Pure & Appl. Chem., Vol. 65, No. 6, pp. 1309–1318, 1993.
Printed in Great Britain.
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Biosynthesis and synthesis of bioactive natural lactams

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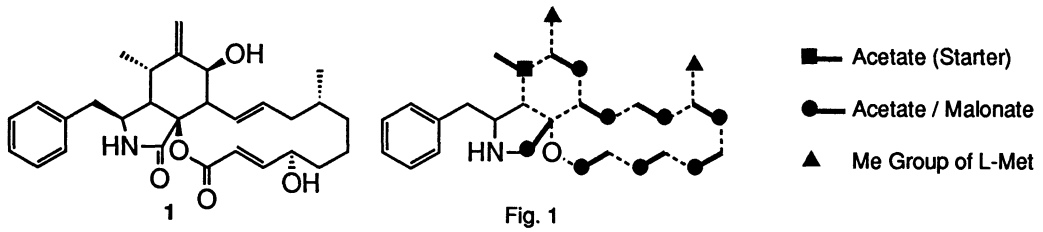
Abstract: A lactam group is a characteristic structural element of many microbial secondary metabolites. Examples of own biosynthetic studies are the cytochalasans and pseurotins which are γ-lactams. Detailed results are presented for the β-lactam tabtoxin (wildfire toxin) and tabtoxinine β-lactam. The biosynthetic building blocks were established. The methyl group of L-methionine provides the carbonyl-C-atom of the β-lactam moiety. In the course of approaches to the synthesis, 1-aminocyclopropanecarboxylic acid, 1-aminocyclopropanol (coprinol) and coprine, which are both inhibitors of aldehyde dehydrogenase, were synthesized. In view of the formation of the β-lactam ring a new α-benzyloxyacryl Iron(II) complex for the synthesis of α-hydroxy-α,α-dialkylcarbonyl derivatives was prepared. Finally, studies on the biosynthesis of the spirostaphylotrichins, a family of spirocyclic γ-lactams, are presented. Having established the building blocks a series of minor metabolites of the wild type and of mutant strains were isolated and their structures elucidated.

The secondary metabolites of micro-organisms are characterized by their enormous variety of chemical structures. Many of these compounds exhibit interesting biological properties. In general the biological features cannot be assigned to a single specific molecular structure or functionality. However, many bioactive microbial metabolites contain a lactam group as a characteristic structural element. The classical examples are the penicillins and cephalosporins as well as the series of related antibiotics.

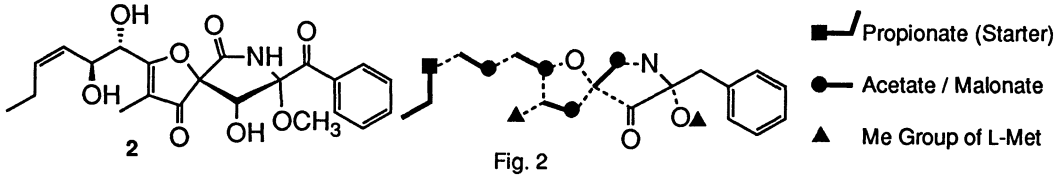
A γ-lactam moiety is an important structural element of the cytochalasans (ref.1,2), e.g. cytochalasin B (**1**). Having elucidated the structures of the first compounds isolated we have carried out detailed biosynthetic studies by means of classical incorporation experiments with isotope labelled potential precursors. The building blocks were found to be a natural amino acid, in most cases L-phenylalanine, acetate / malonate and L-methionine.The latter provides extra methyl groups (ref.3,4) (Fig. 1). It is anticipated that the α-amino acid condenses with a polyketide chain forming an amide bond. Subsequent ring closure leads to the bicyclic system. The γ-lactam is the result of a mixed biogenetic pathway involving a ketide and an α-amino acid. A *Baeyer-Villiger* oxidation provides the lactone oxygen of cytochalasin B (ref.5).



