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HALOGEN-CONTAINING ANTIBIOTICS FROM STREPTOMYCETES

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ABSTRACT. Thousands of new structures of organic compounds have so far been described in the genus *Streptomyces,* the best-known producer of antibiotics. Streptomycetes are the most thoroughly studied microorganisms producing biologically active compounds; out of them several tens contain a halogen atom in their molecules. Mainly is chlorine, however, brominated and fluorinated compounds have also been described. The main attention was devoted to strains producing industrially manufactured antibiotics such as chloramphenicol, chlortetracycline, or the group of antibiotics of vancomycin type. However, other halogenated metabolites also exhibit interesting biological effects. It will be the aim of this chapter to describe the occurrence, isolation, structure and occasionally also biosynthesis of these metabolites.

INTRODUCTION

For a long time it was assumed that halogen containing compounds are only exceptionally detected in living organisms. This assumption began to change at the end of the sixties when bioorganic chemistry experienced a stormy progress due to the development of modern physico-chemical methods. As a result numbers of compounds discovered during the last 30 years increased by several orders of magnitude. Halogenated metabolites found in both terrestrial and aquatic organisms were studied mainly with respect to their potential pharmacological activity. Unfortunately, the number of newly described compounds steadily increases, whereas that of compounds applied in the practice grows only relatively slowly and the difference between the two groups of compounds permanently increases.

Chlorinated derivatives are the most frequent halogenated metabolites found in the nature. This is mainly due to the fact that chlorine is the most common halogen found on the Earth. Chloramphenicol, isolated from the soil bacterium *Streptomyces venezuelae,* was the very first halogenated metabolite detected. This important commercial antibiotic will be dealt with in chapter 1. Other chlorinated metabolites, *e.g.* chlortetracycline, will be discussed in chapter 2.

The three remaining halogens, *i.e.* fluorine, bromine and iodine, are only rarely present in soil microorganisms, *e.g.* streptomycetes. Compounds with those halogens were mainly prepared by directed biosynthesis by adding a respective anion to the cultivation medium (*e.g.* addition of bromine yielding bromotetracycline). Fluorine derivatives such as fluoroacetate and 4-fluorothreonine were also detected. Iodine-containing metabolites have not yet been described in streptomycetes. The chemical structure of halogenated metabolites found in streptomycetes is highly varied. The simplest derivative (*e.g.* fluoroacetate described above) contains only two carbon atoms, whereas certain chemically complex antibiotics contain a substantially higher number of carbon atoms.

In the following subchapters we tried to describe individual compounds according to their chemical structure with a special attention paid to important industrial antibiotics.

Chloramphenicol

In 1947 a strain of *S. venezuelae* [1-4] was isolated from a soil sample collected in Venezuela. It produced a wide-spectrum antibiotic called chloramphenicol (**1**) according to its chemical structure. Due to its simple chemical structure it is still used. It is the only antibiotic produced by the chemical synthesis rather than biosynthetically. Nevertheless, *S. venezuelae* is used as a model organism in biosynthetic and genetic experiments.

Thus for instance its biosynthesis [5] was investigated with the aid of stable isotopes (^{2}H, ^{13}C), in experiments in which acetic acid or dichloroacetic acid served as precursors. In streptomyces it was found that the addition of amino acids such as phenylalanine, or P-nitrophenylserine increases the chloramphenicol production. In further experiments NaCl was replaced with NaBr and bromo and chloroderivatives could be isolated from the cultivation broth [6].

Chloramphenicol is produced by *S. venezuelae via* the following pathway: chorismic acid → *p*-aminophenylalanine → *p*-aminophenylserine → dichloroacetyl → *p*-aminophenylserine → chloramphenicol.

Chloramphenicol is a relatively simple compound containing two asymmetric carbon atoms (4 isomers exist). Biological activity of all of them was investigated. The results obtained can be summarized as follows:

1. Nitro group is not important for the biological activity.
2. Substitution of the phenyl group by another aromatic or heterocyclic ring decreases the activity.

3. There is at least one hydrogen on the nitrogen atom. Substitution of this hydrogen atom by any other group results in the loss of the biological activity.

4. Esterification of the primary alcohol group also leads to the loss of the biological activity.

5. Out of four possible isomers (**1-4**) only the D-threo isomer (**1**) is active. The activity of the remaining isomers is about 1 %.

Chloramphenicol has an outstanding bacteriostatic effect against a number of Gram-positive and Gram-negative bacteria. It inhibits growth of the genera *Aerobacter, Staphylococcus, Streptococcus, Diplococcus, Proteus, Bacillus, Vibrio, etc.* It also inhibits rickettsiae, including those causing louse-borne typhus, and some large viruses (causing trachoma, veneral lymphogranuloma, atypical pneumonia, and some other diseases). The antibiotic is a specific drug for the treatment of typhoid and paratyphoid fevers, dysentery, brucellosis, toxic dyspepsia, trachoma, and other diseases. Chloramphenicol is very effective against bacillary dysentery in children. When given perorally administered, it is well absorbed and is carried by blood to tissue and tissue fluids.

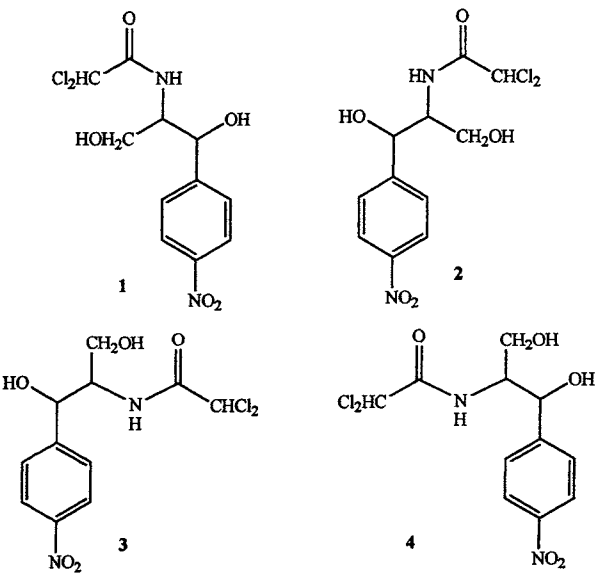


Fig. (1). Four possible isomers of chloramphenicol

Tetracyclines

Tetracyclines [7-12] are probably the most important group of halogen containing antibiotics. They are produced primarily by *S. aureofaciens*, however, further streptomycetes producing these antibiotics were described. Similar structures have recently been found also in other microorganisms (see below). Chlortetracycline (**5**) is the most common derivative. About one hundred of other halogenated tetracyclines are known at present. In addition, organic chemists prepared thousands of synthetic and semisynthetic tetracyclines. Chlortetracycline is used as a wide-spectrum antibiotic. Microorganisms sensitive to chlortetracycline develop resistance, but this resistance develops only slowly, much more slowly than resistance to streptomycin. The excessive use of chlortetracycline in medicine increases the occurrence of resistant microorganisms. Chlortetracycline should preferably be used to treat diseases whose causative agents are resistant against penicillin or streptomycin. It is successfully used for the treatment of bacterial pneumonia, brucellosis, tularemia, pertussis, scarlet fever, anthrax, and other bacterial diseases. Moreover, chlortetracycline can be used to treat various forms of louse-borne typhus, various rickettsioses, and also some viral diseases (trachoma, lymphogranuloma). Doses not exceeding 500 mg are applied perorally three- to four-times a day.

