Pure & Appl. Chem., Vol. 68, No. 3, pp. 745–748, 1996.
Printed in Great Britain.

Microbial metabolites—an infinite source of novel chemistry

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Abstract
Biodiversity is a precious source for modern biotechnology. It is a source which potentially holds innovative and sustainable solutions to a broad range of important problems for modern society. Improved cooperation between the natural product chemists and the microbiologists is an important step forward for speeding up the process of evaluating these potentialities. Further, microbiologists and natural product chemists in tropical countries, with the richest flora and fauna placed right at their door step have a very central position. They are essential for building up international scientific cooperation, with the objective of expanding our understanding of biological and biochemical diversity, and based on this bringing forward more biological solutions. The entire process should build on a principle of fairness and equity in sharing of the benefits and respecting the State´s sovereign right to its own resources.

Introduction

Microbial diversity is constituting an infinite pool of novel chemistry, making up a valuable source for innovative biotechnology. So far we have only scratched the surface of it. The most recent estimates suggest that by now we only know approx. 5% of the total species of fungi and may be as little as 0.1% of the bacteria. And among the ones already described, only a small fraction has been examined for metabolite profile.

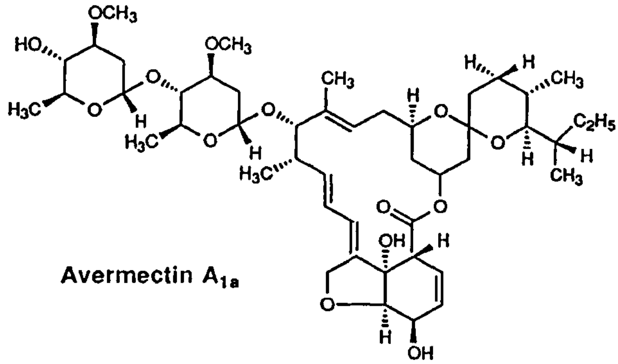
The microbial secondary metabolites can be brought in use in three different ways: the bioactive molecule can be produced directly be fermentation; or the fermentation product can be used as starting material for subsequent chemical modification (derivatization); or thirdly the molecules can be used as lead compounds for a chemical synthesis.

However, the chemical composition of bioactive microbially produced compounds are often intriguingly complicated, including stereochemical diversities which in many instances will make chemical synthesis close to impossible -at least seen from an economical perspective.

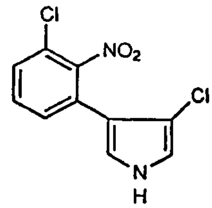
Examples on products which are produced by fermentation and used both with and without chemical modification in agriculture are Avermectins, Blasticidins, Kasugamycins, Validamycins etc. Examples on products which can not be used directly as fermentation product are Strobilurins and Pyrrolnitrine as both these products (in the form produced by the microorganism) are too highly affected by UV degradation. Various chemical modifications, however, stabilize the molecule. The hope is that such stabilization does not on the other hand lead to unacceptable residue problems.

New Methodologies

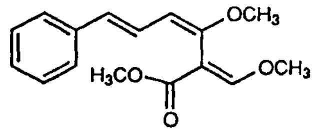
Data bases on natural products have become an important tool in the discovery process of novel metabolites. Examples on the most well known data bases are: Berdy Natural Product Data base (23.000 compounds, in English, DOS compatibility, no chemical structure given); Kitasato Microbial Chemistry Data Base (16.000 compounds, in Japanese, NEC compatibility, no structures given); New Kitasato Chemistry Data Base (16.000 compounds, in English, DOS compatibility, no structures); Chapmann and Hall (15.000 compounds, in English, DOS compatibility, including chemical structures); Actfund (10.800 compounds, in English, DOS and NEC compatible, no structures given).



