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Nontraditional Microbial Bioactive Metabolites

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ABSTRACT. Microorganisms produce low-molar-mass secondary metabolites exhibiting different biological activities, which are used, *e.g.*, in medicine as antimicrobial and antifungal agents, alkaloids and toxins. Some of these substances have highly diverse biological activities and unusual structures. They are produced by streptomycetes, fungi, and bacilli, but interesting products have also been obtained from microorganisms growing in extreme conditions. Several thousands of microbial products have so far been discovered and many other, which can be potentially useful and/or prospective for human use, can still be in the offing.

CONTENTS

1 INTRODUCTION

The present-day medicine is unthinkable without antibiotics which are used to treat the majority of diseases provoked by microbial pathogens. Several hundreds of antibiotics are widely used in medicine while thousands of others are unsuitable for use because of their side effects. In spite of the fact that several thousands compounds isolated from microorganisms and having some biological activity are known, new substances are still being sought by major pharmaceutical companies due to the continuous appearance of resistant pathogen strains which can be suppressed only by much higher antibiotic concentrations or are completely resistant to known antibiotics. In parallel with the search for new antibiotics and other bioactive products, know natural products are being modified chemically, semisyntheticaly or enzymically. New derivatives of penicillin, cephalosporin, tetracyclines, macrolides, active against resistant pathogens, have been prepared. Structural genes of the producers of bioactive substances are combined and new substances are obtained. Another important source of new compounds comes from mutants of the producers of known active substances (Běhal 2000).

In addition to antibiotics, a broad spectrum of natural products having other effects on living organisms were found in microorganisms: coccidiostatics used in poultry farming, antiparasitic compounds with a broad spectrum of activity against nematodes, substances with antitumor activity, immunosuppressants, thrombolytics, compounds affecting blood pressure, herbicides and pesticides, pheromones, pigments, compounds with antiviral activity, growth promoters of animals and plants, *etc.* (cf., *e.g.*, Žižka 1998).

Another special group of natural products includes enzyme inhibitors (Umezava *et al.* 1976). These compounds can inhibit antibiotic-degrading enzymes, as well as certain enzyme activities in human metabolism that cause illness. Many enzyme inhibitors are proteinase inhibitors, variously active against pepsin, papain, trypsin, chymotrypsin, cathepsin, elastase, renin *etc.* Inhibitors of different glycosidases, esterases, kinases, phosphatases, cAMP phosphodiesterases have also been isolated. Hence microorganisms provide a virtually unlimited source of novel chemical structures with many potential therapeutic applications.

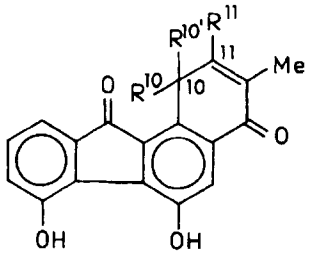
This review introduces representatives of some interesting groups of microbial metabolites with nontraditional biological activities used mainly in medicine.

2 INHIBITORS OF ENZYMES SPLITTING ANTIBIOTICS

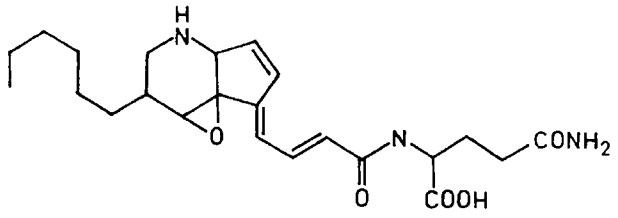
One of the mechanisms of microbial resistance against antibiotics is the action of antibiotic-splitting enzymes. β-Lactam antibiotics are degraded by β-lactamase, which hydrolyzes the β-lactam ring of penicillins and cephalosporins. The discovery, that clavulanic acid produced by *Streptomces clavuligerus* inhibits enzymes splitting β-lactam antibiotics (Reading and Cole 1977) was followed by successful search for next inhibitors (Aldridge 1983; Zhou *et al.* 1993; Adam *et al.* 1987; Kang *et al.* 2000).