Biosynthesis of tetracycline was studied extensively and a number of various intermediates were isolated from the cultivation broth (**6-37**) [13-16], *e.g.* isochlortetracycline (**6**). High amounts of chlortetracycline are used in agriculture, *e.g.* in food additives on fish farms or also in pig, calf, sheep and poultry breeding.

The addition of NaBr to the medium leads to the production of a brominated derivative (bromotetracycline) (**7**) instead of the chlorinated one [17]. Other chlortetracyclines such as demethylchlortetracycline (**8**) [18] or anhydrotetracycline (**9**) [19] were isolated from mutants of *S. aureofaciens* and have similar antibiotic effects.

The structure-function relationships can be summarized as follows.

1) Substitution in positions 5, 6 and 9 is not important. For instance, the exchange of chlorine with bromine in position 7 yields compounds with very similar antibiotic effects.

2) Substitutions in positions 2, 11a and 12a result in biologically active compounds only if the original compound can be easily released in the target organism.

3) Replacement of the dimethylamino group leads to a decreased biological activity or its complete loss.

4) Changes in the spatial arrangement (5-epimer) give rise to biologically inactive compounds.

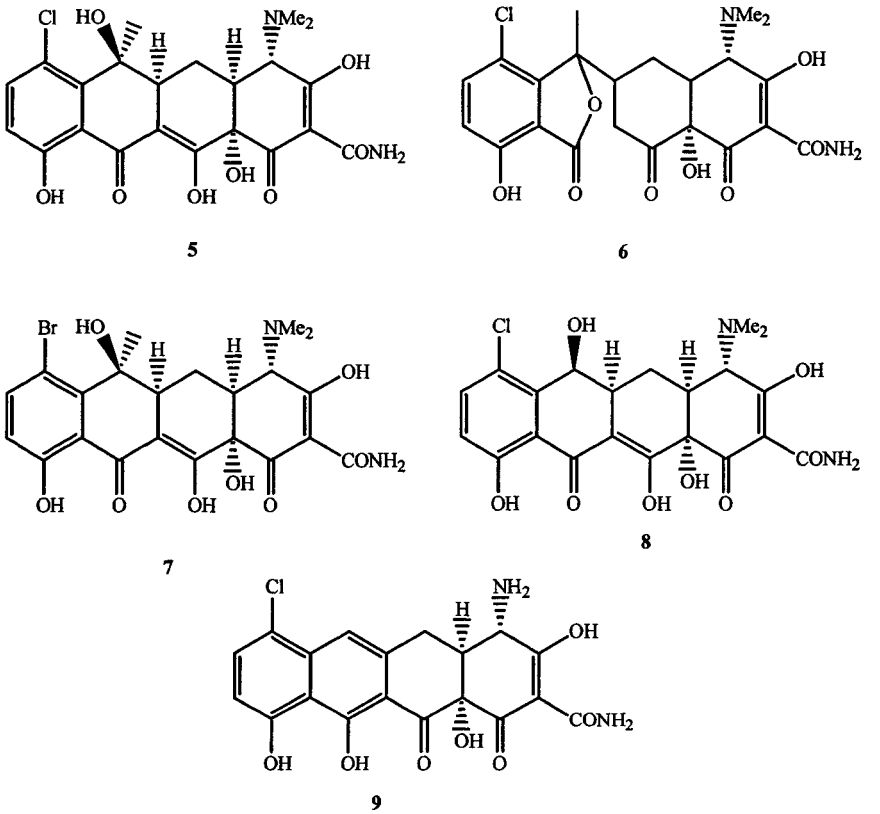
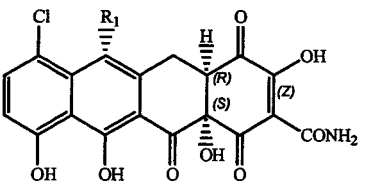
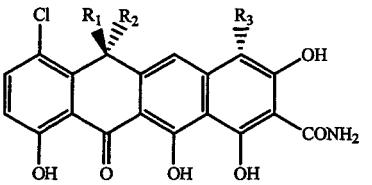


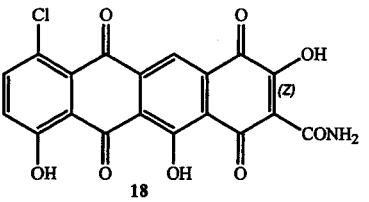
Fig. (2). Tetracyclines



10 R_1 = Me
11 R_1 = H



12 R_1 = H, R_2 = Me, R_3 = H
13 R_1 = OH, R_2 = Me, R_3 = H
14 R_1 = OH, R_2 = Me, R_3 = OH
15 R_1 = OH, R_2 = Me, R_3 = NMe_2
16 R_1 = H, R_2 = H, R_3 = OH
17 R_1 = R_2 = R_3 = H



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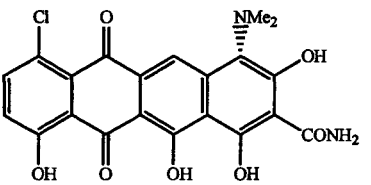

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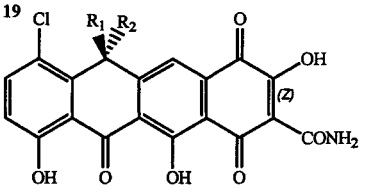
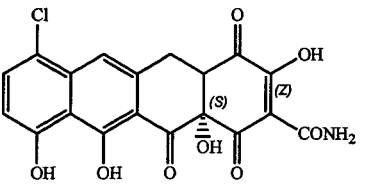