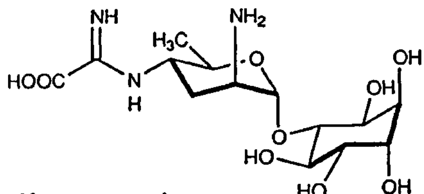
Avermectin A_{1a}



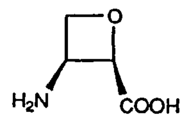
Pyrrolnitrin



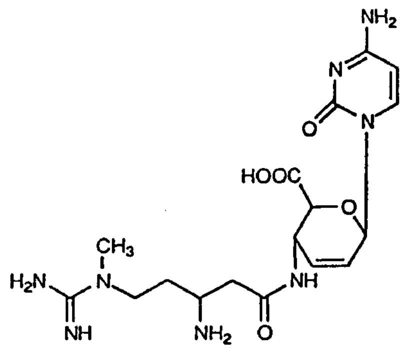
Strobilurin A

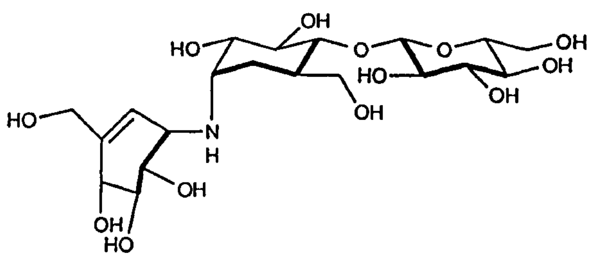


Kasugamycin



Oxetin





Validamycin A

Blasticidin S

Natural product chemists have made good use of new and refined equipment and methodologies -all in all making it possible to isolate, purify and structure elucidate more novel bioactive molecules, including also the more difficult ones (e.g. occurring in extremely low concentrations).

A recent finding of a fungal strain which can produce Taxol stimulates to a discussion of whether some of the plant metabolites eventually will be found also to be produced by microorganisms (as a result of a convergent development) or whether some of the natural products described to occur in low concentrations in plants actually are produced by (previously unnoticed) endophytic microorganisms.

Need for interdisciplinary cooperation

Traditionally microbiologists and natural product chemists have not been very closely cooperating. However, due to recent developments in both disciplines it has become obvious that synergistic benefits can be achieved by establishing a closer cooperation. The obvious fields where the natural product chemist can harvest benefits from a cooperation with the microbiologists are *development of bioassay for efficient monitoring of isolation and purification of new compounds; *bioassay fingerprinting to help early de-selection of known compounds (hereby supplementing the chemical data and giving additional avenues for tapping into the computerized data bases); *activity spectrum to help de-selecting the very toxic compounds; *obtaining a sharper focus in the natural product chemistry work on biologically active compounds. Novel and potentially useful may be of more interest than to go exclusively for just novelty.

Another way of integrating chemistry and biology and hereby achieving a synergistic effect is to use microorganisms as an overlayer to TLC plates. Wherever you identify a clearing zone on top of a TLC spot you directly know which compound is having a specific biological effect.

A similar list could be prepared for the areas of potential benefit for the microbiologist gained from a cooperation with the natural product chemists (e.g. design of selective bioassay, improved understanding of microbial interaction; and identification of strains via metabolite profiles).

A total of 6-10,000 bioactive natural products have been described. Only a very small fraction of the discovered products have been clarified as to what role they play in microbial interactions and ecology and even fewer have been found useful for practical purposes (approx 100 patents are filed for natural products annually being of interest for application in agriculture, silviculture or horticulture).

The ratio of the discovered products which add to the understanding of our surrounding nature and the ones we can bring in use would be much higher if a closer contact was built between the (micro)biologists and the chemist.

But also the plant pathologists may benefit from such cooperations: An obvious topic is within the area of biocontrol. Much too many scientific publications and presentations limit the observation regarding characterization of the organism used for bio control to "producing antibiotics" or "not producing antibiotics". A helping hand from the natural product chemist in the neighbouring department could mediate that the level of understanding would be highered (e.g. clarifying whether the metabolite had a role to play in the control mechanism and therefore could be used to monitor and quality test the large scale production of the biocontrol agent or if the metabolite in question actually was a toxic compounds and therefore of concern seen from safety and registration point of view).