1

Fig. 1

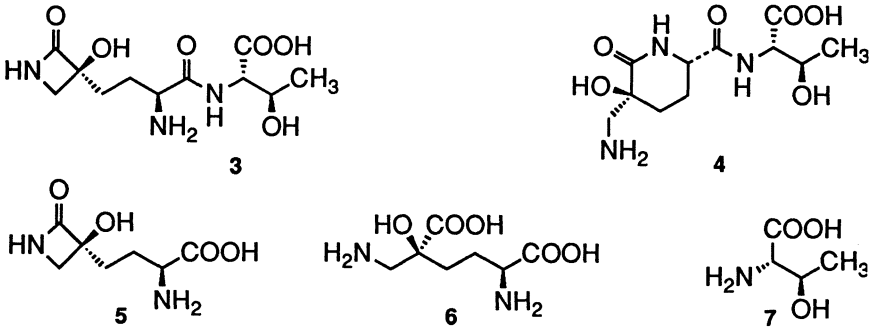
A similar situation was found in the course of our studies of the pseurotins, a small family of metabolites isolated from cultures of *Pseudorotium ovalis*. The major compound is pseurotin A (**2**) (ref.6). After the structural elucidation, the biosynthesis was studied (ref.7). Incorporation experiments revealed the nature and distribution of the building blocks of **2** as shown by Fig. 2. For the biosynthesis a polyketide with propionate as starter unit combines with L-phenylalanine. L-Methionine provides a C-methyl and the O-methyl group. Again the γ-lactam which includes an unusual aminal group, is formed by combining an α-amino acid with a polyketide moiety.



2

Fig. 2

Unexpected results were obtained in the case of the β-lactam tabtoxin (wildfire toxin, **3**) and tabtoxinine β-lactam (**5**). **3** is an exotoxin of the phytopathogenic bacterium *Pseudomonas syringae* pv *tabaci* which induces wildfire disease, a leaf-spot disease, on tobacco plants (ref.8,9). It is a potent irreversible inhibitor of glutamine synthetase. The biologically active β-lactam **3** readily undergoes intramolecular transacylation to the stable, but inactive δ-lactam isotabtoxin (**4**). Acid catalyzed hydrolysis of both **3** and **4** leads to tabtoxinine (**6**) and L-threonine (**7**). In order to investigate the biosynthesis of tabtoxin (**3**) *Pseudomonas syringae* pv *tabaci* was reisolated from its host, the tobacco plant. It led to an activation of the bacteria in order to produce **3**.



3 4 5 6 7

Incorporation of several ^{14}C-labelled amino acids as well as L-[methyl-^{14}C]methionine, L-[1,2-$^{13}C_2$]- and L-[3,4-$^{13}C_2$]aspartate, *rac*.[1,2-C_2]glycerol, [2,3-$^{13}C_2$]pyruvic acid, and [1,2-$^{13}C_2$]acetate into isotabtoxin (**4**) demonstrated that the building blocks of **3** are L-threonine (**7**), L-aspartate, the methyl group of L-methionine and a C_2-unit derived from the C_3-pool (ref.10). The results obtained by the incorporation experiments involve the entire skeleton of tabtoxin (**3**) and are summarized in Fig. 3.

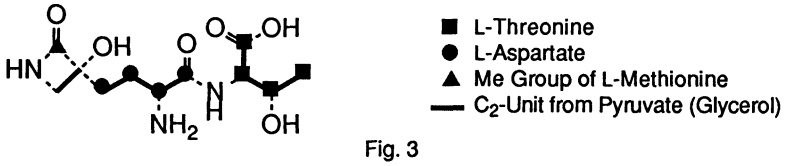


Fig. 3

To establish those building blocks which are derived from L-aspartic acid, a 1:1 mixture of L-[1,2-$^{13}C_2$] and L-[3,4-$^{13}C_2$]aspartic acid was synthesized using ethyl [1,2-$^{13}C_2$]bromoacetate as starting material. The desired mixture of doubly labelled L-aspartic acid was obtained from [1,2-$^{13}C_2$]fumaric acid and NH_3 using aspartase which is present in immobilized cells of *E. coli* ATCC 11'303. *Rac.*[1,2-$^{13}C_2$]glycerol was prepared from diethyl [1,2-$^{13}C_2$]malonate. The synthesis of [2,3-$^{13}C_2$]pyruvic acid started from [1,2-$^{13}C_2$]acetic acid via [2,3-$^{13}C_2$]acetyl bromide, [2,3-$^{13}C_2$]pyruvonitrile, and [2,3-$^{13}C_2$]pyruvamide (ref.11). The incorporation experiments established L-threonine (**7**) as a direct precursor of the threonine moiety of **3**. The methyl group of L-methionine provides the carbonyl C-atom of the β-lactam moiety. These findings represent a novel pathway in β-lactam biosynthesis. An explanation may be the cyclization of a N-hydroxymethyl or N-formyl derivative of an open-chain amine. A synthetic equivalent to such a cyclization of a N-formyl-α-amino ketone is known (ref.12). A hypothetical mechanism for the β-lactam ring closure in the biosynthesis of tabtoxinine β-lactam (**5**) may be formulated, a biradical being the intermediate (Fig. 4).

