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Slime moulds (*Myxomycetes*) as a source of new biologically active metabolites

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Abstract - An investigation of the chemistry of acellular slime moulds revealed the presence of several types of metabolites. Tetramic acid derivatives and physarochrome A are responsible for the pigmentation of plasmodia, whereas naphthoquinones occur in fruiting bodies of *Trichia* and related genera. Two unusual pyrone derivatives have been isolated from plasmodia of *Fuligo* and *Ceratiomyxa* species. *Arcyria denudata* produces a whole family of biogenetically related bisindolylmaleimides, some of them possessing unusual structures and exhibiting phosphorescence phenomena.

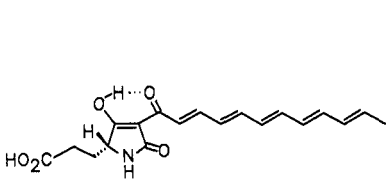
The acellular slime moulds or Myxomycetes are unique organisms which take an intermediate position between the plant and animal kingdoms (ref. 1). In a certain stage of their life cycle they form jelly-like plasmodia which feed on bacteria and are able to move by a synchronized perpendicular flow of their protoplasm.

After some time, the plasmodium changes within a few hours into small, fungus-like fruiting bodies (peridia) which often exhibit delicate structures and colours. They release spores from which protozoe-like amoeba originate which mate and finally aggregate again to the plasmodial stage.

During this life cycle light plays an important role (ref. 2). Thus it has been found that blue light causes young plasmodia to move away from the light source whereas it has an attractive effect to aged plasmodia, guiding them to exposed sites suitable for spore propagation. Light is also necessary to induce sporulation in several species (ref. 3). Obviously, the light response is connected to the presence of yellow pigments in the plamodia, at least in some cases.

In spite of their interesting physiology and biochemistry, the secondary metabolism of Myxomycetes remained virtually unknown. In this lecture I would like to present first insights into the chemistry of these organisms.

Let us begin with compounds from plasmodia. One of the most common slime moulds is *Fuligo septica*. Its yellow plasmodia can be found on hips of old bark after rainfall. We were able to isolate one of the main pigments, fuligorubin A (1) (ref. 4, 5) after chromatography of the crude extract on Sephadex LH-20. The compound exhibits an absorption maximum at 450 nm in accord with its pentaene chromophore. The acyltetramic acid structure explains the strong tendency of 1 to bind calcium and magnesium ions. The 3-(*R*) configuration of fuligorubin A was proved by comparison with a synthetic model compound. This points to a biosynthesis in which D-glutamic acid is condensed with a heptaketide chain.



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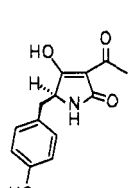

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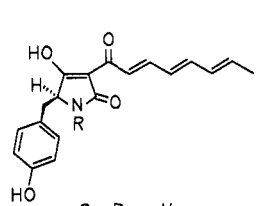
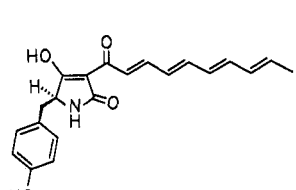


3, R = H
4, R = CH_3



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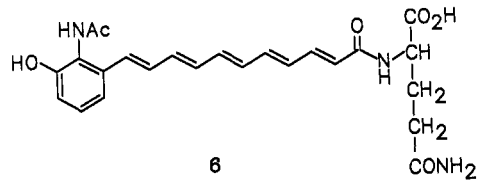
Acyltetramic acids are also responsible for the orange-yellow colour of plasmodia from *Leocarpus fragilis*. This common slime mould forms insect egg-like fruiting bodies which are found in autumn attached to dead conifer needles, grass or similar substrates. We have isolated four compounds **2-5** from this organism which are biogenetically derived from L-tyrosine.

