A New Role for Polyketides

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Recent studies have shown that natural products are still not replaceable in the broad field of drug discovery and development.[1] Polyketides, which are produced by various organisms such as bacteria, fungi, or plants, form perhaps the most interesting subgroup of natural products, since they exhibit the largest diversity in chemical structures and biological activities. Between 5000 and 10000 polyketides are known and about 1% (!) of these possess drug activity, which is five times as many as the average. Pharmaceutically important polyketide drugs include antibiotics, cancer chemotherapeutics, immunosuppressants, cholesterol lowering agents, and antifungals; their sales exceed 15 billion dollars. Thus, an enormous value is attached by the pharmaceutical industry to polyketide compounds. More and more new polyketides are created through the developing advanced techniques of combinatorial biosynthesis,[2] and new directions can be observed, such as attempts to link the biosyntheses of modular polyketides and nonribosomal peptides[3] and the attachment of important deoxysugar moieties to various polyketide-derived aglycones.[4]

Recently, a previously totally unknown role of polyketides has been discovered by Small and co-workers, in the laboratories of the National Institutes of Health in Hamilton, Montana, USA; this is in the role of a toxin which is required for virulence in context with Buruli ulcers caused by *Mycobacterium ulcerans*.[5] The latter is a chronic and progressive necrotizing ulcer for which no medical treatment exists. The discovery might provide a target for future therapeutic strategies to treat this severe human skin disease which primarily occurs in Australia and Africa. In addition to this potential direct benefit of the research, there is evidence that this polyketide toxin might represent the first of a family of virulence factors associated with other, even more dangerous mycobacterial diseases, such as leprosy or tuberculosis. However, the presence of a toxin has been suggested only in the context of Buruli ulcers,[6] since this is unique among the mycobacterial diseases because of its small acute inflammatory response.

Although a toxin in the culture supernatant of *M. ulcerans* was reported to cause a cytopathic effect on mouse fibroblasts,[6] and immunosuppressant properties of the filtrate of *M. ulcerans* were even detected,[7] it seemed to be impossible to isolate and characterize the compound associated with this effect. More recently, a method for the isolation of the toxin from intact bacteria was developed, which allowed its partial purification, and evidence for a lipophilic compound was published.[8, 9] Then Small and co-workers succeeded in isolating and identifying two polyketides, unusual macrolides with two long side chains, as the toxic principals. They were subsequently called mycolactones A (**1**) and B (**2**), and it was proven through intradermal injections of the purified mycolactones into the backs of guinea pigs that the mycolactones are directly responsible for the necrosis associated with the Buruli ulcer.[5, 10]

The chemical structures and physicochemical data of the mycolactones A (**1**) and B (**2**) were published about six months later (Scheme 1).[11] The suggested structures reveal that these compounds are constructed from two type 1 polyketide chains, a decaketide (consisting of five acetate and five propionate units), in which the last five building blocks fold into the twelve-membered lactone moiety, and an octaketide (consisting of five acetate and three propionate units), which forms a hexadecanoic side chain linked through an ester bond to the lactone moiety (Scheme 2).

It is assumed that the structures of mycolactones A and B differ only in their ester side chains, in which the 4′,5′ double bond is *Z*-configured in **1**, and *E*-configured in **2**. Both structures seem to be in an equilibrium, since separation and reanalysis (HPLC, NMR spectroscopy) always yields the same mixture (**1**:**2** = 3:2). This *E*/*Z*-isomerization obviously complicated the structure elucidation,[11] however, it might have been avoided with light exclusion, since it is likely to be light-induced. Although the reported structures of **1** and **2** show an *E*-configured 8,9 double bond, the authors assign a *Z* configuration in their text as well as in their deduced IUPAC name.[11] The indicated nonobservation of an NOE correlation between the 22-CH_3 methyl protons and 9-H also supports an *E* rather than a *Z* configuration of this double bond. Thus, the missing stereochemistry of the ten chiral centers and the questionable configuration of the 8,9 double bond make the structure elucidation incomplete; however, there is no doubt about the macrolide structures and, thus, about their polyketide origin.