3 INHIBITORS OF PEPTIDASES

Bones serve not only as the supportive system of the body but they also play an important role as reservoirs of minerals such as calcium and phosphorus. Even fully formed, bone is not a permanent structure but is constantly undergoing formation and reabsorption under strict control, which ensures bone integrity and homeostasis. An imbalance between bone formation and reabsorption, results in bone diseases such as osteoporosis, osteopetrosis, hypercalcemia and hypocalcemia. Osteoporosis is a chronic bone disease, frequently occurring in postmenopausal women, who often have aberrant bone reabsorption by osteoclasts; the bone mass then first decreases and finally the bone breaks easily. Inhibition of bone reabsorption by peptidase inhibitors may prevent the onset and the progress of osteoporosis. To date, several tens of peptidase inhibitors have been isolated from streptomycetes, *e.g.*, fluostatins A and B (**1a,b**; Akiyama *et al.* 1998*a,b*), epostatin (**2**; Akiyama *et al.* 1998*c*), A-75943 (Morishita *et al.* 1998), and also from moulds (Woo *et al.* 1995; Yamada *et al.* 1998; Otsuka *et al.* 1999).



		R^{10}	$R^{10'}$	R^{11}
1a	fluostatin A		=O	H
1b	fluostatin B	H	OH	OH



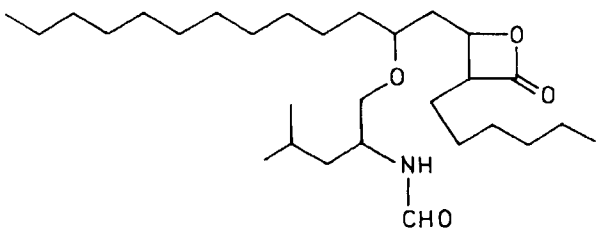
2 epostatin

The activity of elastase, which hydrolyses the elastin in elastin-containing tissues, is also controlled by inhibitors. Elastase dysbalance can evoke acute and chronic arthritis, inflammations or organ damage such as pancreatitis. Elastase inhibitors are used as prophylactics of these against diseases. Several elastase inhibitors such as elastatinal (Umezawa *et al.* 1973), or elasnin (Omura *et al.* 1978) were isolated from actinomycetes. *Penicillium vermiculatum* was formal to produce another elastase inhibitor, vermilutin (Šturdíková *et al.* 1995).

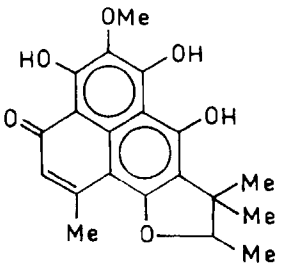
4 INHIBITORS OF LIPASES

Treatment of obesity is an important therapeutic goal for reducing secondary risks in patients with a variety of disorders such as hypertension, diabetes mellitus or atherogenic heart, and central nervous system or peripheral vascular disease. In some individuals, obesity may be associated also with psychological abnormalities due to a distortion of self-image. The average, so-called Western adult consumes 100 g of triacylglycerols and 4–8 g of phospholipids per day about ⅔ of which are of animal origin (Thompson *et al.* 1997). Lipids are hydrolyzed in the intestinal lumen by gastric lipase, pancreatic lipase and cholesterol esterase. Cholesterol in the intestinal lumen is both free and esterified. Cholesterol-esterhydrolase hydrolyzes esterified cholesterol to free sterol, in which form the cholesterol is absorbed. *Streptomyces toxytricini* was found to contain lipstatin which irreversibly inhibits the above lipases. Lipstatin is hydrogenated to tetrahydrolipstatin (**3**) and used for curing obesity (Borgstrom 1988). The absorption of lipids is limited by inhibition of their hydrolysis. Tetrahydrolipstatin covalently binds to pancreatic lipase (1 mol of tetrahydro-

lipstatin to 1 mol of enzyme; Hadvary *et al.* 1991). Obese people, cured by tetrahydolipstatin, lost 10 % of their body mass and the in level of cholesterol decreased by 6–10 % (Scheen *et al.* 1999).



3 tetrahydrolipstatin



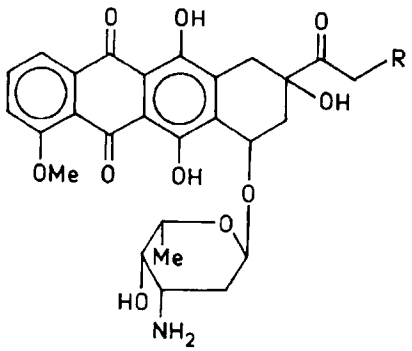
4 erabulenol A

Phospholipases are lipolytic enzymes which catalyze the hydrolysis of ester bound of phosphoglycerols. Inhibitors of phospholipase A2, which can be used for curing various afflictions and allergies, were isolated from *Streptomyces violaceusniger* (Yoshimura *et al.* 1998).