Acyltetramic acids have been isolated before from streptomycetes and fungi. They exhibit remarkable antibiotic and cytotoxic activities and some of them act as tremorgenic mycotoxins (ref. 6). The possibility that these compounds may protect the vulnerable plasmodia of slime moulds against the attack of microorganisms has to be considered as well as their possible role as photoreceptors or metal chelating agents.

Physarum polycephalum is one of the few species of Myxomycetes which can be easily cultivated. Its physiology and biochemistry has therefore been well investigated. Many investigators were intrigued by the possible role of its yellow plasmodial pigments as photoreceptors and it had been claimed that these pigments are carotenoids, pteridines, flavins, flavones, peptides or nitrogen-containing polyenes (ref. 7).



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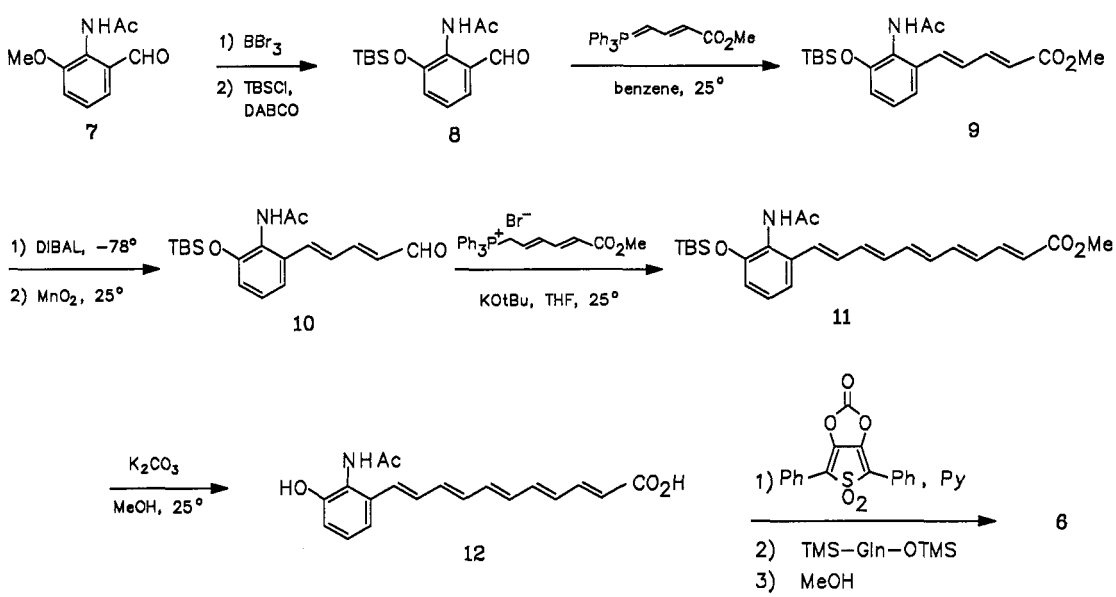
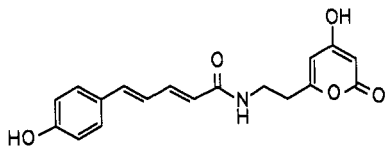
Recently, we were able to determine the structure of one of the main pigments which we have named physarochrome A (6) (ref. 8). It is a rather unique combination of L-glutamine with a penta-unsaturated carboxylic acid carrying a 2-acetylamino-3-hydroxyphenyl end group. Because this pigment can only be obtained by a tedious procedure in amounts of a few milligrams from the crude extract, we have developed a total synthesis. It starts from 2-acetylamino-3-methoxy-benzaldehyde (7) which is transformed into the *tert*-butyldimethylsilyl (TBS) derivative **8** by cleavage of the methyl group and subsequent treatment with TBS-chloride. Wittig reaction then leads to the diene ester **9** which after conversion into the aldehyde **10** is transformed into the pentaene ester **11** by a second Wittig reaction. As indicated by the NMR spectra, the formation of the unsaturated chain proceeds mainly to the all-(*E*)-configuration. During mild saponification of ester **11** an unexpected loss of the TBS protecting group was observed. The free acid **12** was then coupled with per-trimethylsilylated L-glutamine and after hydrolytic work-up a yellow pigment was obtained which was identical with physarochrome A (6). The synthesis of **6** is at present being optimized and should provide sufficient amounts for physiological investigations.