The structures of the mycolactones indicate an unusual biosynthesis. Two independent type 1 polyketide synthases (PKS) are required, since both PKS-derived moieties differ

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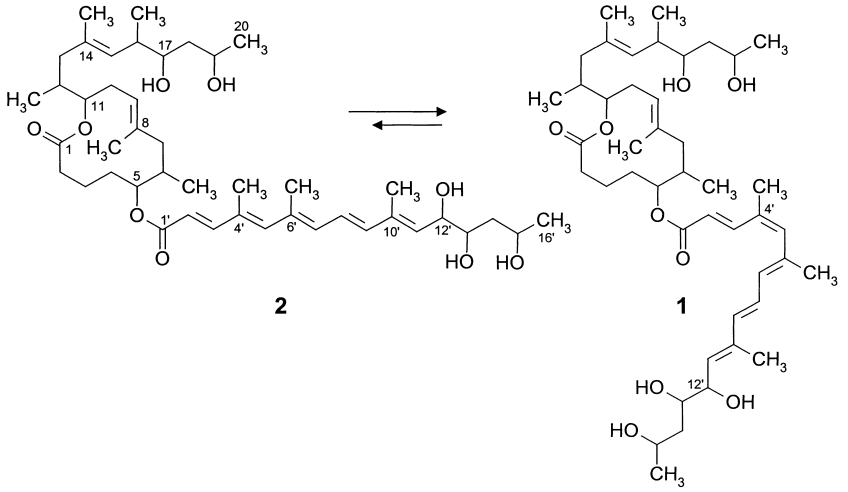
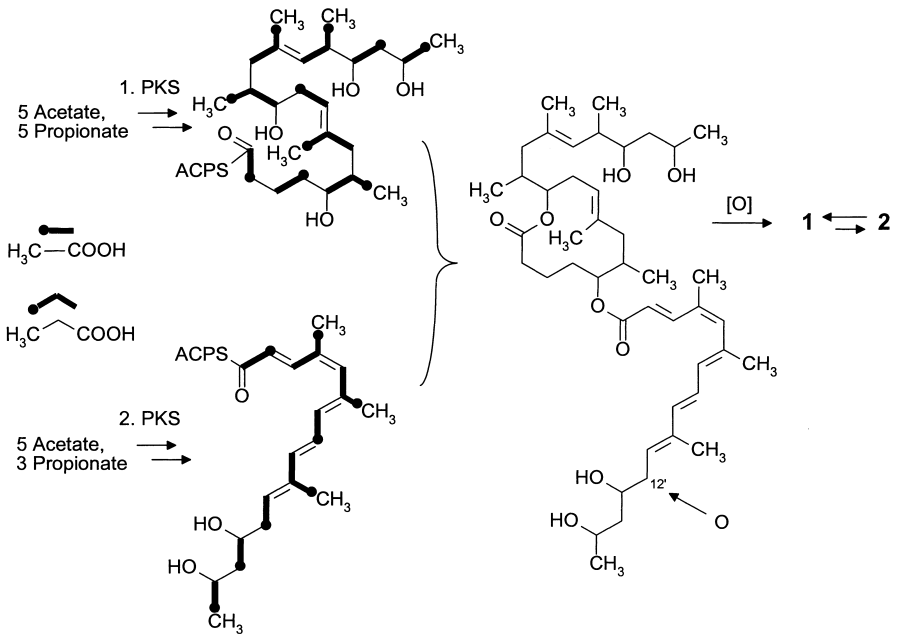


Scheme 1. Suggested structures for the two mycolactones A (**1**) and B (**2**), which seem to be in equilibrium.



Scheme 2. Plausible biosynthetic hypothesis for the formation of the mycolactones which involves two different type 1 polyketide synthases (PKS) and only one post-PKS enzyme (a monooxygenase).

