cl$



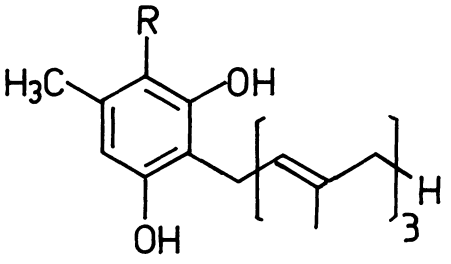
23

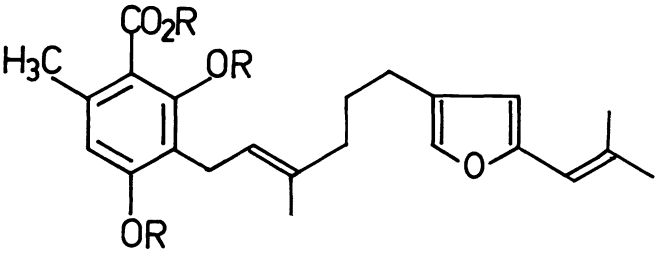
Strobilurins are produced by several species of *Strobilurus* and *Mycena*, and the recent isolation of oudemansin (23) from cultures of *Oudemansiella mucida* adds another member to this interesting group of fungal metabolites (Ref. 17). An antibiotically active substance, mucidin, has been obtained before from the latter fungus (Ref. 18). It is used in the CSSR for the treatment of dermatomycoses. Strobilurin A has been synthesized recently in four steps from 2-oxo-butanoic acid and cinnamaldehyde (Ref. 19):



21

Only few antibiotically active compounds have been isolated from fruiting bodies of Higher Fungi. In 1950 Japanese chemists isolated grifolin (24) from sporophores of *Albatrellus confluens* (= '*Grifola confluens*') (Ref. 20). The hemiterpenoid nature of this slightly bacteriostatic metabolite was established by Goto, Kakisawa and Hirata (Ref. 21).
Recently we investigated the sporophores of the related *Albatrellus cristatus*, specimens of which had been collected in the U.S.A. and in Bavaria. The American variety contained only a small quantity of grifolic acid (25). The Bavarian variety, however, yielded large amounts of cristatic acid (26) by simple extraction with petrol ether (Ref. 22). 26 is easily recognized on TLC plates by the development of a green colour on exposure to vapours of acids. This colour reaction is caused by the 2,4-disubstituted furane ring present in the modified farnesyl side chain. The same moiety seems to be responsible for the antibiotic and cytotoxic activity of 26. Whereas the antibacterial activity is completely lost on permethylation to (27), this





24, R = H

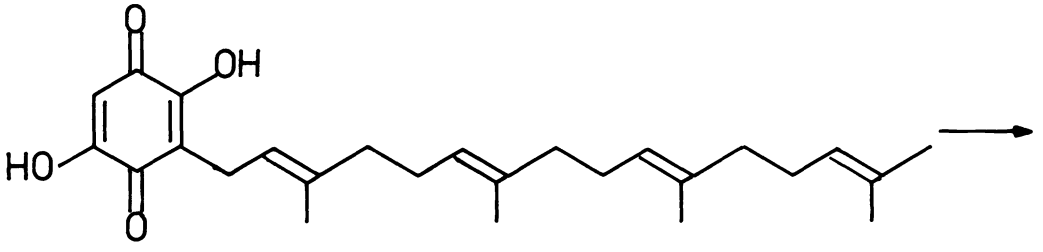
25, R = CO_2H

26, R = H

27, R = CH_3

compound still shows a strong inhibitory effect against cells of the ascites form of Ehrlich carcinoma.

A further modification of the side chain in a hemiterpenoid mushroom metabolite is shown by tridentoquinone (30), the beautiful red pigment of the fruiting bodies of *Suillus tridentinus* (Ref. 23). It may be easily obtained by extraction of the mushrooms with petrolether and filtration over a polyamide column. The ansaquinone structure of (30) could be derived biogenetically from boviquinone-4 (28) via epoxidation to (29). (30) may then be formed by intramolecular electrophilic attack of the terminal epoxide on the hydroxybenzoquinone with subsequent formation of the dihydrofurane ring. After we have developed a syntheses of (29) feeding experiments can be started to prove this hypothesis.