20 R_1 = OH, R_2 = Me
21 R_1 = H, R_2 = H



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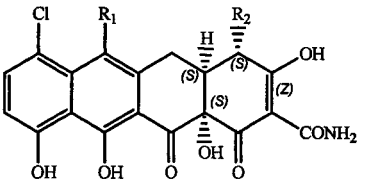
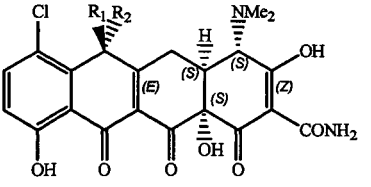
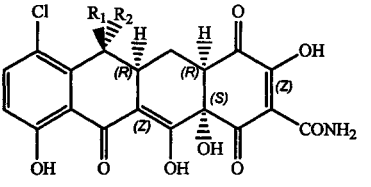


23 R_1 = H, R_2 = NH_2
24 R_1 = Me, R_2 = NH_2
25 R_1 = Me, R_2 = NMe_2



26 R_1 = OH, R_2 = H
27 R_1 = OH, R_2 = Me

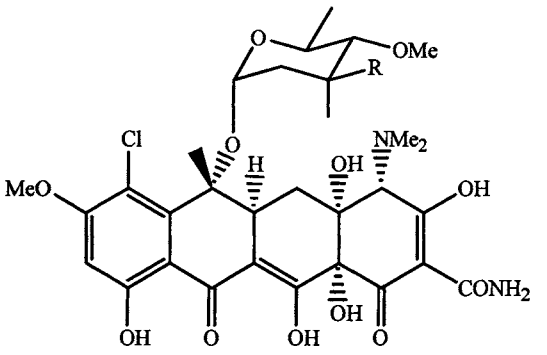


29 R_1 = OH, R_2 = H
30 R_1 = OH, R_2 = Me

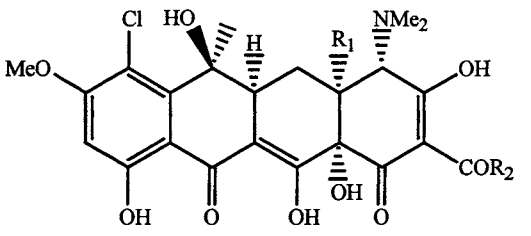
Fig. (3). Some important tetracycline metabolites

No	Name
10	4-oxoanhydrochlortetracycline
11	Methylchlorpretetramide
12	Methylhydroxychlorpretetramide
13	Chloraureovocidin
14	methylchlortetramide-blue
15	6-demethyl-6-deoxy-7-chloraureovocidin
16	Chlorpretetramide
17	chloro-A-C-diquinone
18	chlortetramide-blue
19	chlortetramide-green
20	4-oxoanhydrodemethylchlortetracycline
21	6-demethyl-6-deoxy-7-chlortetramid-green
22	4-oxoanhydrodemethylchlortetracycline
23	4-aminoanhydrodemethylchlortetracycline
24	4-aminoanhydrochlortetracycline
25	anhydrochlortetracycline
26	dehydrodemethylchlortetracycline
27	dehydrochlortetracycline
28	4-oxodemethylchlortetracycline
29	4-oxochlortetracycline
30	4-oxodehydrochlortetracycline

More recently, dactylocyclines A (**31**), B (**32**), D (**33**) and E (**34**) have been isolated from a *Dactylosporangium* strain ATCC 53693 [20-24]; these have the opposite stereochemistry at C6. Dactylocyclinones *i.e.* 8-methoxy-chlortetracycline (Sch 36969) (**35**), 2′-*N*-Methyl-8-methoxychlortetracycline (Sch 33256) (**36**) and 4a-hydroxy-8-methoxychlortetracycline (Sch 34164) (**37**) are the parent members of the family that are the 6-epimers of 8-methoxychlortetracycline from *Actinomadura brunnea* [25,26]. The dactylocyclinones contain unusual sugar moieties, with dactylocycline A being the biologically most active member. A group of tetracyclines including SF 2575 (**38**) has been isolated from *Streptomyces* species containing C-glycosidic bond at C9 and a salicyloyl ester at C4.



31 R = NHOH
32 R = NO_2
33 R = NHOAc
34 R = OH



35 R_1 = H, R_2 = NH_2
36 R_1 = H, R_2 = NHMe
37 R_1 = OH, R_2 = NH_2

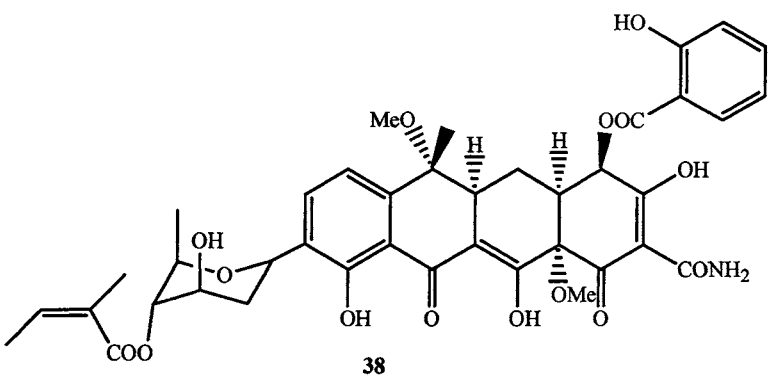


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Fig. (4). Dactylocyclines and dactylocyclinones.

Oxygen compounds

S. malachitofuscus produces X14766A, the only chlorine containing polyether antibiotic (**39**) described so far. The producer was isolated from the Mexican soil in 1976. The antibiotic is active against Gram-positive bacteria forming strong complexes with both monovalent and divalent metals (K, Na, Rb, Cs, Ba, Sr, Ca, Mg, Li) [27,28].