not only in chain length and sequence of the building blocks (AAPPPAPPAA and AAAPAPPA for the decaketide- and octaketide-derived chains, respectively; A = acetate, P = propionate), but also in the degree of reduction. It is known that modular type 1 PKS[12] can catalyze a different degree of reduction after each condensation step, either: no reduction at all, only ketoreduction (KR), KR followed by dehydration (DH), or complete reduction to a saturated CH_2 group through KR, DH, and enoyl reduction (ER). Here we find either KR, or KR-DH, or KR-DH-ER reduction cycles, in a different sequence for the octaketide- and the decaketide-derived moieties of the mycolactones. Besides the PKS reactions, post-PKS modifications, such as glycosyl transfer steps or introductions of additional oxygen atoms through oxygenases, are typical for most known polyketides. Here, post-PKS enzymes seem to be scarcely involved. Only the oxygen at C-12′ necessitates an oxygenase reaction due to its position in the molecule (Scheme 2). Gene sequence analysis studies revealed a close relationship between *M. ulcerans*, *M. tuberculosis*, and *M. marinum* genes.[13a] The genes encoding the mycolactone biosynthesis have not been discovered or described yet, however, type 1 PKS genes were found in the course of the *M. tuberculosis* genome project,[13b] and, therefore, it is speculated that polyketides may also play a role in tissue destruction and immunological modulation characteristic of tuberculosis or leprosy.[5]

In conclusion, the mycolactones possess interesting novel structures due to an unusual biosynthesis requiring a cooperation of two PKS. More important, the mycolactones are the first discovered macrolides from *Mycobacterium*, and the first identified virulence determinants of a bacterial human pathogen, which means an entirely new role for bioactive polyketides.

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D-Serine as a Modulator in the Nervous System

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D-Amino acids occur rarely in nature. The genetic process only has codes, which consist of nucleotide triplets, for the 20 "standard" L-amino acids. On the other hand, D-amino acids do exist in the cell wall of gram negative bacteria, in numerous peptide antibiotics, and in other natural systems. Unlike the L-amino acids, they are not taken up with food or synthesized stereospecifically. They are formed by racemization from their L congeners, a process which is enzymatically catalyzed by racemases.

Unexpectedly, two Japanese laboratories reported some time ago the existence of D-serine and D-aspartate in mammals as well, in the brains of rats and humans.[12] Over the years, evidence accumulated for the fact that D-serine in mammalian brains is not an artefact, but is, rather, an important signalling molecule.[3] This hypothesis was recently strengthened by the findings of Snyder's group at the Johns Hopkins University in Baltimore.[4] They succeeded in cloning and sequencing a serine racemase from a mammalian brain. This protein represents a novel enzyme, possibly a first member of a whole family of enzymes, without any significant homology to other known amino acid racemases from lower organisms (with the exception of a short consensus sequence forming the binding site for pyridoxal phosphate). The enzyme is 339 amino acids long, has a calculated relative molecular mass of 36.3 kDa, and operates with the coenzyme pyridoxal phosphate (Figure 1).

Several amino acids, such as L-glutamate, glycine, and γ-aminobutyric acid (GABA) serve in the nervous system as neurotransmitters by transmitting nerve impulses from cell to cell. D-Serine does not seem to be such a transmitter, but rather a "neuromodulator", an essential regulator at synapses which use L-glutamate as an excitatory transmitter. D-Serine is not released from neurons, like a "normal" transmitter, but from astrocytes, a type of glia cells. In these cells Snyder and his co-workers localized the serine racemase.

To appreciate this discovery one has to take a closer look at the neurochemistry of glutamatergic synapses. L-Glutamate is the most important excitatory transmitter in the central nervous system (CNS) of vertebrates. (Insects use L-glutamate peripherally, that is, at their nerve – muscle synapses.) L-Glutamate is released presynaptically and triggers an effect postsynaptically, after it has bound to receptor proteins. Glutamate receptors comprise a large and complex group of

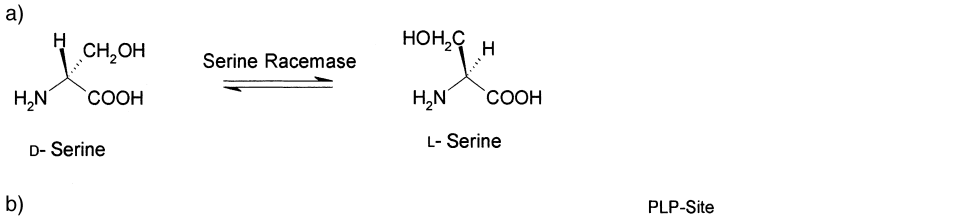

Figure 1. a) The reaction catalyzed by serine racemase. b) The amino acid sequence of serine racemase (single letter codes). The binding site for the coenzyme pyridoxal phosphate, close to the N terminus, is underlined.

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