7 8 9 10 11 12 6

The light response of Myxomycetes with colourless plasmodia could be caused by compounds which absorb in the UV region. We were able to isolate from plasmodia of *Fuligo septica* an unusual pyrone derivative, fuligopyrone (**13**). From *Ceratiomyxa fruticulosa* ceratiopyrone (**14**) was obtained in which the pyrone ring is connected with a long aliphatic side chain. Both compounds contain a dienyl chromophore which is conjugated to a benzene ring or the pyrone residue, respectively.



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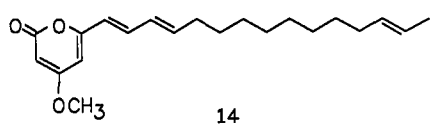

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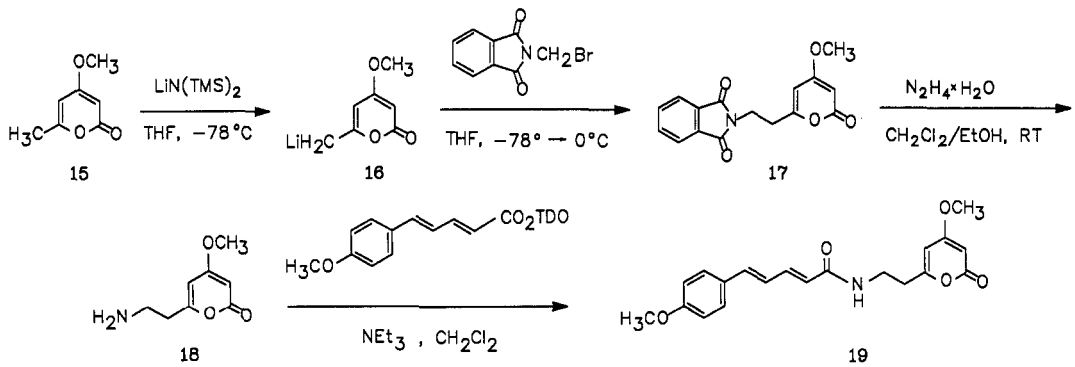
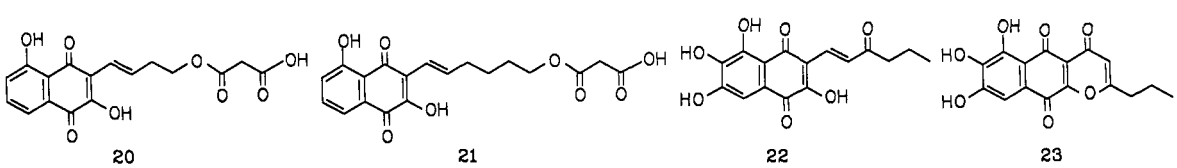
The structure of fuligopyrone was secured by a synthesis of its dimethyl ether. The synthesis starts from 4-methoxy-6-methyl-2-pyrone (**15**) which can be lithiated exclusively at the methyl group with lithium hexamethyldisilazane in THF at -78°C. Reaction of the resulting anion **16** with *N*-bromomethylphthalimide yielded the phthaloyl derivative **17** which was transformed into the free amine **18** by treatment with hydrazine hydrate. Coupling of **15** with 5-(4-methoxyphenyl)-2,4-pentadienoic acid was achieved in high yield by the TDO ester method (ref. 9). The product was identical with fuligopyrone dimethyl ether (**19**) obtained from the natural compound by treatment with diazomethane. The photochemical and biological properties of these pyrones are under investigation.