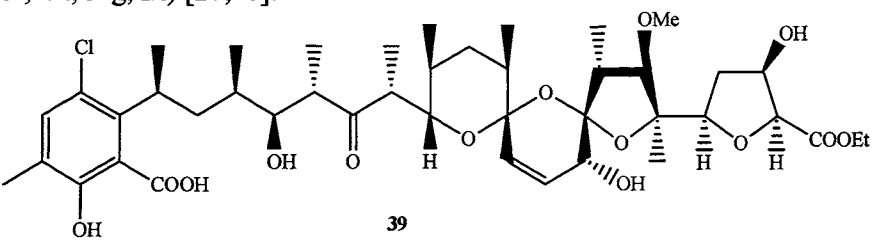

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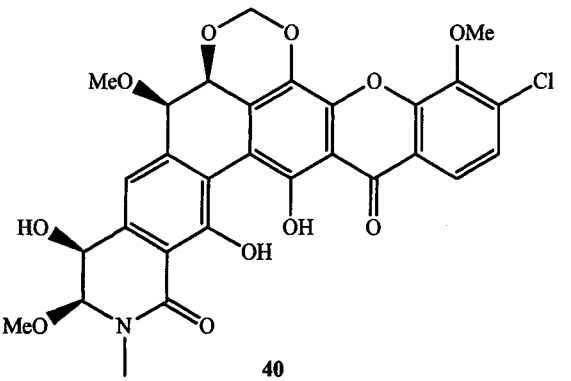
Fig. (5). Structure of polyether antibiotic-X14766A

Lysolipins I (**40**) and X (**41**) [29] were isolated from *S. violaceoniger*. The compounds inhibit both Gram-positive and Gram-negative bacteria inducing probably the lytic activity of bacterial cells. The antibiotic was one of the first antibiotics whose biosynthesis was studied with the aid of labeled stable precursors. A highly interesting experiment was performed including the cultivation of *S. violaceoniger* in the atmosphere of isotopically labeled oxygen (^{18}C) as shown in Fig. 6. Compounds BE19412A (**47**) BE19412B (**48**) active against implanted Ehrlich cancer cells were isolated [30] from *Streptomyces* sp.



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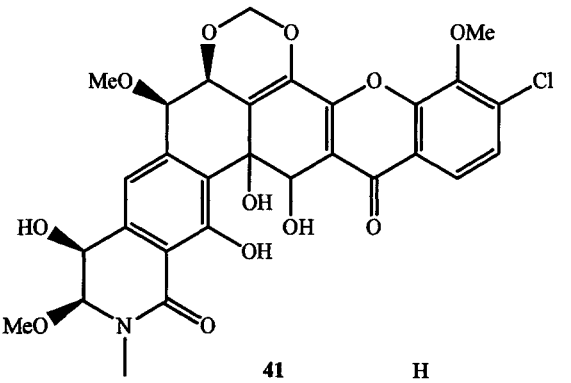

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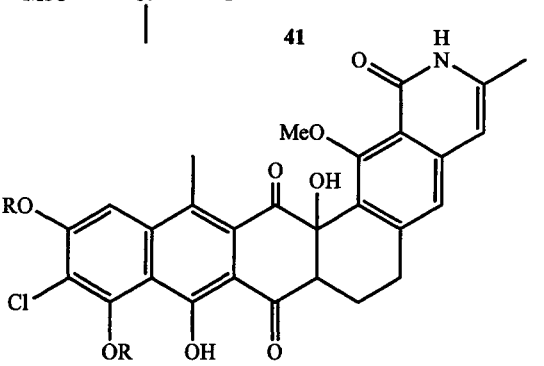


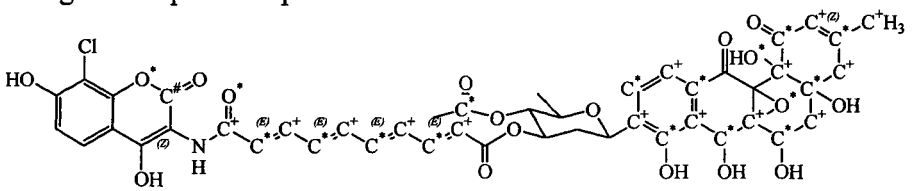
42 R = H
43 R = Me

Fig. (6). Lysolipin like compounds

A known strain of *S. antibioticus* was found to produce a compound designated simocyclinon D8 (**44**) [31]. The compound exhibits antibiotic activity against Gram-positive bacteria and cytostatic effects against various cancer cells. Its structure and biosynthesis were clarified on the basis of physical-chemical methods, mainly by 1D and 2D NMR and ESI-MS. It was found that during its biosynthesis the incorporation of ^{18}O into the aminocoumarine residue is probably catalyzed by a monooxygenase.

Naphtocyclinone (**45**) was isolated from the mycelium of *S. arenae* [32]. Another similar metabolite (naphtomevalin, *i.e.* **46**) was isolated from an Australian soil sample at 2.5 μg/ml.

S. aculeolatus produces a group of antibiotics [33] with biological activity against Gram-positive bacteria (**47-50**). The structure of these antibiotics [34] was clarified by physico-chemical methods, mainly by 1D and 2D NMR. It was found that the production in a 50 L fermentor reaches up to several tens of milligrams of pure compounds.



C^* [1,3-$^{13}C_2$]malonic acid, C^+ [2-^{13}C]malonic acid, $C^\#$ L-[1-^{13}C]tyrosine, $^{*}{}^{18}O_2$

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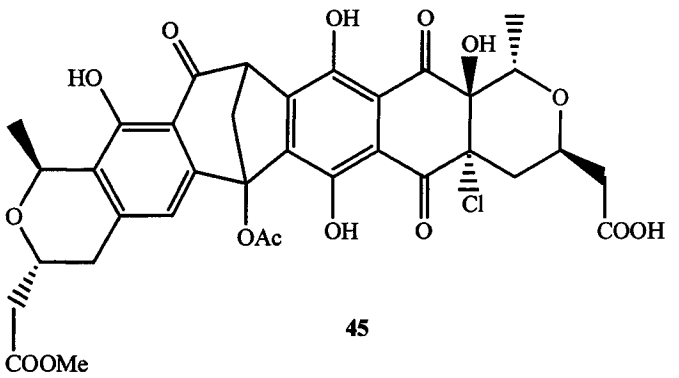
Fig. (7). Precursors in biosynthesis of simocyclinon D8



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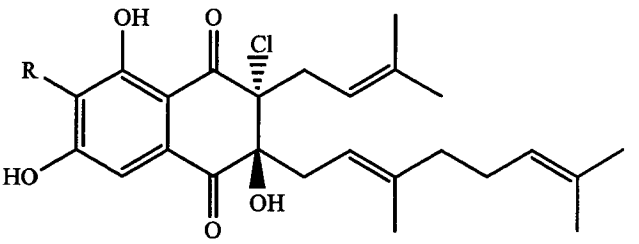
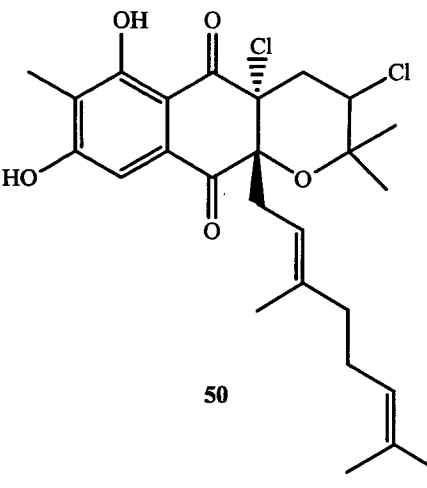


