Big Effects from Small Changes: Possible Ways to Explore Nature's Chemical Diversity

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Fungi or bacteria that produce secondary metabolites often have the potential to bring up various compounds from a single strain. The molecular basis for this well-known observation was confirmed in the last few years by several sequencing projects of different microorganisms. Besides well-known examples about induction of a selected biosynthesis (for example, by high- or low-phosphate cultivation media), no overview about the potential in this field for finding natural products was given. We have investigated the systematic alteration of easily accessible cultivation parameters (for example, media composition, aeration, culture vessel, addition of enzyme inhibitors) in order to increase the number of secondary metabolites available from one microbial source. We termed this way of revealing nature's chemical diversity the 'OSMAC (One Strain – Many Compounds) approach' and by using it we were able to isolate up to 20 different metabolites in yields up to 2.6 g_L^{-1} from a single organism. These compounds cover nearly all major natural product families, and in some cases the high production titer opens new possibilities for semisynthetic methods to enhance even more the chemical diversity of selected compounds. The OSMAC approach offers a good alternative to industrial high-throughput screening that focuses on the active principle in a distinct bioassay. In consequence, the detection of additional compounds that might be of interest as lead structures in further bioassays is impossible and clearly demonstrates the deficiency of the industrial procedure. Furthermore, our approach seems to be a useful tool to detect those metabolites that are postulated to be the final products of an amazing number of typical secondary metabolite gene clusters identified in several microorganisms. If one assumes a (more or less) defined reservoir of genetic possibilities for several biosynthetic pathways in one strain that is used for a highly flexible production of secondary metabolites depending on the environment, the OSMAC approach might give more insight into the role of secondary metabolism in the microbial community or during the evolution of life itself.

KEYWORDS:

chemical diversity · natural products · OSMAC approach · polyketides · secondary metabolism

1. Introduction

The search for bioactive compounds (for example, to overcome the danger of increasing microbial resistances) is one of the central subjects of industrial and academic natural products chemistry.[1] Various methods to achieve this goal have been described in the literature (such as combinatorial chemistry or high-throughput screening of different biological sources).[2–4] An improved strategy is the search for biomolecules (that is, antibiotics, enzymes) by recombinant approaches that bypass the limitations imposed by the traditional requirement for isolation and cultivation of potent microbial producers of such compounds by capturing genes and complete pathways from the metagenome of different environments.[5–7] However, most of these approaches can be performed only by the pharmaceutical industry or biotech companies with a good financial background, leaving academic research behind. With this problem in mind, one has to think about alternatives for academia to compete with 'big money'. One possible way is to focus on a reasonably small number of organisms that are investigated in every detail and bear the chance to bring up several new products from one source. Not just since the recent projects of whole genome sequencing of microorganisms has it become obvious that most fungi or bacteria that produce secondary metabolites have the potential to generate more than one compounds. A good example is the complex metabolite pattern of *Streptomyces* sp. Gö 40/10 (Scheme 1).[8, 9] Five ansamycins (ansatrienine A (**1**), naphthomycin B (**2**) and K (**3**), diastovaricin I (**4**) and II (**5**)), 14-membered macrolides of the cineromycin-B-type **6** – **12**, collinolactones **13** and **14** (as a 1:3 mixture of *cis* – *trans* isomers), and the two different butyrolactones **15** and **16** can be isolated from this strain from one single fermentation.

Compared to this strain with its mainly reduced type I polyketides, *S. cellulosae* ssp. *griseorubiginosus* (strain S 1013) produces only four compounds but originated from three different biogenetic pools (Scheme 2).[10–13] The gabosines D (**17**) and E (**18**) are derived from intermediates of the sugar pathway, hexacyclinic acid (**19**) represents one of the rare polycyclic type I polyketides, and compound **20** is an angustmycin A deriva-