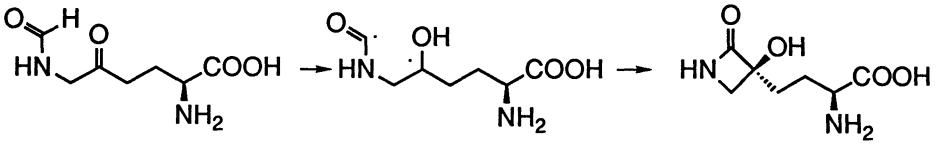
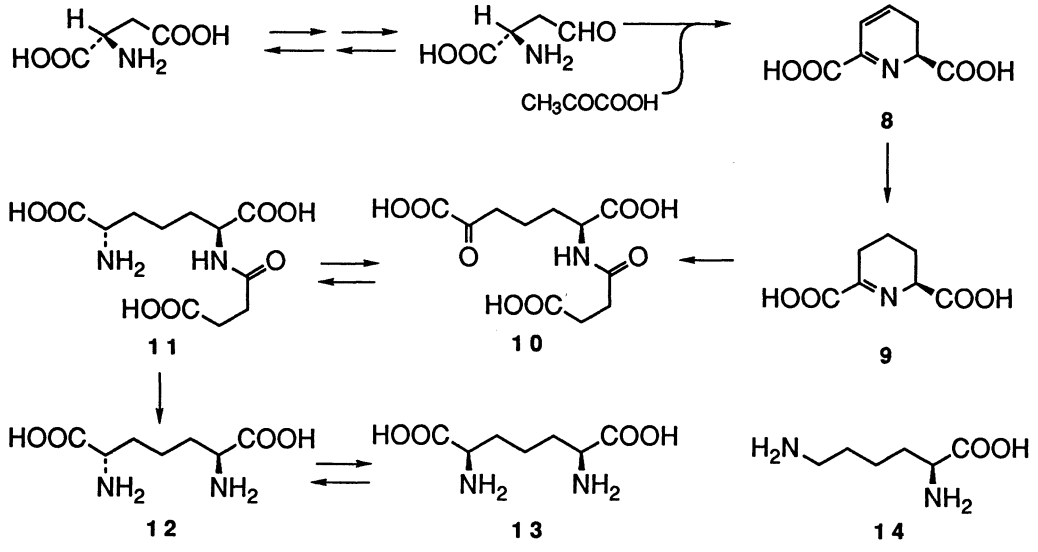


Fig. 4 **5**

Regarding the remaining two ring C-atoms incorporation of [2,3-$^{13}C_2$]pyruvate shows that these must be derived from a C_2-unit which is metabolized *via* the C_3-pool. Acetate can be ruled out as an intermediate. The incorporation of L-lysine (**14**) indicates, that the biosynthesis of tabtoxinine (**6**) proceeds along the lysine pathway (ref.13) until an unknown intermediate is reached. The intact incorporation of L-aspartic acid as well as the C_2-unit derived from pyruvate but not from acetate are explained by this assumption.

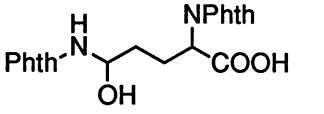


8 **11** **10** **9** **12** **13** **14**

A detailed analysis of the lysine pathway reveals six possible intermediates. Lysine itself may probably be precluded, because in an earlier feeding experiment, [^{14}C]lysine was incorporated to a much lesser extent than [^{14}C]aspartate. One of the six intermediates **8**-**13** shows a C_2 axis of symmetry, i.e. (*S*,*S*)-2,6-diaminopimelic acid (**12**). Since the intermediates **8** and **9** are unstable, an

incorporation experiment was carried out with (±)-[2,6-$^2H_2$]diaminopimelic acid which was prepared from 2,6-diaminopimelic acid. ^{2}H-NMR revealed that no deuterium was incorporated into tabtoxin (**3**). This result is consistent with the assumption that the biosynthesis of **3** is branching off from the lysine pathway before the formation of **12** (ref.11).