46 R = H
49 R = Me



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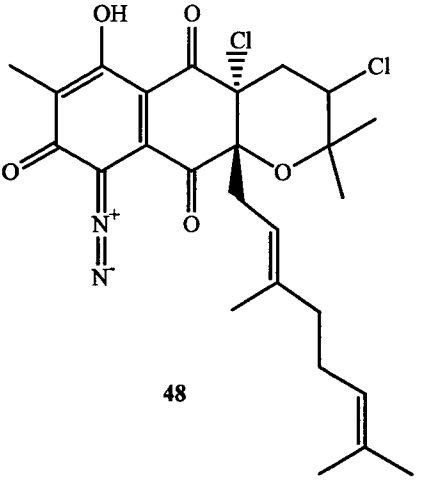

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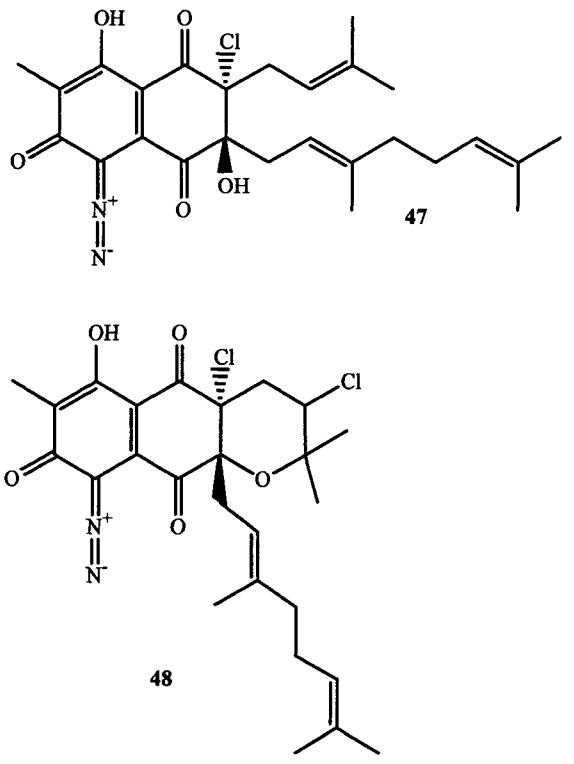


Fig. (8). Naphtocyclinones

Macrolide antibiotics are frequent in the genus *Streptomyces*. During the last 50 years several thousands of new antibiotics were described [35]. However, macrolides with heteroatoms in their structure, *e.g.* a halogen, are much less frequent. A 34-membered macrolide antibiotic colubricidin A (**51**) isolated from a new *Streptomyces* species can serve as example. The compound exhibits a very low activity on Gram-positive and Gram-negative bacteria. On the other hand, it has an excellent nematocidal activity against *Caenorhabditis elegans*.

Another compound, ansamitocin P3 (**52**), was isolated [36] from lichens of the family *Thuidiaceae*. However, it may be assumed that the lichens do not synthesize this compound but that it is rather a product of streptomycetes living on surface of these lichens. Ansamitocin P3 exhibits an excellent cytotoxicity against human tumors, leukemia and carcinoma in cell cultures.

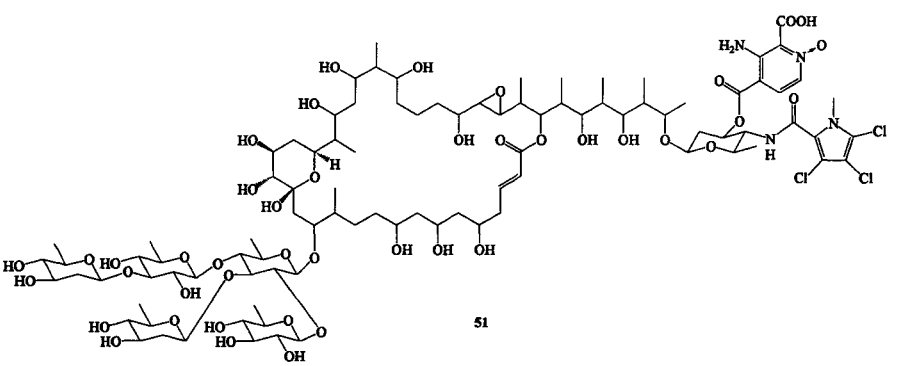
Roseophilin (**53**) was isolated [37] from *S. griseoviridis* and found to exhibit cytotoxicity against leukemic and epidemoid cells at a concentration of IC=0.5 μM.

Ansamycin antibiotics are probably the most complex organic compounds produced by the genus *Streptomyces*. In addition to the antibacterial, they also exhibit antiviral effects. Ansamitocin P-3 shows a potent cytotoxicity against the human solid tumor cell lines A-549 and HT-29. The compound exhibits a significant activity against P-388 lymphocytic leukemia in mice and both 9PS (murine lymphocytic leukemia) and 9KB (human nasopharyngeal carcinoma) in cell culture systems. [38].

Naphtomycin A (**54**) was isolated from *S. collinus* and its structure was clarified by means of spectral methods. Naphtomycin A is a strong vitamin K antagonist. In addition to naphtomycins A and H (**55**), a geometric isomer of naphtomycin B (**56**), was produced by a streptomycete isolated from the Indian soil [39].

Naphtomycins B and C, *i.e.* thio- and chloroansamycins, were isolated [40] and found to exhibit the antibiotic activity [41]. As already mentioned above, their structure is very complex and has been revised several times [42,43]. Their biosynthesis was investigated using labeled isotopes [44].

S. antibioticus produces another unusual macrolide antibiotic [45] called chlorothricin (**57**) containing, in addition to the aglycone (modified methylsalicylic acid), saccharides, dideoxyhexoses in the first place. Their biosynthesis was investigated by the incorporation of stable isotopes [45-47]. The compounds were only active in a synthetic medium and inactive in a complex one [48]. Compounds designated MC031-034 (**58-61**) are similar to chlorothricin mentioned above and were isolated from the cultivation broth of *Streptomyces* sp. collected in Japan. Another compound, 2-hydroxychlorothricin (**62**), with antitumor activity, was isolated [66] from *Streptomyces* K818.