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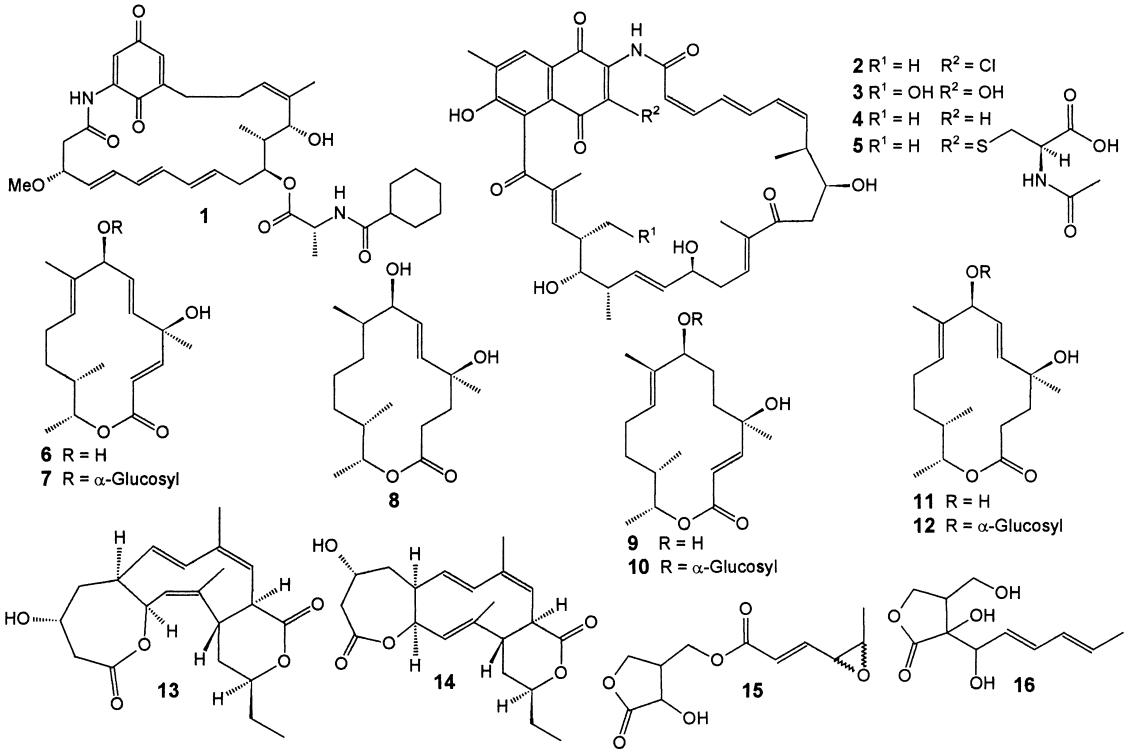
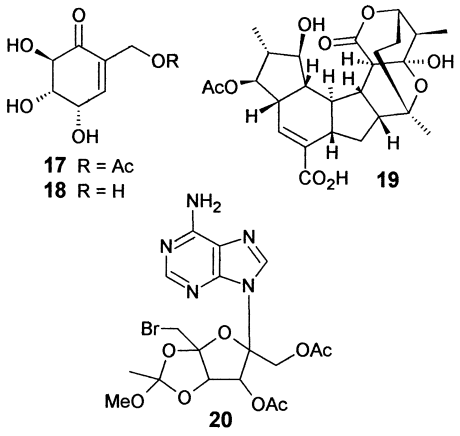


Scheme 1. *Isolated metabolites from* Streptomyces *sp. Gö 40/10.*



Scheme 2. *Isolated metabolites from* Streptomyces cellulosae *S 1013.*

tive.[14] The corresponding ethyl *ortho*-ester of **20** was synthesized previously from angustmycin A, EtOH, and Br_2 in $CHCl_3$ in good yield.[15] The production of **19** was increased 13-fold by the addition of 1 gL^{-1} of NaBr to a standard cultivation medium.

Further examples are the myxobacteria that also produce various polyketides, nonribosomal peptides, and hybrides of both.[16] These observations were confirmed at the genomic level by the identification of several biosynthesis gene clusters in these organisms.[17] Furthermore, the identification of such a gene cluster for a so-far unknown siderophore from *Stigmatella aurantiaca* Sga15 led to a more detailed analysis of the fermentation broth and the identification of myxochelin A that was isolated previously from other myxobacteria.[18] Similar surprises were obtained from the *Streptomyces coelicolor* A(3)2 genome project (http://www.sanger.ac.uk/Projects/S coelicolor/).[88] Although this strain can be designated as the best-known secondary metabolite producer, several formerly unknown gene clusters (polyketide synthase (PKS) type I and II, nonribosomal peptide synthetase (NRPS)) have been found in the genome besides the known clusters for methylenomycin, prodigiosin, actinorhodin, and calcium-dependent antibiotic (CDA). Sequencing of the avermectin producer *Streptomyces avermitilis* ATCC 31267 revealed 24 further gene clusters for siderophores, spore pigments, and secondary metabolites of polyketide or nonribosomal peptide origin with so-far unknown structure and function.[19] Why have the corresponding compounds been able to hide for almost 50 years? Maybe simply due to detection and analytical reasons or because these clusters are not functional. Another possibility is that these gene clusters are silenced under the fairly unnatural standard cultivation conditions and that one can possibly obtain the corresponding products under altered cultivation conditions, as was assumed for the new siderophore coelichelin from *S. coelicolor*, whose NRPS gene cluster has been identified without seeing the final tripeptide itself.[20]

The biosynthesis of secondary metabolites follows the usual metabolic pathways. The enzymes that bring up a desired compound are determined by the corresponding mRNA which itself is based on the complementary DNA. One result of this multistep information flowchart is the possibility to manipulate the desired biosynthesis at different steps (Figure 1). On the DNA level, for example, mutagenesis or combinatorial biosynthesis offers an easy possibility to generate new enzymatic activities resulting in modified products,[21, 22] whereas precursor-directed biosynthesis and mutasynthesis act in vivo using the lack of specificity of some biosynthetic enzymes to introduce different

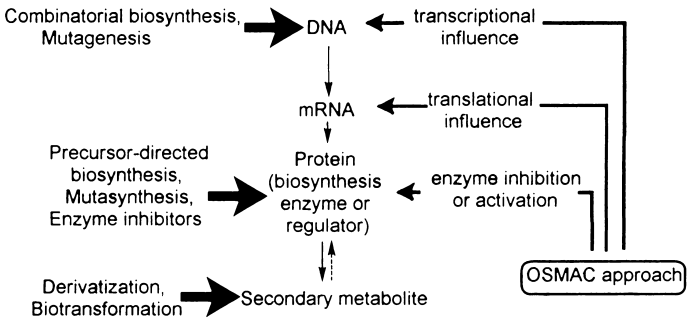


Figure 1. *Possible ways to influence the biosynthesis of secondary metabolites.*

precursors into the target molecule.[23] Finally, biotransformation and derivatization deal with chemical or biological modifications of the intermediates or end products of a given biosynthesis.[24]