Sporophores of Myxomycetes often exhibit yellow, orange, brown or red colours. These objects have to be collected in their natural habitat which creates problems because of their small size. This may explain the paucicity of data on their chemical composition (ref. 10).

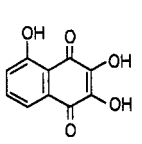


TDO=2,3-dihydro-3-oxo-2,5-diphenylthiophen-1,1-dioxide

The delicate sporophores of *Trichia* and *Metatrichia* species can be found on dead logs and branches. They contain as main pigments naphthoquinone derivatives like trichione (**20**) and homotrichione (**21**) (ref. 11). All compounds incorporate a 2,5-dihydroxynaphthoquinone chromophore to which an unsaturated side chain of four or six carbon atoms is attached at 3-position. In some cases a terminal hydroxy group is present which carries a malonic acid half 8ester residue. Treatment of the plasmodia of *Lindbladia tubulina* with mineral acid induces a colour change from dark brown to red, for which the pigments lindbladione (**22**) and lindbladiapyrone (**23**) are responsible, which are present in the slime mould in the form of salts.



20 21 22 23



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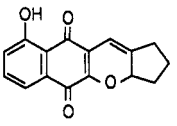

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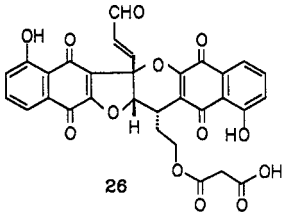

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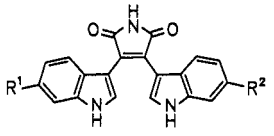
The main pigments are accompanied by a number of biogenetically closely related minor compounds. Thus, 2,3,5-trihydroxynaphthoquinone (**24**) from *Trichia floriformis* apparently has lost its side chain by oxidative cleavage. Vesparione (**25**) (ref. 12) from *Metatrichia vesparium* exhibits antibiotic properties and may originate from **21** by cyclisation of the side chain (ref. 8). The more complex pigment TF 1 (**26**) from *T. floriformis* may be formed from two molecules of trichione.

The most interesting group of compounds so far has been found in sporophores of *Arcyria* species, especially the beautifully red *Arcyria denudata*. A TLC of the crude extract reveals the presence of numerous red, purple, green and yellow spots, some of them exhibiting intense fluorescence under UV light. Unfortunately, the organism is not very common and one has to be lucky to collect it in quantities sufficient for a chemical investigation.

The main pigments of *Arcyria denudata* and related species are bisindolylmaleimides (ref. 13) which we have named arcyriarubins (**27**) and arcyriaflavins (**28**) (ref. 14, 15). According to the number of hydroxy groups present in the 6- and 6'-positions, they are classified as types A, B and C. The only exception is arcyriaflavin D (**29**) from *Dictydiaethalium plumbeum* which has a 5,6'-dihydroxylation pattern. The arcyriarubins and arcyriaflavins exhibit moderate antibiotic activities against bacteria and fungi.



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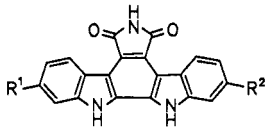

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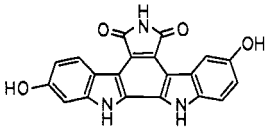
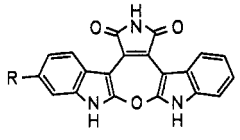


29

	R^1	R^2		
Arcyriarubin A	H	H	Arcyriaflavin A	
B	OH	H	B	
C	OH	OH	C	

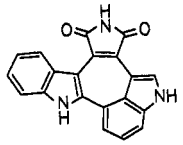
Recently, chlorine containing analogues of arcyriaflavin A have been isolated from cultures of *Nocardia aerocoligenes* (ref. 16). Rebeccamycin and its 11-dechloro derivative are *N*-glycosides with tumor inhibiting properties. Structurally related are the complex glycosides staurosporine (ref. 17) and antibiotic SF-2370 (ref. 18) which are produced by streptomycetes. These compounds show a number of interesting biological properties and act as inhibitors of protein kinase C.