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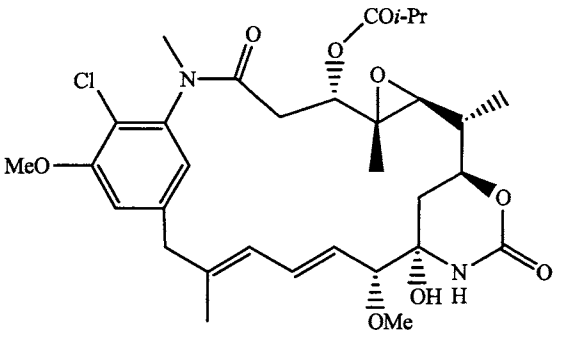

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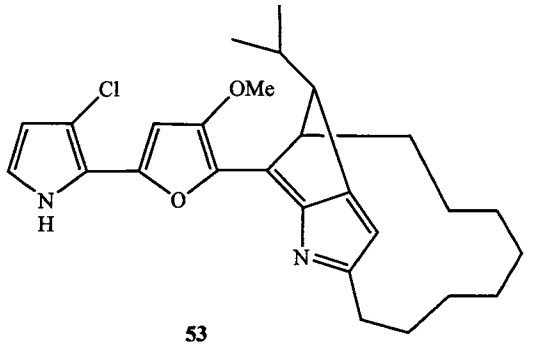

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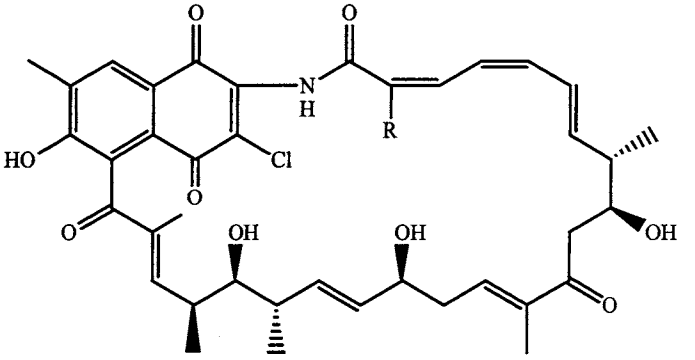
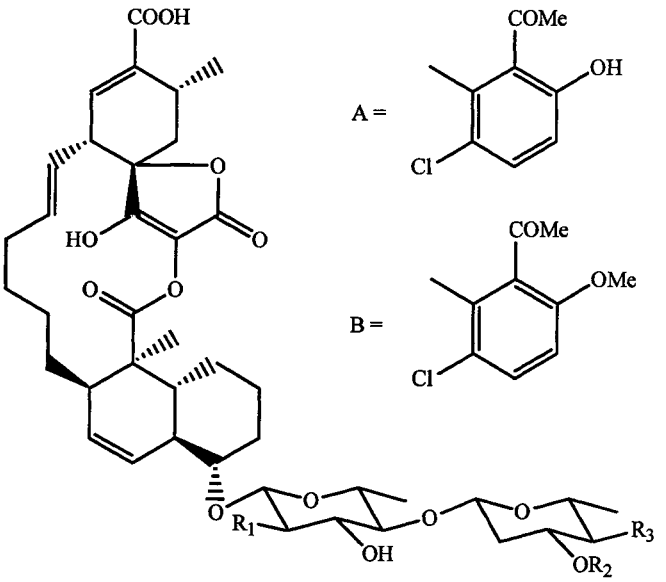


54 R = Me
55 R = H



57 R_1 = H, R_2 = B, R_3 = OH (may be also Br)
58 R_1 = H, R_2 = A, R_3 = OH
59 R_1 = OH, R_2 = A, R_3 = OH
60 R_1 = H, R_2 = H, R_3 = OA
61 R_1 = OH, R_2 = H, R_3 = OA
62 R_1 = OH, R_2 = B, R_3 = OH

Fig. (9). Chemical structures of macrocyclic antibiotics

A large group of antibiotics called orthomycins was described as a product of different streptomycetes [50]. The group includes flambamycin, curamycin and avilamycin. These antibiotics exhibit a very low if not negligible activity against Gram-negative bacteria, yeasts and fungi. On the other hand, they have a significant effect on staphylococci and streptococci.

Flambamycin exhibits a very low toxicity and shows an interesting activity against Gram-positive bacteria, *Neisseria,* Gram-negative bacteria and some Gram-positive bacilli. It is practically inactive against Gram-negative bacilli, yeasts and filamentous fungi. This selectivity coupled with excellent therapeutic activity in mice infected experimentally with *Staphylococcus aureus, Streptococcus pyogenes haemolyticus*, or *Neisseria meningitidis* encouraged structural investigation of flambamycin.

In the course of studies [51-53] on the production of antimicrobial agents by microorganisms, a new antibiotic flambamycin (**57**) (also known as 21190 RP), was discovered in the culture broth of *S. hygroscopicus* DS 23230. The strain was isolated from a soil sample collected in Great Britain and its antibiotic properties were demonstrated by classical methods. It exhibits all the main morphological and biochemical characteristics of the species *S. hygroscopicus*, especially the tight spiral sporophores, the dark grey color of the normally sporulated mycelium, and also in ageing cultures the production of dark patches and of an exudate on surface of colonies on an agar medium.

South-American soil was a good source [54] of *S. curacoi* producing the antibiotic curamycin (**58**). This antibiotic also contains unusual saccharides, *i.e.* L-lyxose and 4-O-methyl-D-fucose.

Avilamycin A (**59**) isolated [55] from *S. viridochromogenes* collected from a Venezuelan soil sample, exhibits similar effects. Its structure was elucidated by NMR methods and two unusual saccharides – evalose and evermicose – were identified in the sugar moiety.

Antitumor antibiotics of the enediyne group, containing a highly unusual structure of the 9-membered unsaturated ring, were isolated from an unidentified streptomycete [56,57] designated L585-6 (**60**). These compounds were found to be highly active primarily against known cell lines [58] but almost inactive against Gram-negative bacteria. They form strong complexes with DNA preventing its replication and, as a result, exhibit a high activity against different cancer types.