It is well known that media composition can have a great impact on the production of microbial products. High glucose, phosphate, or ammonium concentrations are generally regarded as repressors of secondary metabolism, and several examples of the production of secondary metabolites in media with low contents of these components are described in the literature.[25–27] Contrary to these observations, high phosphate concentrations might induce the production of selected metabolites.[28–30] Even usual amino acids are described as potential inducers of secondary metabolites; this underlines once more the random character of finding the optimized production media.[31, 32] In general, variation of cultivation parameters to induce the production of formerly unknown compounds is a very similar but even more random approach to the improvement of fermentations to obtain maximum production titers of desired compounds.[33–36]

We have used the systematic alteration of easy accessible cultivation parameters (for example, media composition, pH value, temperature, addition of enzyme inhibitors, oxygen supply, culture vessel), probably the most simple and natural approach to increase the number of secondary metabolites from one single organism.[37] In theory every single biosynthesis step can be influenced either at the transcriptional, the translational, or the enzyme level; this would possibly result in a vast number of permutations of new natural products (Figure 1). In nature, where a different environment results in a different transcriptome, proteome, and finally a different metabolome which allows an organism to survive, one can speculate that different secondary metabolites might be the result of these special requirements.[38] These can be as simple as the production of siderophores after iron deficiency, but one can speculate further about the role of secondary metabolites in even more complex situations (for example, signaling, communication, predators).[39] Due to our lack of knowledge of the complex biosynthetic and regulative crosstalk in a single cell and between cells, all levels of secondary metabolite biosynthesis can be influenced by this random approach imitating natural environmental changes. We have termed this way of releasing nature's chemical diversity the 'OSMAC (One Strain – Many Compounds) approach', and it resulted from the observation that very small changes in the cultivation conditions can completely shift the metabolic profile of various microorganisms.[11] In the following sections we present examples, mainly from our group, that focus on different aspects of this random but efficient way of improving the metabolic diversity. Furthermore, the biosynthetic pathways that are prerequisite to this diversity will be discussed.