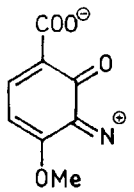
The cholesteryl ester transfer proteins promote exchange and transfer of neutral lipids such as cholesteryl ester and triacylglycerols between plasma lipoproteins. Evidence is accumulating for involvement of these protein in atherosclerosis. Some novel active compounds such as eranbulenols (*e.g.*, eranbulenol A, **4**) isolated from *Penicillium* sp. and *Aspergillus terreus* were shown to be inhibitors of cholesteryl ester transfer protein and might be used in medicine (Tomoda *et al.* 1998). The level of cholesterol in blood is also reduced by mevinolin, which is produced by *Aspergillus terreus* and is a specific inhibitor of hydroxymethylglutaryl-CoA reductase (Alberts *et al.* 1980).

5 CANCEROSTATICS

The not very important anthracycline antibiotics daunorubicin (**5a**; *syn.* daunomycin, rubidomycin) and doxorubicin (**5b**; *syn.* adriamycin) which had been known for 20 years, proved to be excellent antitumor agents, and are widely used in the treatment of a number of solid tumors and leukemias in humans (Hutchinson 1995). Other cancerostatics were found in different microorganisms. Topoisomerase II has been shown to be the primary cellular target for a number of clinically important agents with diverse and unrelated chemical structures. It is now believed that the ability of these agents to form a cleavable complex with topoisomerase II is responsible for the antitumor activity. New compounds, especially from streptomycetes, were found in the course of the screening program for specific topoisomerase II poisons. These include diazoketones (*e.g.*, cremeomycin, **6**) (Ehrlich *et al.* 1956; McGuire 1995), terpentecin and UCT4B (Kawada *et al.* 1995), UCH9 (Ogawa *et al.* 1998) and topostatin (Suzuki *et al.* 1998). Another target for cancerostatics is aromatase, an enzyme of cytochrome p_{450} class, which catalyzes the conversion of androstens to estrogens. Inhibitors of aromatases can be used for curing breast cancer and prostate cancer. Such inhibitor was isolated from *Bacillus* sp. 3072 (Oohata *et al.* 1995). Promising cancerostatics were obtained when *Streptomyces galilaeus* ATCC 31133 was hybridized with the gene for aklavinon 11-hydrolase (Kim *et al.* 1996).



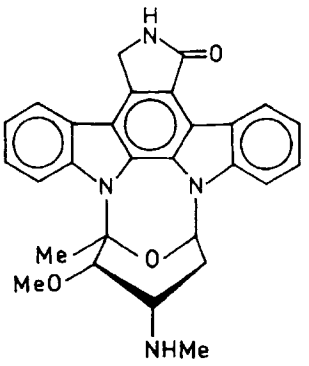
		R
5a	daunorubicin	H
5b	doxorubicin	OH



6 cremeomycin

6 INHIBITORS OF PROTEIN KINASES

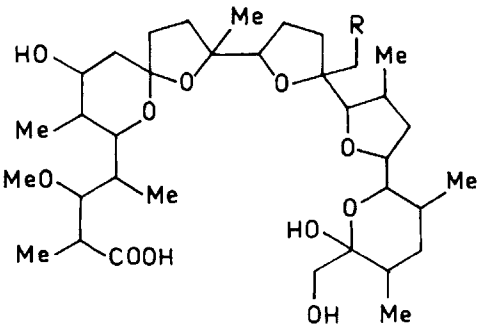
Resistance to antitumor agents is a major problem in the treatment of cancer. When tumor cells aquire resistance to antitumor agents such as anthracyclines, they generaly show cross-resistance to other antitumor agents having different structures and different modes of action. This multidrug resistance is often associated with the presence of a transmembrane glycoprotein, which is an ATP-dependent drug pump that reduces the intracellular concentration of drugs and it can export a broad range of commonly used chemotheraputic drugs from the cells. Several tumor-cell lines of the multidrug-resistance phenotype were shown to exhibit increased protein kinase activity (Ma *et al.* 1991). Staurosporin (7) produced by *Saccharotrix* sp., inhibitor of protein kinases, including serine/threonine and serine/tyrosine kinases, enhances accumulation of vincristine in multidrug-resistant cells (Sato *et al.* 1990; Omura *et al.* 1995) (other interesting bioactivities of staurosporin are intensively studied.). A semisynthetic analog of pyripyropene, 7-*O*-benzoylpyripyropene A, effectively reverses phosphoglycoprotein-related multidrug-resistance by interacting directly with the phosphoglycoprotein in drug resistant cells (Rho *et al.* 2000). Andrastin A, the product of *Penicillium* sp. FO-3929 enhanced the cytotoxicity of vincristine in vincristine-resistant KB cells (Rho *et al.* 1998).