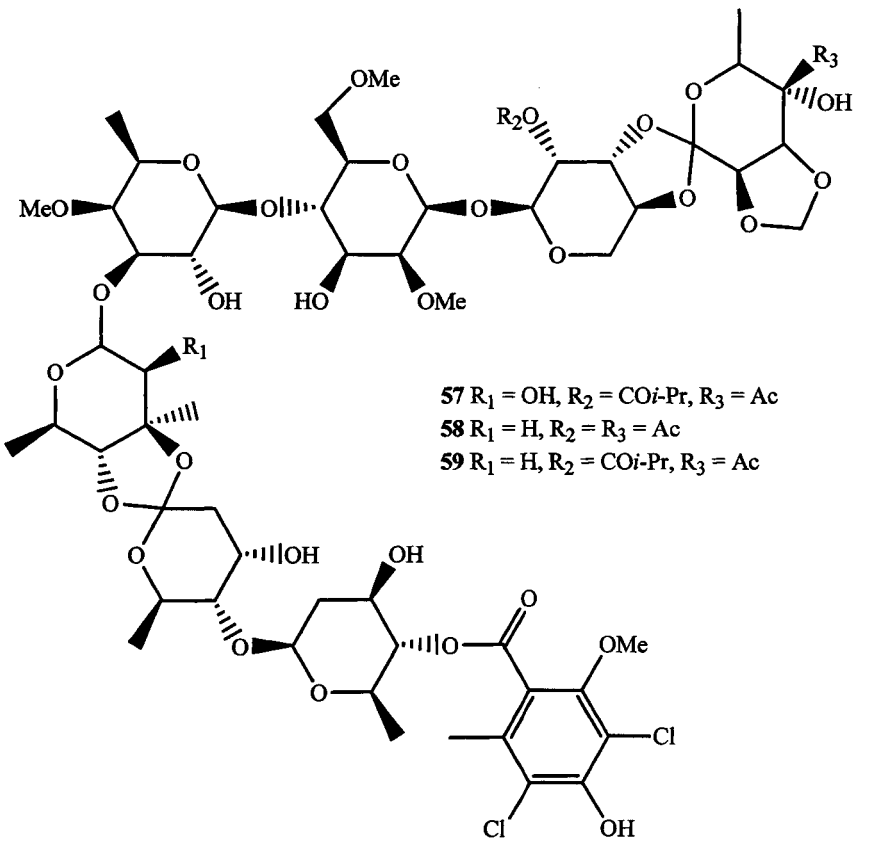


Fig. (10). Chemical structure of orthosomycin antibiotics.

Antibiotic C-1027 (**61**) was isolated from the cultivation broth of *S. globisporus* [59] and its molecular weight was determined to be 15 000 [60]. The chromophore part of this antibiotic [60] contains a 9-membered ring incorporated into the 16-membered macrocycle [61]. Complexity of its structure was described several times [61,62] and its absolute stereochemistry was determined on the basis of not only 2D NMR but also of CD spectra. Its high cytotoxicity exceeds even that of doxorubicin [63]. *S. graminofaciens* was several times erratically described [64,65], as a producer of chlorinated flavone derivatives (**62,63**).

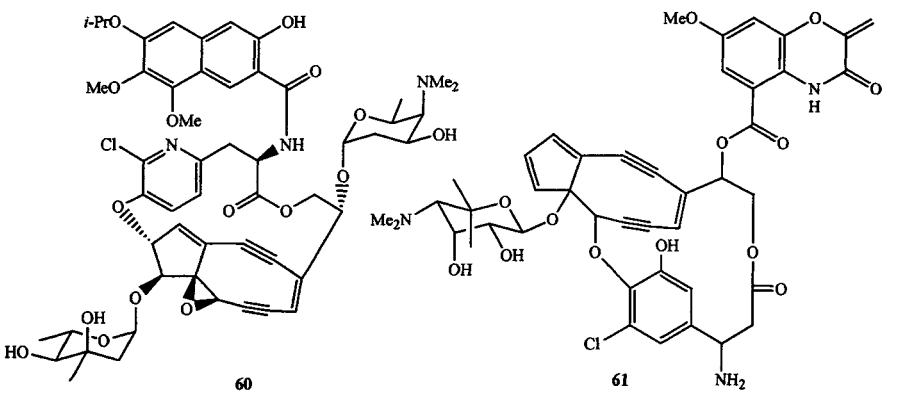
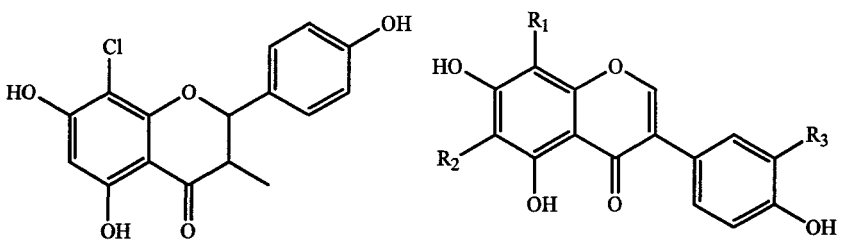


Fig. (11). Unusual structures of antibiotics with enediyne group

It was shown later that the streptomycete can only modify rather than biosynthesize these compounds [66] and that all such derivatives, *i.e.* even genisteins (**64,65**) come from the soy meal or cotton seed and are not produced by streptomycetes. These results demonstrate clearly that flavonoids isolated from streptomycetes cultivated on media containing plant components, such as soy meal or cotton seed, always come from the cultivation medium and are not of microbial origin. The same effect was observed in *S. griseus* [65] producing two additional chlorinated genisteins (**66,67**). This also holds true [67] for isoflavone (**68**) isolated from Japanese soil.

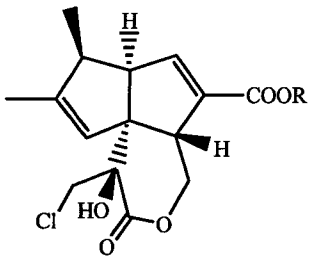


62 2(*S*):3(*S*)
63 racemic mixture of 2(*S*):3(*R*) and 2(*R*):3(*S*)

64 R_1 = Cl, R_2 = H, R_3 = H
65 R_1 = Cl, R_2 = Cl, R_3 = H
66 R_1 = H, R_2 = Cl, R_3 = H
67 R_1 = H, R_2 = Cl, R_3 = Cl
68 R_1 = Cl, R_2 = H, R_3 = OH

Fig. (12). Derivatives of flavones metabolized by *Streptomyces*.

Acidic lipophilic antibiotics pentalenolactones (**69,70**), active against Gram-positive and Gram-negative bacteria including acid-fast bacteria, were isolated [68] from *Streptomyces* sp.



69 R = H
70 R = Me

Fig. (13). Pentalenolactones from *Streptomyces*

Nitrogen compounds

An L-leucine antimetabolite called AL-719 was isolated [69] from the cultivation broth of different *Streptomyces* species. In this metabolite one of two methyl groups is substituted by chlorine (**71**).

A glutamic acid analogue (**72**) was isolated [70] from a production *Streptomyces* strain. It was found to be partially effective against *Micrococcus luteus*.