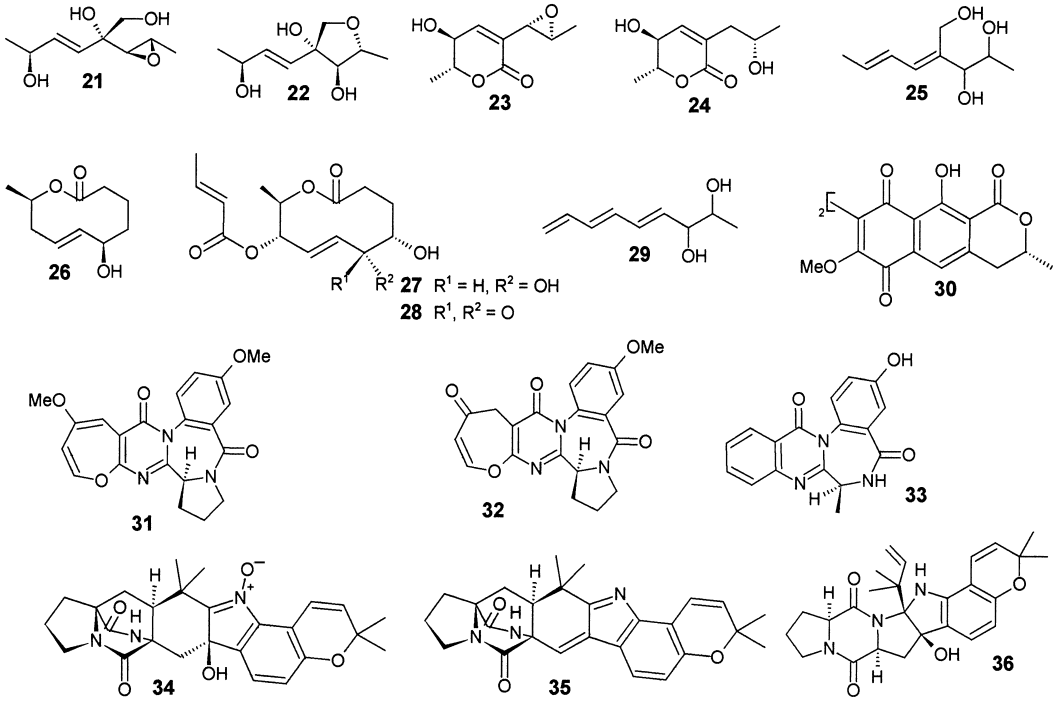
2. Fungi

2.1. *Aspergillus ochraceus* DSM 7428

Before the OSMAC approach was used to release the chemical diversity of *Aspergillus ochraceus* DSM 7428, the only known product aspinonene (**21**) was designated as the main metabolite, produced in yields of up to 8 mg L^{-1}.[40] However, variation of the culture conditions and detailed analysis of the culture broth led to the isolation of 15 additional compounds in yields of up to 94 mg L^{-1}; these compounds result from five different biosynthetic pathways (Scheme 3). This increase in the number and amount of compounds was due to extensive use of different culture vessels (for example, shaking flasks, static liquid cultures in P (*Penicillium*) flasks, different fermentors) as described previously.[41, 42] Most of these new metabolites are based on different polyketide synthases. Isoaspinonene (**22**), aspyrone (**23**), dihydroaspyrone (**24**), and dientriol (**25**) represent putative variations of the aspyrone biosynthesis which was studied in detail by the groups of Simpson and Staunton.[43, 44] The aspinolides A – C (**26** – **28**) and triendiol (**29**) are unbranched pentaketides of a different biosynthetic origin. Compounds **26** – **28** belong to the well-known class of decanolides (such as decarestricines, cephalosporolides, pyrenolides) that have been isolated from various fungi.[45, 46] Xanthomegnin (**30**), a common fungal pigment, was isolated after cultivation in shaking flasks for one week and was shown to be the main pigment of the yellow conidiospores.[47, 48] The benzodiazepines asperloxin A – C (**31** – **33**) could be isolated exclusively after cultivation in P flasks. Although benzodiazepines are common fungal metabolites, their biosynthesis is still unclear. One can postulate a fungal NRPS to generate the tripeptide backbone, as was postulated in the biosynthesis of asperlicin.[49] Epoxidation of the left benzene ring followed by a rearrangement would led to the oxepine ring in **31** and **32**, as was shown by cultivation of the strain in an $^{18}O_2$ atmosphere.[50] Further amino acid derived compounds are the aspergamides A – C (**34** – **36**) which also have been isolated exclusively from static liquid cultivations. The biosynthesis of the paraherquamides and brevianamides, highly similar compounds from different *Penicillium* sp., was studied in detail, and an intramolecular Diels – Alder reaction was postulated as the central biosynthetic step leading to the bridged diketopiperazine structural element.[51] Following this hypothesis, **36** can be considered as an additional derivative of the postulated uncyclized intermediate.[47]

2.2. *Sphaeropsidales* sp. F-24'707

When we started with the fungus *Sphaeropsidales* sp. F-24'707, the strain was described as the producer of the antifungal



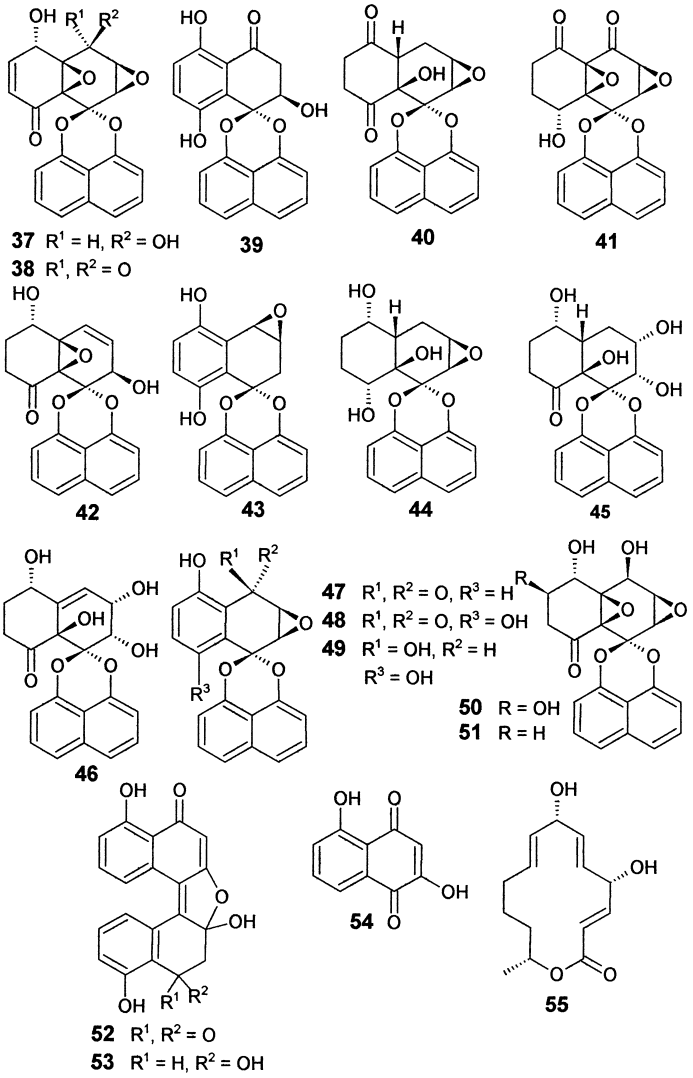
Scheme 3. *Secondary metabolites of* Aspergillus ochraceus *DSM 7428.*