In order to test whether $^{\varepsilon}$N-formyl-5-oxolysine (**18**) is a biogenetic intermediate its synthesis was attempted *via* 5-oxolysine (**17**). The preparation of the latter was improved by using N-diphthalyl-hydroxylysine (**15**) as starting material. But the formylation was unsuccessful. *Vice versa* the oxydation of $^{\varepsilon}$N-formyl-δ-hydroxylysine (**19**) to **18** was not achieved in a satisfactory manner.



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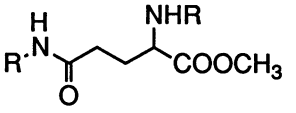
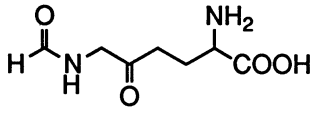
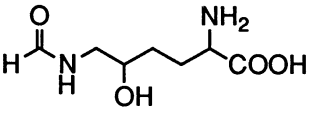


16 R = Phthalyl
17 R = H



18



19

In connection with the biosynthetic studies we became interested also in a chemical total synthesis of tabtoxin (**3**). Our concept was based on a retrograde synthesis which leads to synthons A ((*S*)-malic acid), B and C (L-threonine, **7**) (Fig. 5).

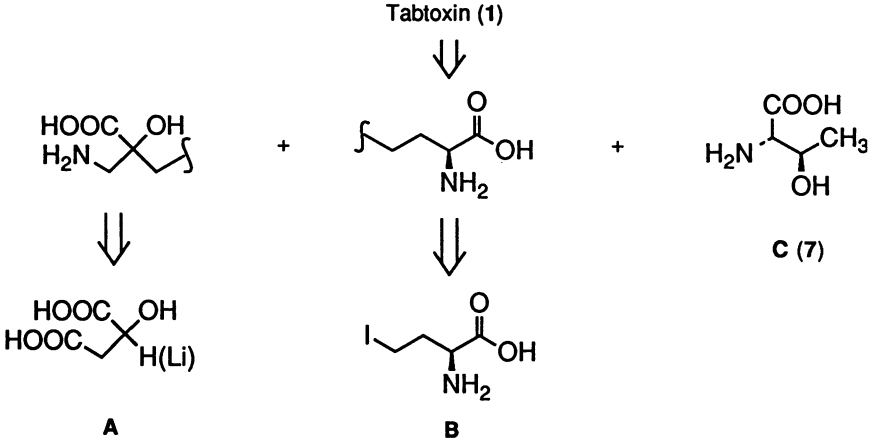
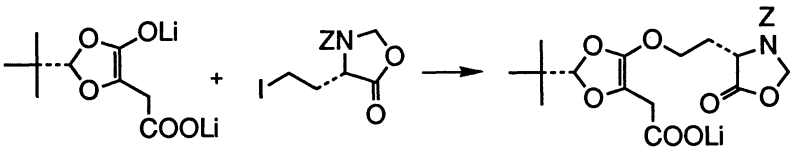
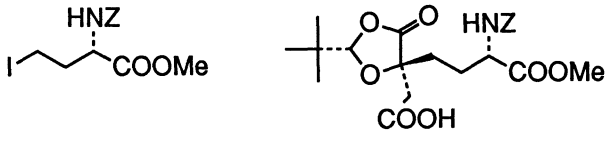


Fig. 5

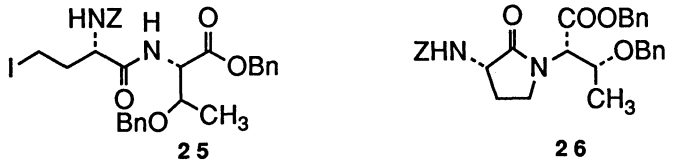
The Li-enolate **20** is available from (*S*)-malic acid in optically pure form (ref.14). It is known that **20** undergoes C-alkylation with electrophiles (ref.16). However, when **20** was treated with the iodide **21**, which is a precursor of synthon B, exclusive formation of the O-alkylated product **22** was observed instead of the C-alkyl derivative, probably due to steric hindrance. This ist the first example of an O-alkylation instead of the expected C-alkylation under the conditions used. The reaction of the iodide **23** with **20** finally led to the C-alkyl derivative **24** under special conditions. But the yield was