A closer inspection of the *Arcyria* compounds revealed the presence of several new structural types which have not yet been found in streptomycetes.



30 a, R = H

30 b, R = OH



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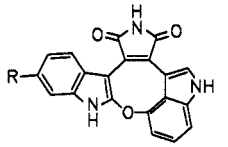
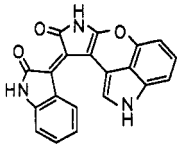


32 a, R = H

32 b, R = OH



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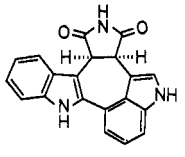

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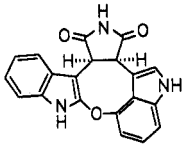

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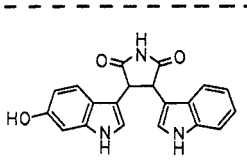

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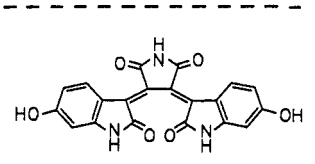


37

An interesting variation is represented by the red arcyroxepins A and B (**30a**, **30b**) (ref. 14). Both compounds show strong broadening of the 4-H signals in the ^{1}H-NMR spectrum at room temperature and may be detected on thin layer chromatograms by their characteristic colour change to violet on exposure to ammonia.

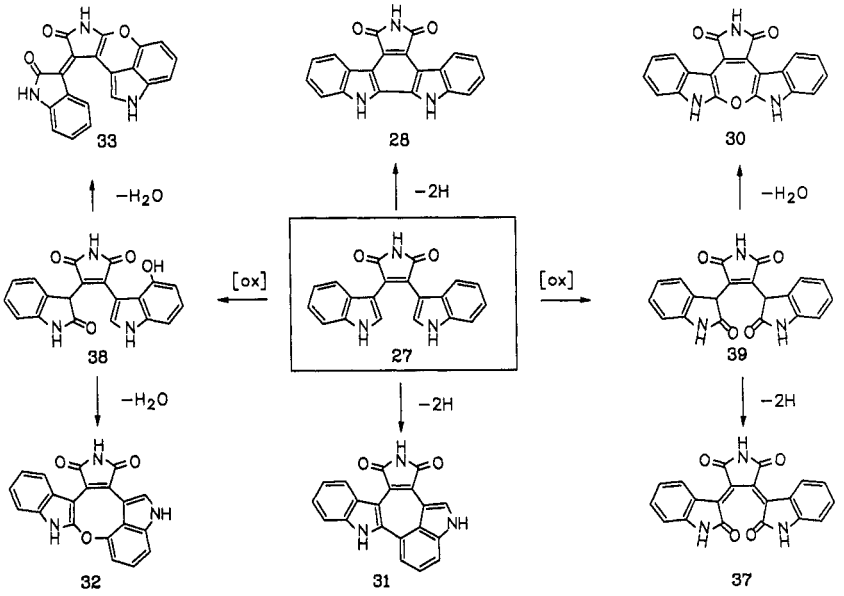
Recently, we have elucidated the structures of arcyriacyanin A (**31**) and the acyroxocins A and B (**32a**, **32b**). As one can easily see, these compounds are variations of the arcyriaflavin and arcyroxepin types respectively, in which the connection of the two indole moieties has occurred between the 2-position of one ring and the 4-position of the other. The structure of **32b** has been confirmed by a single crystal X-ray analysis.