spirobisnaphthalene compound cladospirone bisepoxide (**37**).[52–54] A combination of different media and cultivation vessels resulted in the isolation of eight new spirobisnaphthalenes (cladospirones B – I (**39** – **46**)) and six known members of this class of compounds (palmarumycins C_2 (**47**), C_3 (**48**), and C_{12} (**49**), diepoxins σ (**38**), η (**50**), and δ (**51**)) in yields of up to 2.6 g L^{-1} (Scheme 4).[55] For this strain the major breakthrough was a solid-phase cultivation in P flasks with wet oat grains as a single substrate, probably due to the more natural living conditions compared to standard liquid cultures. Furthermore, enzyme inhibitors were used to block distinct steps in the postulated biosynthetic pathway and some astonishing results could be obtained. Besides the expected accumulation of reduced intermediates of the cladospirone bisepoxide biosynthesis (for example, palmarumycins C_2 (**47**) and C_3 (**48**)) after addition of ancymidole, a well-known inhibitor of P_{450}-dependent monooxygenases, an almost threefold increase in the production of cladospirone bisepoxide was observed instead of the expected decrease.[56] The reason for this is unclear but underlines the complexity and our lack of knowledge of cellular biochemistry. An additional example for such a 'nonlinear effect' is the inhibition of the 1,8-dihydroxynaphthalene (DHN) biosynthesis in strain F-24′707 by tricyclazole, a commercially available antifungal agrochemical that inhibits 1,3,8-trihydroxynaphthalene reductase.[57] The expected breakdown of natural DHN melanin and spirobisnaphthalene biosynthesis is accompanied by the production of two new bisnaphthalenes, named sphaerolone (**52**) and dihydrosphaerolone (**53**), and 2-hydroxyjuglone (**54**) from the accumulated 1,3,8-trihydroxynaphthalene.[58] Furthermore, a totally different metabolite could be isolated in yields of up to 70 mg L^{-1}; this turned out to be one of the rare fungal macrolides (Scheme 4).[59] It was named mutolide (**55**) and was initially isolated from a UV mutant of strain F-24′707 that additionally produces cladospirone bisepoxide (**37**). Is the biosynthesis of **55** regulated by intermediates of the spirobisnaphthalene pathway, and is this mechanism leaky in the UV mutant? The answers to these questions remain open. However, this unexpected result demonstrates the usefulness of enzyme inhibitors to influence the metabolite pattern of suited strains. In total three structurally distinct classes of natural products could be isolated from strain F-24′707, two of them belonging to the huge number of fungal compounds derived from hydroxylated naphthalene. A nearly custom-made production of several members of these class of compounds could be achieved through a combination of enzyme inhibitors, different culture conditions, and UV mutagenesis; this was a much simpler and faster method than combinatorial biosynthesis.[56] Additionally, some of these compounds were produced in very high yields that allow further semisynthetic approaches that may enhance even more the structural diversity within this type of metabolites.

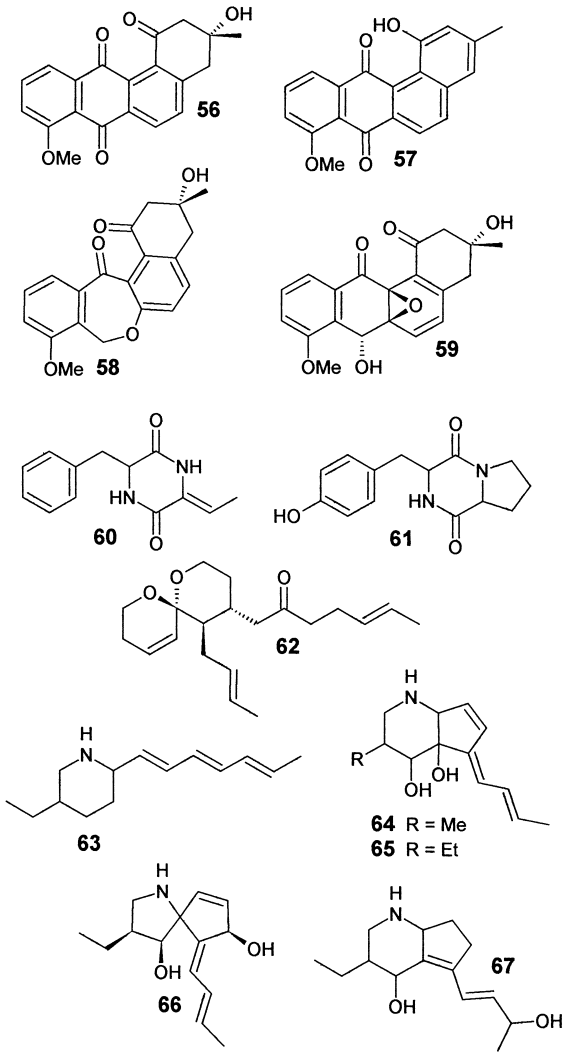
3. Actinomycetes

3.1. *Streptomyces* sp. Gö 40/14

All metabolites from *Streptomyces* sp. Gö 40/14 could be isolated from a combination of different fermentors (10 L, 50 L, and 10 L airlift fermentor), media composition, and control of the pH value (Scheme 5).[37] The angucyclinones **56** – **59**, typical products of a type II PKS and the diketopiperazine **60** were produced in varied yields under nearly all conditions, whereas the diketopiperazine **61** and the spiroketal **62** could be isolated only from cultivations in special fermentors. However, whereas



Scheme 4. Isolated metabolites of Sphaeropsidales *sp. F-24'707. Compounds* ***40*** *–* ***42*** *and* ***44*** *–* ***46*** *have been isolated exclusively from a solid-surface cultivation.*