7 staurosporin

7 COCCIDIOSTATICS

Poultry now accounts for 30 % of all meat consumed. Parasites are a problem wherever poultry are raised, and economic losses can be significant. Streptomycetes produce compounds which are potent coccidiostatics and are used in agriculture, especially in large-scale poultry production. The well known compounds are monensins (**8a,b**) (Westley 1977) produced by *Streptomyces cinnamonensis*, lasalocid produced by *S. lasaliensis* and salinomycin produced by *S. albus*. Lasalocid is licensed for use in poultry but not for use in egg-laying birds. It persisted in eggs for 10 d after withdrawal of medicated feed and replacement with lasalocid-free feed. It was shown that the relative ability of monensin, salinomycin and lasalocid to accumulate in eggs was in the ratio 0.12 : 3.3 : 63 ng/g egg per mg/kg of feed consumed, respectively. This indicated that the potential for monensin and salinomycin to cause residues in eggs was very low as compared with lasalocid (Kennedy *et al.* 1998). Residues of monensin, salinomycin and lasalocid in tissues are generally low, are dependent on their concentration in feed and decline rapidly after withdrawal of medicated feed and replacement with drug-free feed. Monensin, salinomycin and lasalocid were also efficacious against spontaneous *Eimeria* infection in rabbits (Pacandl 1986). These compounds are oligoethers which possess the ability to form lipid-soluble complexes that provide a vehicle for a wide variety of cations to traverse lipid barriers. Four anticoccidial agents, diolmycins, which inhibited the growth of *Eimera tenella*, were also isolated from *Streptomyces* sp. WK-2955 (Tabata *et al.* 1993). Coccidiostatics possess a high parenteral toxicity so no clinical applications have emerged.



		R
8a	monensin A	Me
8b	monensin B	H

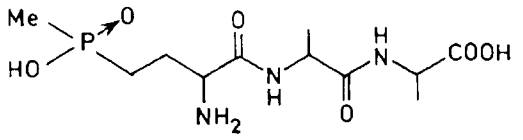
8 ANTIPARASITICS

Microorganisms produce several antiparasitics. The more important ones are avermectins produced by *Streptomyces avermitilis*. Ivermectin (22,23-dihydroavermectin B_1) is used in practice. It is a potent antiparasitic compound active against a broad spectrum of nematode and arthropode parasites (Ikeda and Omura

1995; MacNeil 1995). Ivermectin is believed to act mainly through interactions with invertebrate glutamate-gated chloride channel, but other targets such as spleen cells and 2-aminobutyric acid receptors may also play important roles in the antiparasitic activity of ivermectin (Burkhard 2000). It is used to control internal and external parasites in animals and is also used in human medicine. More than 18 million people are treated with ivermectin each year. Delivery modes include oral, topical and injections. Ivermectin is applied against *Onchocerca volvulus*, which evokes onchocerciasis (river blindness). It shows potent microfilaricidal activity against the major filarial parasites of humans, *Wuchereria bancrofti*, *Brugia malayi*, *Loa loa* and *Mansonella ozzardi*. Ivermectin also has excellent efficacy against both human strongyloidiasis and cutaneous larva migrants for which good alternative treatments have not been available; and it is as effective as currently available drugs against the intestinal nematodes *Ascaris lumbricoides*, *Trichuris trichiura* and *Enterobius vermicularis*; against the human hookworms it shows only partial efficacy (Ottesen and Campbell 1994).