It is within such cooperation between biology and chemistry that the most challenging new break through is to be found.

Metabolites and Taxonomy.
It is interesting to note that the richest profile of biologically active metabolites are described from genera which by numbers and prevalence in nature could be recognized as the most successful of all, e.g. Actinomycetes (65% of all bioactive molecules discovered are produced by Actinomycetes), and filamentous fungi (making up approx 20%, the vast majority coming from just three genera: Penicillium, Aspergillus and Fusarium). This suggests that a rich metabolite profile is important for being competitive in nature. Recently, more interest has been generated around studying biologically active metabolites from higher fungi (Basidiomycetes), endophytic fungi, bacteria and filamentous fungi from marine habitats, the symbiotic lichens etc.

On the other hand no strict correlation between chemical class of secondary metabolites and the taxonomic position of the producing organism exists. Both bacteria and fungi have been found to produce similar class of compounds both in complexity, size and activity. It has e.g. surprisingly been found that even complicated molecules as e.g. the methoxyacrylates are produced by a very diverse selection of microorganisms (representing both white and brown spored Basidiomycetes as well as bacteria (Myxobacteria). A possible explanation for this is that a convergent development has taken place, favouring a similar response to a similar external stimulus.

The structural diversity of the bioactive molecules are also impressive. Let me illustrate it with examples on compounds brought in use for agricultural purposes: Avermectin A (a macrolide, m.w. 873 with anthelmintic and insecticidal activity), Oxetin (an amino acid, m.w. 117, herbicidal activity), Strobilurin A (a benzene derivative, m.w. 342 with fungicidal activity).

Safety. One more good reason for establishing a better cooperation between the biologists and the natural product chemists is the safety concern. Most natural product chemists work with unknown materials and therefore potentially with very toxic materials. A simple biological activity test done at an early stage in the purification work could give valuable guidance in this respect. Such activity test could simply be conducted in microtiter or agar plates, holding inoculum of e.g. a gram positive and a gram negative bacteria (e.g. Bacillus and Pseudomonas), a deuteromycete (Ascomycete) (e.g. Botrytis or Aspergillus) and a Basidiomycete (Rhizoctonia) and a green algae (chlorella). Whenever you find broadly active compounds, inhibiting both types of bacteria, several types of fungi and algae it should be regarded as a warning signal. Then maximum of alertness must be exercised (or the compound de-selected).

Patenting

We need increased investment in R&D in order to bring more biological solutions to a stage where they will not only be discovered and described but also developed and brought in commercial use, hereby being available as realistic alternatives to synthetic chemicals. Discovery, toxicity testing, ecotesting, strain development, production and recovery improvements, formulation, efficacy data etc etc are extremely lengthy and hereby costly data to generate (it takes in average 6-12 years from discovery to marketing of a new metabolite). If industries cannot in some way protect their findings they may choose to invest their R&D capacities elsewhere. Which again results in lack of development of new biological solutions. Seen in this perspective patents are necessary prerequisites for generation of the needed interest and investment.

Unced -92.

Most of the discussion after the Rio Convention in 1992 has dealt with conservation of biodiversity. Almost nil attention has been drawn to the possibility for expanding the sustainable use of the biodiversity. Hopefully this will have a more prominent place on the agenda in the future. Here the microbial diversity is in focus, as the microorganisms provides an infinite source of undiscovered molecules. A source more diverse and potentially holding more creative and innovative solutions to a broad range of important problems (in drugs, plant protection, biocides etc). Microbiologists and natural product chemists in tropical countries, with the richest flora and fauna placed right at their door step has a very central position in building up international scientific cooperation, heading for bringing forward more biological solutions, build on a principle of fairness and equity in sharing of the benefits and respecting the State´s sovereign right to its own resources.