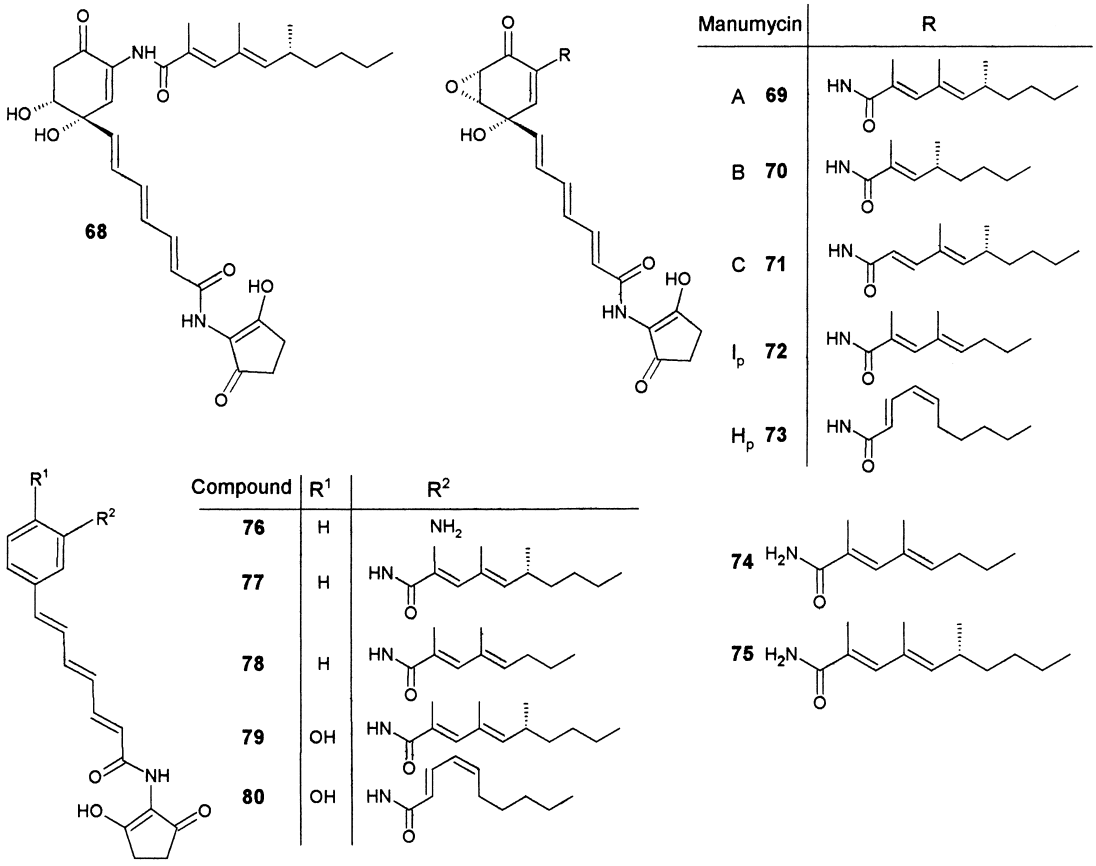
Scheme 5. Natural products from Streptomyces *sp. Gö 40/14.*

63 – **66** were obtained from several experiments, **67** was produced solely in an airlift fermenter under high-aeration conditions (up to 90 L min^{-1}). Compounds **62** – **67** might represent different variations of the late biosynthesis of a PKS I product leading to cyclizations, 1,2-shifts, or hydroxylation of reduced polyketide structures.

3.2. *Streptomyces parvulus* Tü 64

The influence of dissolved oxygen towards the production of secondary metabolites was investigated in detail for the coproduction of the structurally different compounds mimosamycin/chlorocarcin and streptothricin.[60] In the case of the manumycin biosynthesis in *Streptomyces parvulus* (strain Tü 64) the influence of the dissolved oxygen concentration focuses mainly on a distinct compound family. The main metabolite of this strain is manumycin A (**69**) accompanied by manumycins B – D (**70**, **71** and **68**) and the two red antibiotic pigments undecylprodigiosin and metacycloprodigiosin as minor compounds under almost all cultivation conditions.[61–63] The manumycins are potent inhibitors of RAS-dependent farnesyl transferases and they originate from different biosynthetic building blocks. The precursors of the mC$_7$N unit are succinate and glycerol, the C$_5$N unit is a cyclized 5-aminolevulinic acid, and the polyene chains represent typical type I polyketides.[63] Fermentation under enhanced pressure (5 – 6 bar), resulting in increasing dissolved oxygen concentration, precursor-directed biosynthesis with suited C$_7$N analogues as starter units in nonphysiological high concentrations (50 mM), and a combination of both led to the production of more than 20 new manumycines and manumycin analogues.[63] In the following paragraph we will focus only on the high-pressure-induced change of the metabolite pattern of strain Tü 64 (Scheme 6).

With increasing hydrostatic pressure (5 – 6 bar) during the fermentation, the yield of manumycin A (**69**) decreased continuously whereas the new metabolites manumycin I$_p$ (**72**) and H$_p$ (**73**) and 64p A – C (**74** – **76**) are formed instead.[63, 64] Compound **75** represents the upper side chain of the parent metabolite **69**,