9 HERBICIDES

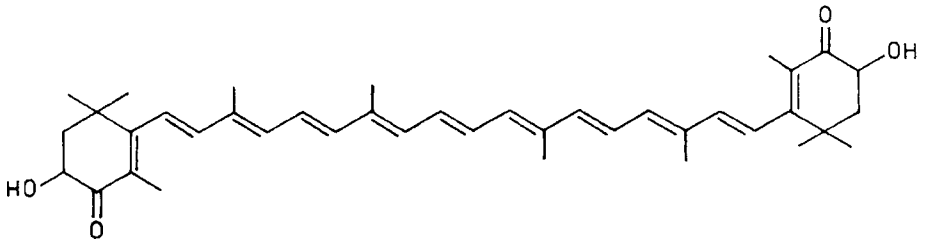
Bialaphos (**9**) is the only natural herbicide used in practice. It is a tripeptide composed of two molecules of L-alanine and an amino acid called phosphinotricin. Bialaphos is produced by *Streptomyces hygroscopicus* and *S. viridochromogenes* (Thompson and Seto 1995). Phosphinotricin is a potent inhibitor of both type-I and type-II glutamine synthetase. Inorganic nitrogen is assimilated by plants and microorganisms *via* glutamine synthetase. For plants this is a dominant pathway, microorganisms have alternative metabolic pathways for assimilation of nitrogen. If we were able to incorporate into cultural plants genes for resistance to bialaphos, we could construct bialaphos resistant crops and destroy selectively weeds.



9 bialaphos

10 ANTIOXIDANTS

Free oxygen radicals, especialy singlet oxygen and peroxyl radicals which are generated during metabolic processes in living organisms, evoke sometimes pathological processes such as arteriosclerosis, cancer, and aging (McCord and Fridovich 1969; Sigler *et al.* 1999). Antioxidants can play an important role in protecting organisms against oxygen radicals. The carotenoid astaxanthine (3,3′-dihydroxy-β,β-carotene-4,4′-dione) (**10**) has attracted interest in recent years because it is an extremely potent antioxidant and



10 astaxanthine

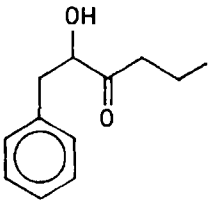
because of its possible role in delaying or preventing degenerative diseases (Schroeder and Johnson 1995), and also because of the economic value of astaxanthin as a pigment source in salmonid aquacultures, lobster cultures and egg production. Astaxanthine is produced by the yeast *Phaffia rhodozyma*. This yeast, which was isolated from sap flues of the birch tree *Betula* (Schroeder and Johnson 1995), can be industrially cultivated just like baker yeast is (Johnson and Lewis 1979). Production of astaxanthine can be increased also by isolating overproduction mutants and optimizing cultivation conditions (An *et al.* 1991; Fang and Cheng 1992).

11 IMMUNOSUPPRESSANTS

The main problem in organ transplantation is preventing rejection of foreign organ transplants, *e.g.*, bone marrow, liver, lung, kidney and heart transplants by the human host and treating autoimmune and infectious diseases. After the discovery of compounds decreasing autoimmunity and following their application, the number of successful transplantations substantially increased. The first active immunosuppressor discovered was the undecapeptide cyclosporin A produced by *Tolypocladium inflatum* (collection name *Trichoderma polysporum* DSM915) (Dreyfuss *et al.* 1976). At present, several tens of immunosuppressives are known which have been isolated mainly from streptomycetes. Important are the macrolides rapamycin and FK506 (Sehgal *et al.* 1994; Meingassner and Stutz 1992), and derivatives of rapamycin (Box *et al.* 1995).

12 ANTIVIRAL COMPOUNDS

Only a few compounds isolated from microorganisms have antiviral activity. From *Bacillus* sp. sattazolin, methylsattazolin (Lampis *et al.* 1995) and sattabacin (**11**) were isolated. They are active against herpes simplex viruses. Another agent active against herpes viruses is fattiviracin A_1 which is produced by *Streptomyces microflavus* strain 2445 (Uyeda *et al.* 1998). An anti-influenza agent, FR198248, was isolated from the culture broth of *Aspergillus terreus*. This compound is a structurally novel tetrahydroxybenzaldehyde (Nashihara *et al.* 2001*a*,*b*). The reason why there are only few known compounds with antiviral activity is the lack of simple methods for detecting their activity.



11 sattabacin

13 CONCLUSIONS

Many bioactive microbial products of different kinds have been discovered to date and many new substances with useful activities will hopefully still be founds. The main problem in discovering a compound with a novel bioactivity is the detection methods, which must be simple and cheap. Approximately 10^5 microorganisms are tested every year world wide but on average only one substance in 10^4 is useful for humans. The probability of obtaining a new useful bioactive product by modification of a known one is much higher. With the increasing spectrum and efficiency of microbial metabolites, new nontraditional sources of such compounds have been tapped, which often include microorganisms living under extreme condition.

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