20 **21** **22**

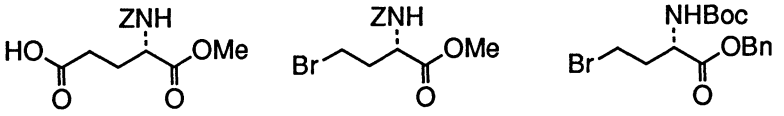


23 **24**

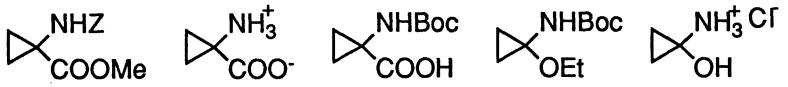


25 **26**

low, the main product still being the O-alkylated isomer. Upon treatment of the Li-enolate **20** with the protected dipeptide **25** a rapid intramolecular reaction to form the γ-lactam **26** occured. When the coupling reaction of **23** was carried out in toluene using 8 equiv. LDA·THF the 1-amino-cyclopropane-1-carboxylic acid derivative **30** was obtained. This observation led to a simple novel synthesis of 1-aminocyclopropane-1-carboxylic acid (**31**), an intermediate in the biosynthesis of the plant hormone ethylene from L-methionine (ref.15). The glutamic acid derivative **27** was converted to the bromide **28** by oxydative decarboxylation. γ-Elimination yielded compound **30** which was deprotected to form the amino acid **31**. In a similar way **29** was transformed into the acid **32**. Electrolytic decarboxylation of the latter led to the ethoxy product **33** (ref.16). Hydrolysis with

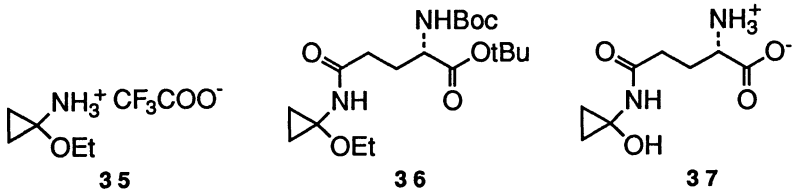


27 **28** **29**



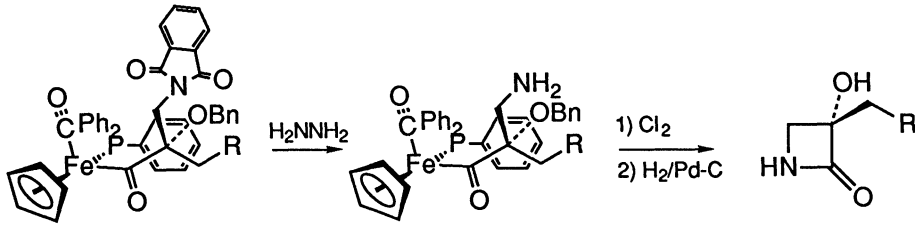
30 **31** **32** **33** **34**

HCl yielded the hydrochloride of 1-aminocyclopropanol (**34**) which is a potent inhibitor of aldehyde dehydrogenase *in vitro* and *in vivo*. The ethoxy derivative **33** also served as starting material for the synthesis of coprine (**37**), a metabolite of the inky cap mushroom (*Coprinus atramentarius*) and also an inhibitor of aldehyde dehydrogenase, *via* the intermediates **35** and **36** (ref.17).



35 36 37

Since our first approach to the total synthesis of tabtoxin (**3**) was not successful the possibility of a β-lactam synthesis *via* **39** was investigated using the iron complex **38** as starting material. These studies led to the asymmetric synthesis of α,α-dialkyl-α-hydroxy-carbonyl compounds by a new chiral α-benzyloxyacryl-iron(II) complex (ref.18).



38 39

Its synthesis is outlined in the following scheme (Fig. 6).