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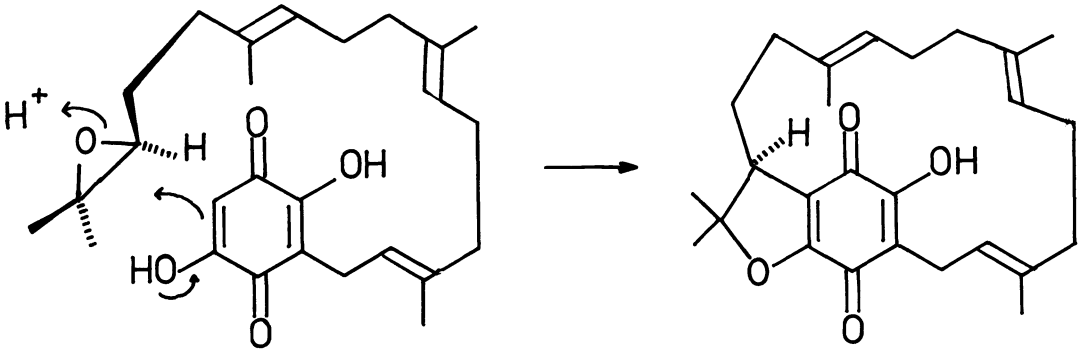
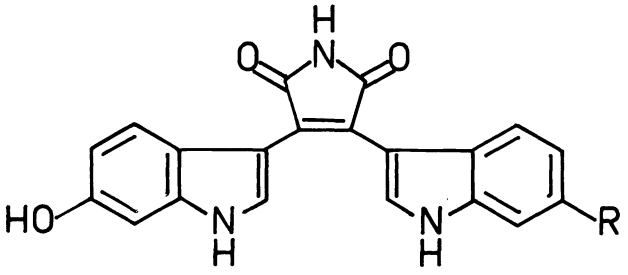


29 30

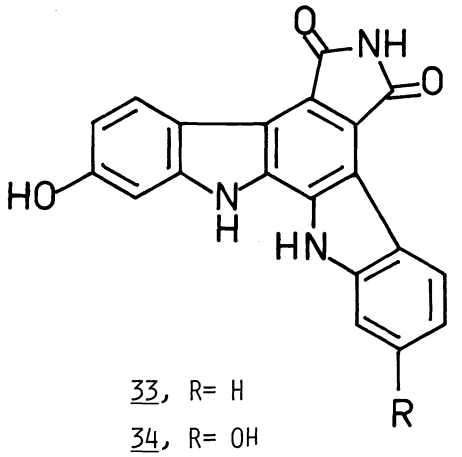
Interesting objects for chemical studies are the Myxomycetes or Mycetozoa, the latter name indicating their taxonomic position between the animal and plant kingdom. At one stage they form a free-living, mobile mass of protoplasm, which suddenly organizes to form small sporophores, which often exhibit beautiful geometrical structures and colours. Myxomycetes may be found together with Higher Fungi on dead wood, decaying leaves and other plant debris. With the exception of a few species used for molecular biological studies not much is known about the chemistry of these unique organisms.

We have recently studied the pigments formed during the fructification of three typical species. The red pigment of *Arcyria denudata* could be chromatographically separated into three red and two yellow components which turned out to belong to a new class of indole pigments (Ref. 24). Arcyriarubin B (31) and C (32) contain the basic system from which the yellow, fluorescent arcyriaflavins B (33) and C (34) can be derived by cyclization and subsequent dehydrogenation. In arcyroxepin (35) the two indole rings are connected by an ether bridge.



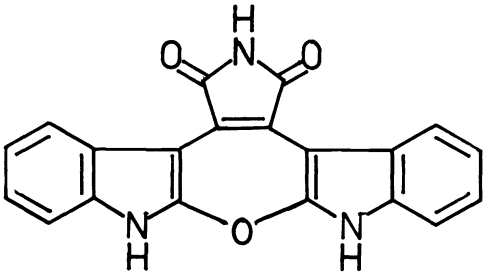
31, R= H

32, R= OH



33, R= H

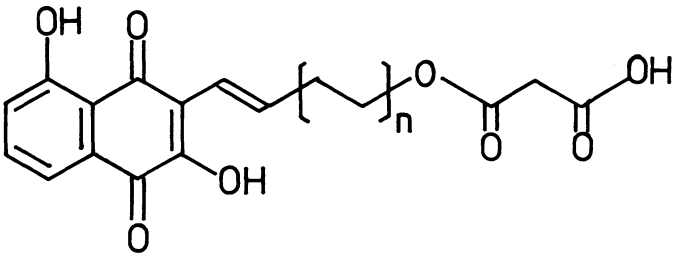
34, R= OH



35

All pigments isolated so far from *Arcyria* exhibit antibiotic activity against several bacteria. Possibly they are used by these fragile organisms as a protection against microbial attack during fructification. The arcyriaflavins are structurally related to staurosporin, an antibiotic and hypertensive agent which has recently been obtained from cultures of *Streptomyces staurosporeus* (Ref. 25).

Completely different pigments are formed during the fructification of *Trichia floriformis* and *Metatrichia vesparium*. The first mentioned species produces high concentrations of an orange naphthoquinone derivative, trichione (36), whereas the second contains as main pigment its homolog homotrichione (37) (Ref. 26). Both compounds are accompanied by minor components which show considerable antibiotic activity.



36, n = 1

37, n = 2

These first results indicate that Myxomycetes produce a number of interesting metabolites, some of them exhibiting biological activity. Despite of the difficulties to obtain sufficient material for structural studies further investigations appear to be highly rewarding.

Acknowledgement - The work described on antibiotics from mycelial cultures would not have been possible without the excellent collaboration with Dr. T. Anke, Professor F. Oberwinkler and their group from Tübingen University. They have carried out the isolation and biochemical evaluation of the antibiotics, and it is a great pleasure for me to express my sincere gratitude to them. Very special thanks go to my dedicated and skillful coworkers, whose names appear in the references. Financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

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