Scheme 6. *Selected metabolites of* Streptomyces *sp. Tü 64.*

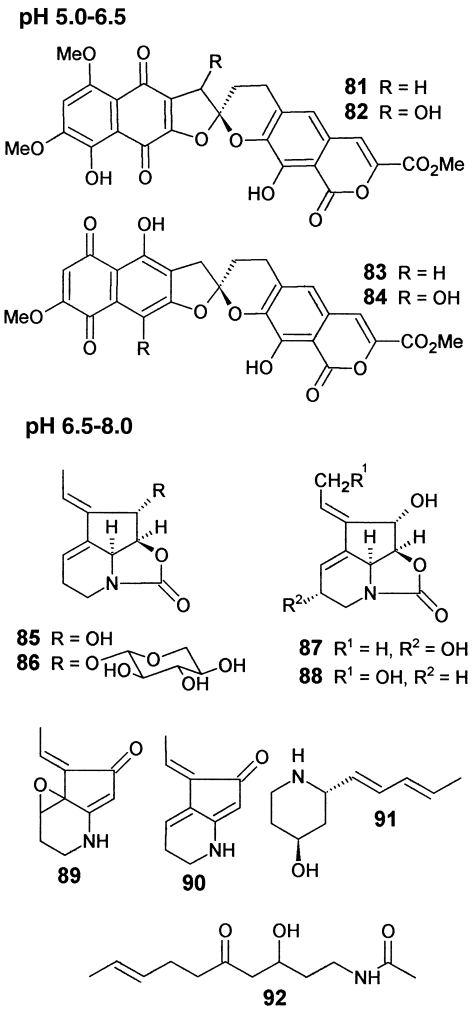
whose intact assembly seems to be disturbed under high-pressure conditions. The 'upper' polyketide chain of **72** and **73** differs from the standard manumycins in chain length and/or methylation pattern. Similar results were obtained from a combination of feeding 3-aminobenzoate and high-pressure cultivation conditions, resulting in the production of the expected compound **77** and the unexpected manumycin I_p analogue **78**. In the case of 3-amino-4-hydroxybenzoate the products were **79** and **80**. The most reasonable hypothesis for the biosynthesis of these five different type I polyketides is the activation and/or regulation of different PKSs (or their corresponding genes) under these different experimental conditions. A second possibility is an induced broad enzyme specificity including effects like 'stuttering' and/or 'skipping' as a result of the high-pressure conditions.[65] Furthermore, the isolation of different manumycins with five different side chains indicated the relaxed substrate specificity of the condensing enzyme and opens up the possibility of increasing the number of these interesting compounds in future experiments by feeding 'unnatural' fatty acids to strain Tü 64.

3.3 *Streptomyces* sp. A1

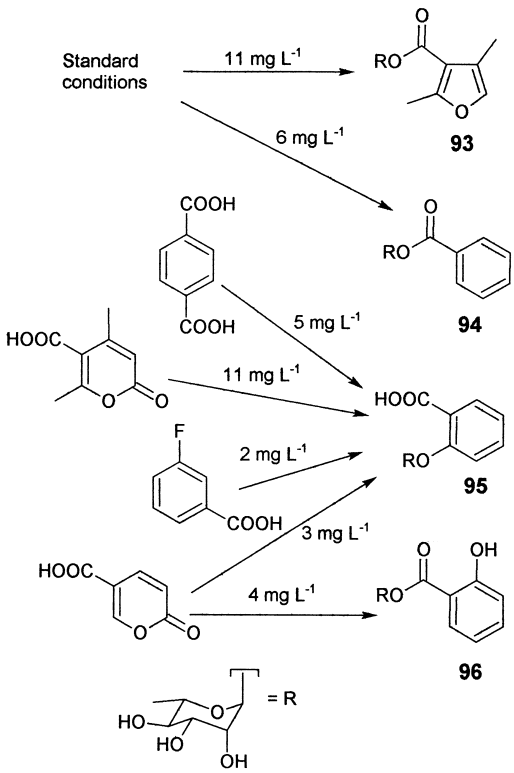
Streptomyces sp. (strain A1) produces the rubromycins **81**–**84**.[66, 67] When different supplements (such as $CaCO_3$, Al_2O_3) were added to the culture medium the metabolite pattern is shifted completely towards the production of streptazoline **85** and similar compounds (Scheme 7).[68] This experiment was driven by the observation that the rubromycins **81**–**84** were only produced when the harvest pH value was slightly acidic, whereas harvest at pH 7.3 led to the isolation of **85** and SS20846A (**91**) instead. In a soybean meal/mannitol medium (harvest pH 8.2) streptazoline (**85**), streptazone A (**89**) and B_1 (**90**) are formed and no rubromycins could be detected.[69, 70] A fourfold increase in the production of **81** could be observed when Celite (30 g L^{-1}) was added to the oat bran medium (harvest pH 5.7) and cultivation of strain A1 in a soil-supplemented medium (30 g L^{-1}, harvest pH 6.5) resulted in the discovery of four new metabolites with only traces of the rubromycins produced. Besides **85** and **89**, 5-*O*-(β-D-xylopyranosyl)streptazolin (**86**), 9-hydroxystreptazolin (**87**), 13-hydroxystreptazolin (**88**), and streptenol E (**92**) could be obtained from a 50-L fermentor.[69, 70] Both, pH shifts and supplements influence the observed biosynthetic pathways, which are based on a PKS I for the streptazolins and their putative precursors and a PKS II for the rubromycins and which can be switched on and off, depending on the cultivation conditions.