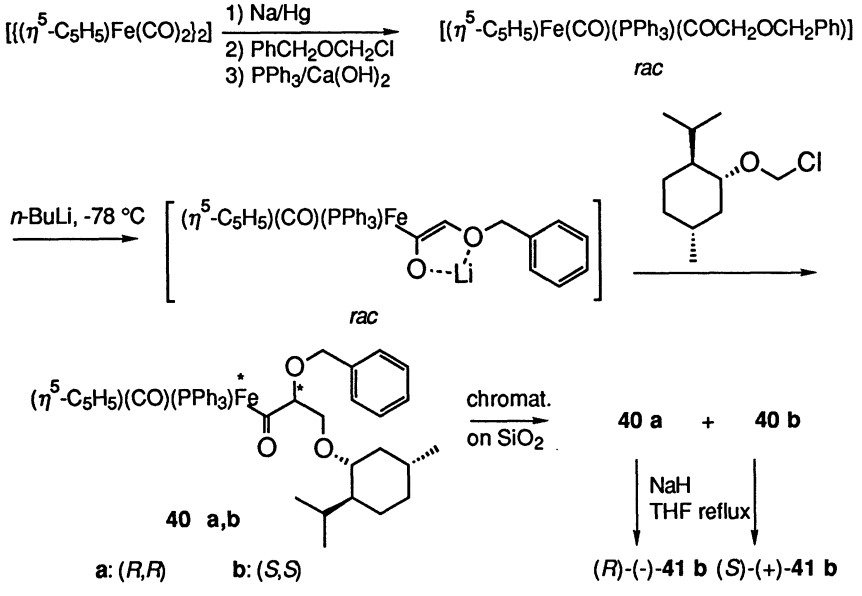


Fig. 6

Compound **41b** was butylated and the resulting Li-enolate alkylated to form **42** with very high diastereomeric selectivity. The oxidative cleavage of the α-benzyloxyacyl ligands from the iron complex with chlorine gas in dichloromethane formed exclusively the ester **43**. Hydrogenolysis of **43** yielded the ester **44**.On the other hand treatment of **43** with iodine led to the benzylmethyl acetal or the ketals **45** respectively (Fig. 7).