A further variation of the general theme is represented by arcyroxindole A (**33**). The structure of this orange coloured pigment is supported by a nuclear Overhauser effect signal enhancement of H-4' in the ^{1}H-NMR spectrum on irradiation at the frequency of H-2 and *vice versa*. Computer calculations indicate that the compound is nonplanar and has a helical structure. The inherent chirality of its chromophore leads to a series of strong Cotton effects in the CD-spectrum.

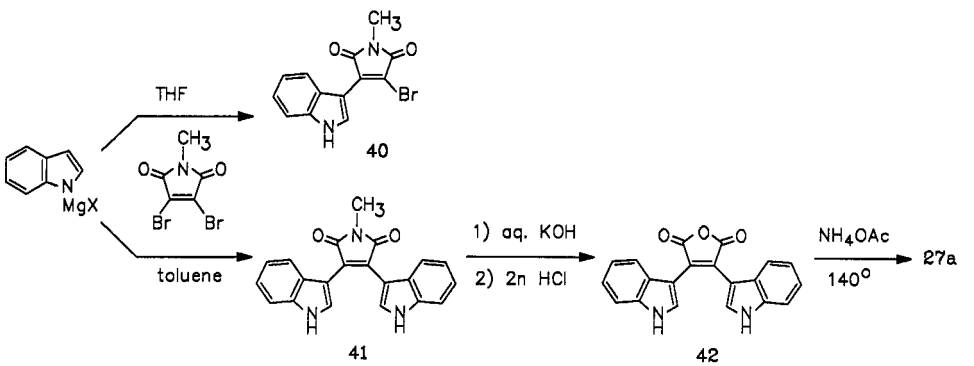
Compounds with further modifications of the basic structure can be found in the yellow sporangia of *Arcyria nutans*. Besides arcyriaflavin A and B this species contains two colourless dihydro derivatives of arcyriacyanin A (**34**) and arcyrioxocin A (**35**). Inspection of molecular models and the NMR spectra point to a *cis*-relationship of the two angular protons in both compounds. Finally, dihydroarcyriarubin B (**36**) and the green arcyriaverdin C (**37**) have to be mentioned which occur in *A. denudata*.

The possible biosynthetic relationships of the *Arcyria* compounds are given in the following scheme. It can be assumed that the arcyriarubins (**27**) may be oxidatively cyclised either to the arcyriaflavin (**28**) or the arcyriacyanin (**31**) type, depending on the conformation of the starting compound. Hydroxylation in 2- and 4'-position may then lead to an intermediate (**38**) which may either cyclise to the arcyroxocin (**32**) or arcyroxindole type (**33**) of pigments. On the other hand, arcyriarubin may be oxidized to the bisoxindole derivative (**39**) which subsequently may undergo ring closure to the arcyroxepin system (**30**) or further oxidation to the arcyriaverdin type (**37**). Of course, other mechanisms including oxidation of **27** or similar intermediates, followed by electrocyclic ring closures (ref. 13), or radical couplings may be postulated to explain the variety of *Arcyria* compounds. In this respect, the recent observation that arcyoxepin A rearranges to arcyroxocin A in refluxing toluene may be relevant.

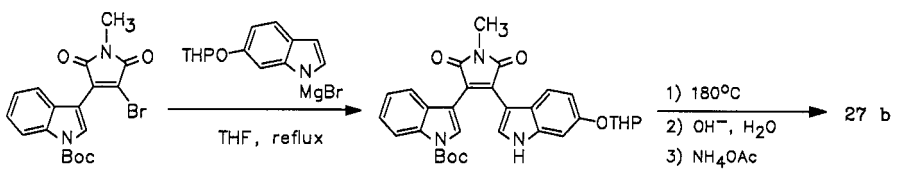
The interesting biological activities of rebeccamycin and the staurosporine group of compounds make the biological evaluation of the different bisindolylmaleimides from Myxomycetes highly desirable. Unfortunately, these compounds can only be isolated in very small quantities from slime moulds and syntheses of the different types of bisindolylmaleimides are therefore necessary.