3.4. *Streptomyces* Tü 3634

Streptomyces Tü 3634 is a well-documented example of using the low specificity of an enzyme system by precursor-directed



Scheme 7. *Metabolite pattern of* Streptomyces *sp. A1.*



Scheme 8. *Metabolites of* Streptomyces griseoviridis *Tü 3634 under standard conditions and induction of metabolites by feeding different nonnatural low-molecular-weight compounds.*

biosynthesis to generate a vast number of 'unnatural' natural products. Compounds **93** and **94** are the main metabolites of *Streptomyces* Tü 3634 under standard cultivation conditions (Scheme 8), but up to 30 further acyl α-L-rhamnopyranosides can be obtained from different feeding experiments with aromatic, heteroaromatic, and α,β-unsaturated carboxylic acids.[71–74] These experiments revealed a strict specificity of the putative glycosyl transferase on the sugar intermediate because only α-L-rhamnopyranosides have been observed.

Most of the isolated metabolites are the rhamnosylated analogues of the carboxylic acids added but additionally, some unexpected metabolites have been obtained as a result of biotransformations (for example, amidation) and the induction of new biosynthetic pathways (Scheme 8).[71] Feeding of salicylic acid to the strain resulted in the production of the unnatural derivatives **95** and **96**. Furthermore, both metabolites could be isolated from feeding experiments with carboxylic acids that are not precursors to **95** and **96**. Possible explanations for the observed results might be the following: The fed carboxylic acids or their degradation products (1) act as signal molecules or imitate natural cofactors resulting in the up or down regulation of silent biosynthesis genes or the stimulation of existing enzymatic activities, respectively, or (2) as xenobiotics they induce an enzymatic detoxification mechanism, as known for the fungus *Neurospora crassa*.[75]

4. Conclusions

We have presented several examples for revealing natures chemical diversity by applying the alteration of cultivation parameters to a limited number of strains. Using the so-called OSMAC approach we were able to isolate more than 100 compounds belonging to more than 25 different structural classes from only 6 different microorganisms. This approach represents a powerful tool to elucidate the secondary metabolome (the overall number of all secondary metabolites of one organism) of different microbes. The number of altered cultivation parameters mentioned in this article is currently being expanded to the influence of light, temperature, and that of other organisms in so-called mixed or pseudomixed cultures.[76] Although a systematic alteration of cultivation parameters was applied to the selected strains, the OSMAC approach is a random approach that does not allow the development of common rules for all microorganisms. But as long as we do not understand the complex network of secondary metabolism and its regulation, everything is allowed to find new natural products and unexpected results can be obtained in any case.

High-quality natural products chemistry is only one way to new pharmaceuticals or agrochemicals. Nevertheless, nobody would deny its importance, which is well documented in the

recent literature.[77–80] With the rapid development of molecular, analytical, and computational tools and with multidisciplinary research its influence will increase even more, and it is in our hands to lead natural product chemistry into the future, not only by continuing with what we have learnt in the past but also with new and exciting research that will bring us to a real understanding of microbial secondary metabolism.

As a matter of course, the next step towards an understanding of the OSMAC approach would be the identification of the molecular triggers that are in charge for the observed induction or change in the metabolite pattern. What compound(s) from an altered medium induce the biosynthesis of the formerly unknown metabolites and how is the biosynthesis regulated by aeration conditions, are there oxygen-dependent promotors? A good example is the change from the 12-membered macrolactone methymycin to the 14-membered narbomycin simply by changing between two cultivation media. Xue and Sherman were able to show that this is the result of an alternative expression of the same gene but the regulation network inducing this change still remains unknown.[81] However, our increasing understanding of the regulation of secondary metabolite biosynthesis will expand the range of the OSMAC approach even more. Several examples for this are described in the literature. Ueda et al. showed the interspecific stimulatory events on antibiotic production and sporulation among different *Streptomyces* species by ethyl ester extractable compounds (probably γ-butyrolactones),[82] and Takano et al. have isolated SCB1 from *S. coelicolor* A3(2), a γ-butyrolactone that elicits antibiotic production of actinorhodin and undecylprodigiosin in the same strain.[83] Nevertheless, unpredictable results, such as the stimulation of antibiotic production by addition of dimethylsulfoxide (DMSO) to the culture broth, open the possibility of 'anything goes'.[84] Every living organism must have the possibility to interact with the environment by so-far unknown mechanisms that result in differential expression and transcription of certain genes and finally in a different metabolome. The main goal would be to find these mechanisms, for example, by use of the increasing quality of bioinformatic tools or by sophisticated analytical and biochemical methods.[85]

With more than 20 000 compounds described in the literature, microorganisms must be called metabolic artists, superior to any metabolic diversity created by man.[86, 87] With a genome size of about 8 Mb in actinomycetes, up to 11 Mb in myxobacteria, and more than 30 Mb in fungi, these three main classes of secondary metabolite producers might possess the genomic prerequisite for more than ten structurally distinct secondary metabolites. Enzymes of the late biosynthesis, encoded within or outside the gene cluster, allow further variations of these metabolites and raise the expectation of the production of up to 50 different compounds from a single strain that can be detected by differential HPTLC or HPLC. The genome itself limits the possible secondary metabolome to a number of compounds being equivalent to the genetic information. However, its visible number of secondary metabolites must be understood as a snapshot of the actual environment of the organism and therefore is likely to be smaller than the maximum number. Nevertheless, the OSMAC approach allows the revelation of the hidden reservoir of chemical diversity and, furthermore, it might be a useful tool to identify several of the 'in silico' natural products coming from the vast number of genome sequencing projects.

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