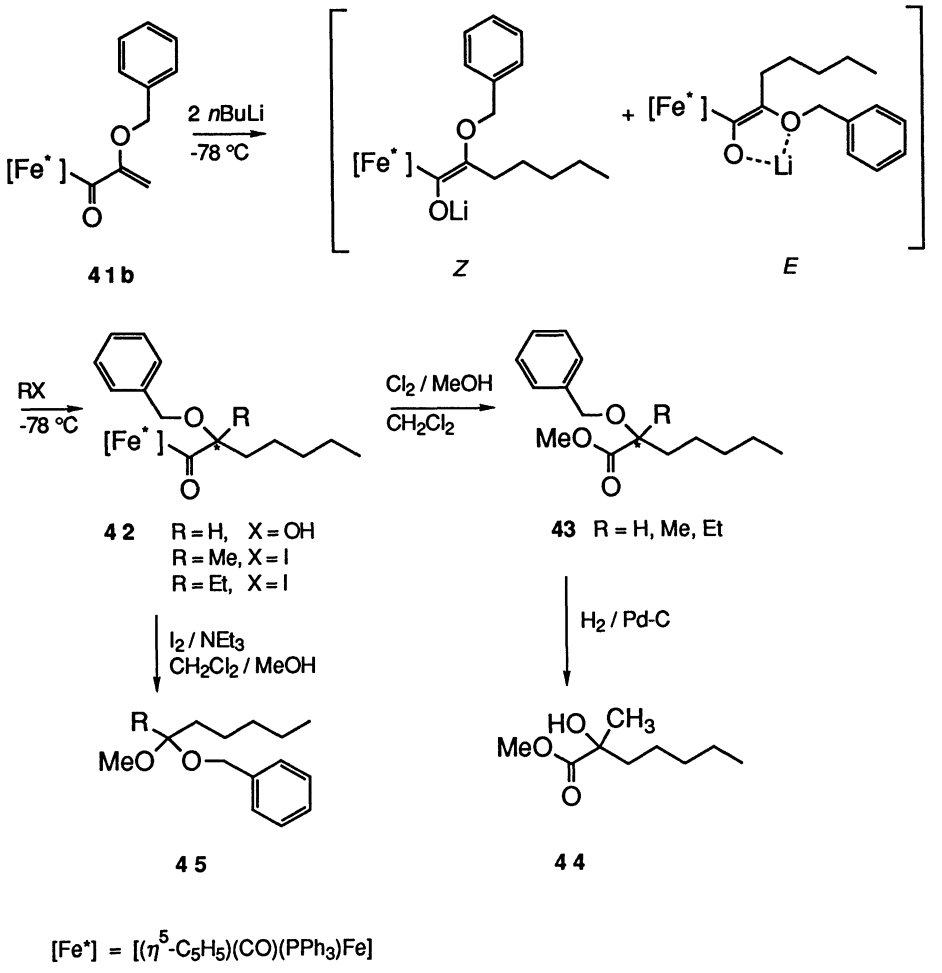


Fig. 7

In the following part, studies on the biosynthesis of the spirostaphylotrichins, a new family of spirocyclic γ-lactams which were isolated from cultures of *Staphylotrichum coccosporum* (ref.19), are presented. Spirostaphylotrichin A (**46**), the first metabolite isolated, possesses lipid lowering activity. Its unusual structure has been established by X-ray diffraction. In order to elucidate the biosynthesis of **46** incorporation experiments were carried out with ^{14}C- and ^{13}C-labelled potential precursors (ref.20). High incorporation of acetate, L-methionine, succinate and aspartate was observed. L-[2,3-$^{13}C_2$]aspartate was synthesized using [1,2-$^{13}C_2$]acetylene as starting material. The latter was converted to [2,3-$^{13}C_2$]fumarate. Enzymatic amination led to the desired labelled L-amino acid. The biogenetic building blocks of spirostaphylotrichin A (**46**) are shown in Fig. 8.

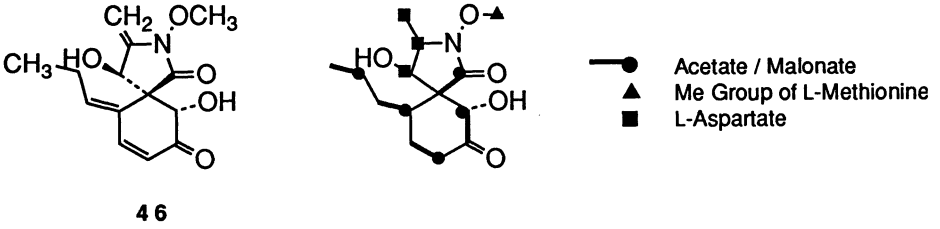


Fig. 8

In order to gain more detailed insight into the biogenetic pathway of **46** the culture filtrates of *Staphylotrichum coccosporum* were analyzed for minor metabolites. It was possible to isolate the spirostaphylotrichins B, C, D, F and R from the wild type (ref.21). F and R may be artefacts. The triticones A and B, phytotoxins from the plant pathogenic fungus *Drechslera tritici-repentis* (ref.22), are probably identical with the spirostaphylotrichins C and D. In addition, mutants were produced by irradiation of the strain with UV-light. After selection of spirostaphylotrichin negative mutants seven additional metabolites, the spirostaphylotrichins E, G, H, I, K, L and M were discovered in mutant P84 (ref.23). Mutant P649 yielded the spirostaphylotrichins N, O and P (ref.24). The structures of the new metabolites were established mainly by NMR-spectroscopy and in some cases by X-ray diffraction. It is interesting to note that metabolite P lacks the methoxy group which is usually attached to the nitrogen atom. Whereas the majority of the new metabolites differ from each other by the state of oxidation or being epimeric, the olefinic double bond in the side chain is shifted to an outer position in some of the compounds. The anticipated biogenetic relationship of the isolated metabolites is presented in Fig. 9.