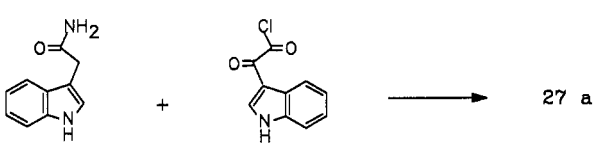
We have developed a simple synthesis of the arcyriarubins **27** by condensation of indolylmagnesium iodide with *N*-methyl-2,3-dibromomaleimide (ref. 14, 19, 20). Depending on the solvent, either the mono- or disubstitution products **40** and **41** can be obtained in high yields (ref. 21). Alkaline hydrolysis of the bisindolyl product **41**, followed by heating of the resulting anhydride **42** with ammonium acetate, yielded arcyriarubin A (**27a**).



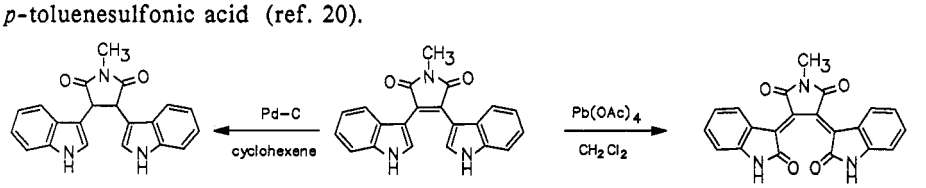
For the synthesis of unsymmetrically substituted arcyriarubins the NH-group at the monosubstituted compound **40** has to be protected. Coupling of the Boc derivative of the monoindolyl compound **43** with 6-(tetrahydropyranyloxy)indole magnesium bromide yielded the arcyriarubin B derivative **44** which was deprotected by simply heating to 180°C. Arcyriarubin B (**27b**) could thus be obtained in high overall yield.



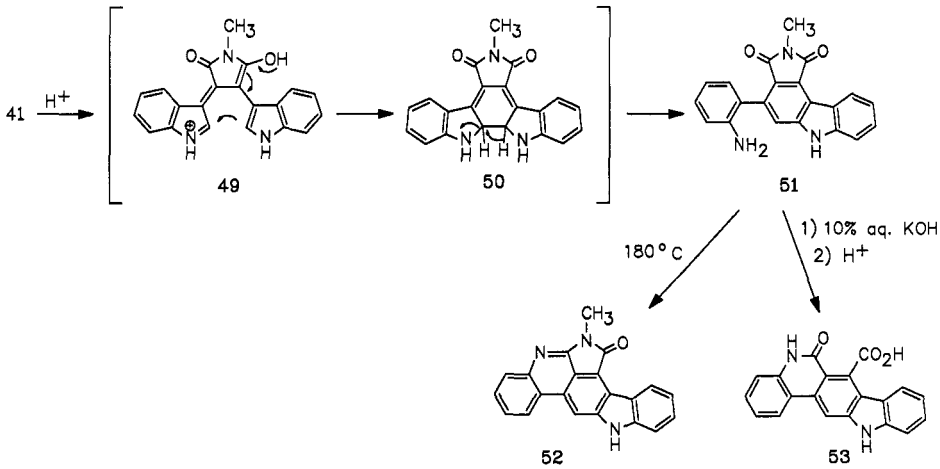
A biomimetic arcyriarubin synthesis should proceed *via* coupling of two indolylacetic acid units at the α-positions of their side chains. Thus, condensation of β-indolyl acetamide (**45**) with β-indolylglyoxylic acid chloride (**46**) leads to arcyriarubin A, albeit in only 11% yield (ref. 22). A related synthesis of arcyriarubin A from β-indolylacetic acid, in which an oxidative coupling of the derived dianion is the key step, has recently been developed by Bergman and Pelcman (ref. 23). From other approaches to bisindolylmaleimides, only Raphael's elegant synthesis of arcyriaflavin B shall be mentioned (ref. 24).