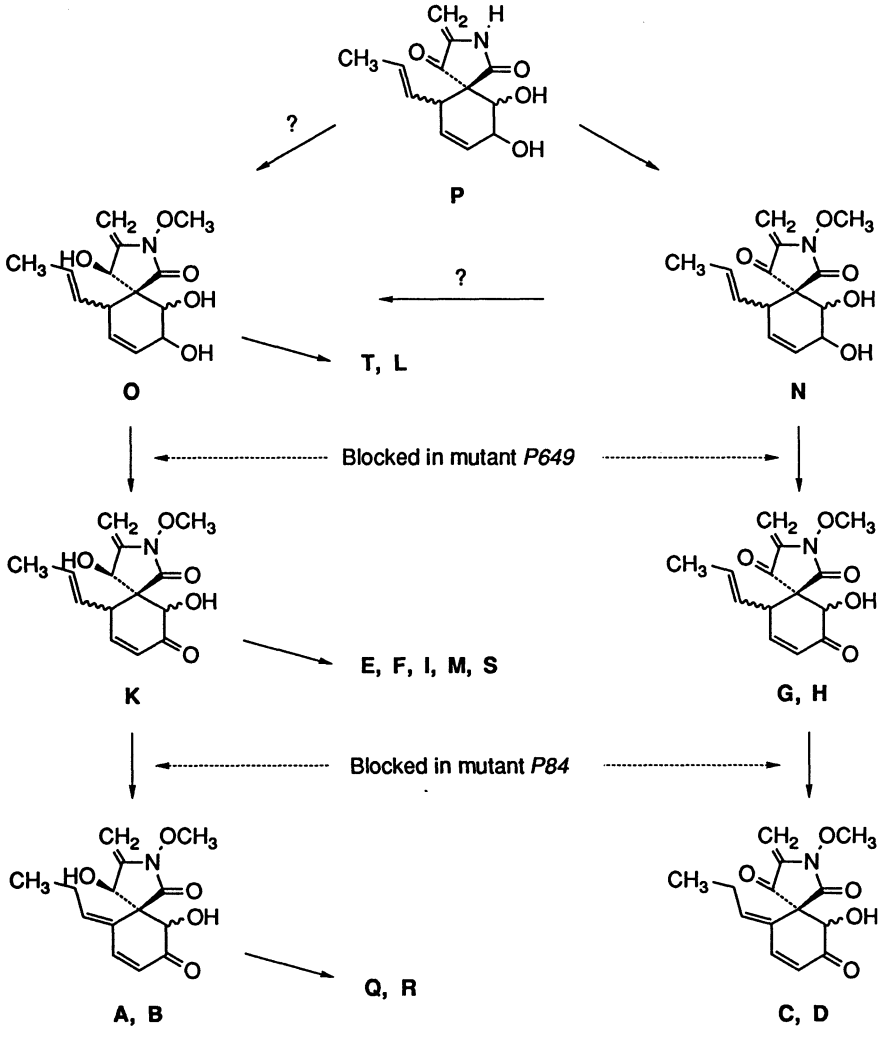


Fig. 9

Proposals for the biosynthetic pathway leading to spirostaphylotrichin P are presented as summarized in Fig. 10. It is assumed that condensation of a pentaketide with L-aspartic acid can take place either before or after formation of the six-membered ring. The formation of the γ-lactam follows in a later stage. The sequence of ring formation may well be reversed. Various mechanisms for the ring closures leading to the spirocyclic system can be envisaged. At one stage a decarboxylation must occur. The oxidation of the nitrogen and the methylation of the hydroxamic acid formed appear to take place in very late stage of the biosynthesis.

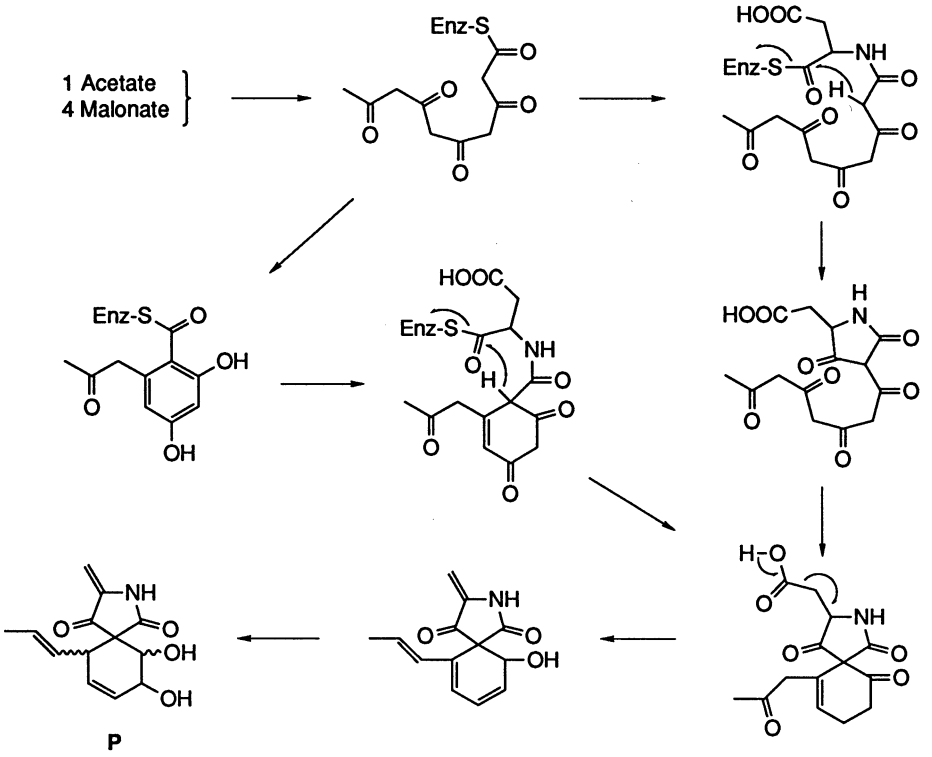


Fig.10

Acknowledgements

The support of these investigations by the *Swiss National Science Foundation* and by *Ciba-Geigy AG*, Basel, is gratefully acknowledged.

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