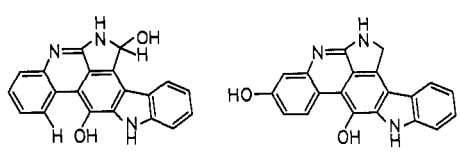
The arcyriarubin chromophore can be easily converted to other types of compounds. Thus, transfer hydrogenation of *N*-methylarcyriarubin A (**41**) with cyclohexene in the presence of palladium on charcoal leads to *N*-methyldihydroarcyriarubin A (**47**). On oxidation of **41** with lead tetraacetate in dichloromethane a dark green compound **48** with the arcyriaverdin chromophore is formed. Treatment of arcyriarubin C (**27c**) under the same conditions yields the natural product **37**. The arcyriarubins may be dehydrogenated to arcyriaflavins by treatment with DDQ in the presence of traces of *p*-toluenesulfonic acid (ref. 20).



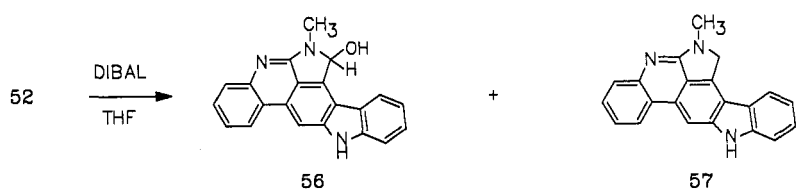
On heating *N*-methylarcyriarubin A (**41**) in toluene in the presence of traces of *p*-toluenesulfonic acid, the formation of an orange-yellow carbazole derivative **51** is observed. Obviously, the intermediate **50**, formed by electrophilic attack of the iminium salt **49** at the neighbouring indole ring, is stabilized by elimination of the amino group. On heating **51** to 180°C, quinoline ring closure takes place and the hexacyclic compound **52** is produced, which exhibits an intense blue UV fluorescence.



During these investigations we became intrigued by the presence of a spot on the TLC plates which showed bright blue fluorescence under UV light and a strongly delayed green phosphorescence after switching off the UV lamp. The afterglow is visible for nearly 30 seconds! This phenomenon is not only visible on silica plates but also in a glycerol matrix at the temperature of liquid nitrogen. We soon found out that the substance responsible for this effect was the quinolonocarbazole carboxylic acid **53**. This compound can be easily prepared by heating amine **51** with aqueous potassium hydroxide. The free carboxylic acid exhibited the most efficient phosphorescence, whereas its amides and esters showed this phenomenon in a less spectacular way.
During these investigations we discovered that extracts of *Arcyria* species contain similar substances with delayed green phosphorescence on TLC plates. We were able to isolate these compounds and elucidate their structure. Arcyrin A (**54**) and arcyrinin B (**55**) possess the same basic skeleton as the synthetic compound **52** but carry an additional hydroxy group and have undergone reduction of the amide function to a hemiaminal or an amine moiety, respectively. The ^{1}H-NMR signals of the hemiaminal part of arcyrin A are in close agreement with those of 7-hydroxystaurosporine (UCN-10), a potent protein kinase C inhibitor which has recently been obtained from a strain of *Streptomyces* (ref. 25).



The structures of arcyrin A and arcyrinin B are further supported by comparison with model compounds **56** and **57** which can be obtained from **52** by reduction with DIBAL. **56** and **57** exhibit the same green phosphorescence on TLC plates as the natural products.



This first exploration of slime mould chemistry demonstrates that these fascinating organisms have developed a rather unique secondary metabolism which offers a wide field for further studies. Only a few of the more than 500 known species have been investigated so far. For further progress, however, the development of methods for the cultivation of Myxomycetes is crucial, to overcome the notorious paucicity of material.

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