Another species of the genus *Streptomyces* [70], identified as *S. xanthocidicus*, was found to produce a compound called FR900148 (**73**). This compound inhibits the growth of both Gram-positive and Gram-negative bacteria. It is assumed to inhibit the cell wall biosynthesis. Using physico-chemical methods its structure was determined as 1-*N*-valyl-3-chloro-2,5-dihydro-5-oxo-1*H*-pyrrole-2-carboxylic acid.

4-Chlorothreonine (**74**) was isolated [71] from the streptomycete designated OH5093. It exhibits the antibiotic activity against the yeast *Candida*.

Armentomycin (**75**) [72] inhibits the growth of bacteria on a synthetic medium. Biosynthesis of this non-protein amino acid is catalyzed by a peroxidase incorporating chlorinated substituents into the molecule without a simultaneous removal of other functional groups.

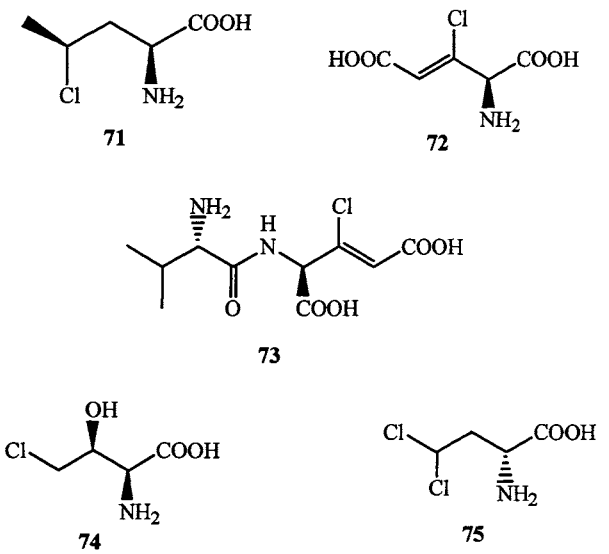


Fig. (14). Amino acid metabolites

The antimetabolic activity of di- tri- and oligopeptides has been generally recognized. Compound (**76**) was isolated [73] from the cultivation broth of *Streptomyces* sp. 372A. The compound inhibits growth of Gram-positive and Gram-negative bacteria, however, only on a chemically defined medium. The addition of L-glutamine abolishes its effect.

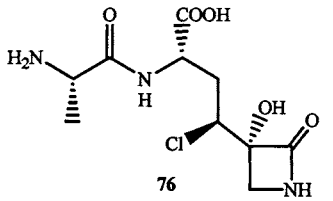
Chlorocardin (**77**) was isolated as a β-lactam antibiotic [74] and exhibits biological activity against bacteria of the genus *Pseudomonas in vitro*.

A new herbicide resormycin (**78**) [75] was isolated from the streptomycete *S. platensis* exhibiting a significant biological effect against the unicellular green alga *Selenastrum capricornutum*. Resormycin inhibits primarily rather the growth in the dark than in the light. The effect of the compound on weeds in crops was also investigated in field experiments.

A complex of actinomycins Z (Z1-Z5) with only two chlorine containing β-depsipeptides (**79**=Z3, **80**=Z5) was isolated from the well-known *S. fradiae* [76]. Both actinomycins (Z3 and Z5) exhibit significant cytotoxicity against cancer cells.

Pepticinnamines (A-F) (**81**=E) inhibiting pharnesyl transfer and belonging to the protein family were isolated [77] from the streptomycete OH 4652. Their structure [78] was determined by means of NMR and found to contain five unusual amino acids and O-pentenylcinnamic acid.

The complete structure of RP 18,631 (**82**), a new chlorine containing antibiotic related to novobiocin, has been determined [79] using a combination of degradative and NMR techniques. Of particular interest is the long-range couplings observed in the pyrrole ring present in the molecule.



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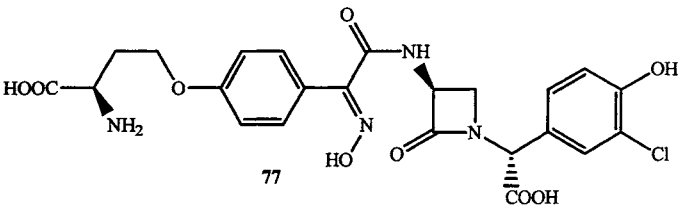

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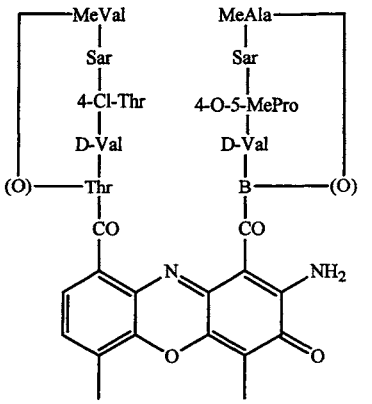
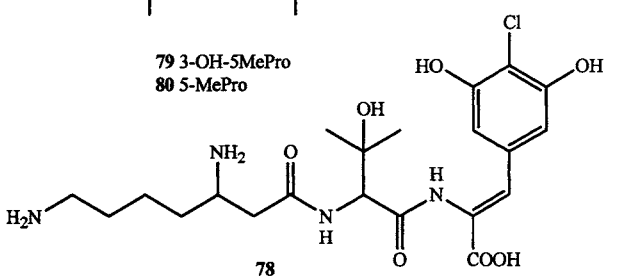


79 3-OH-5MePro
80 5-MePro



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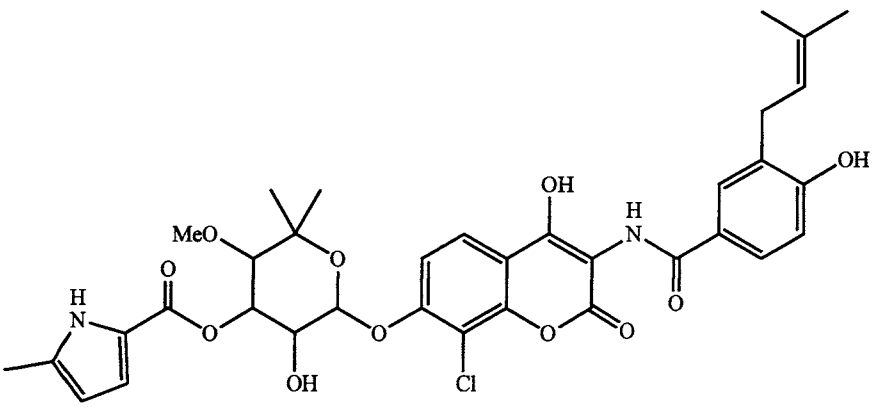


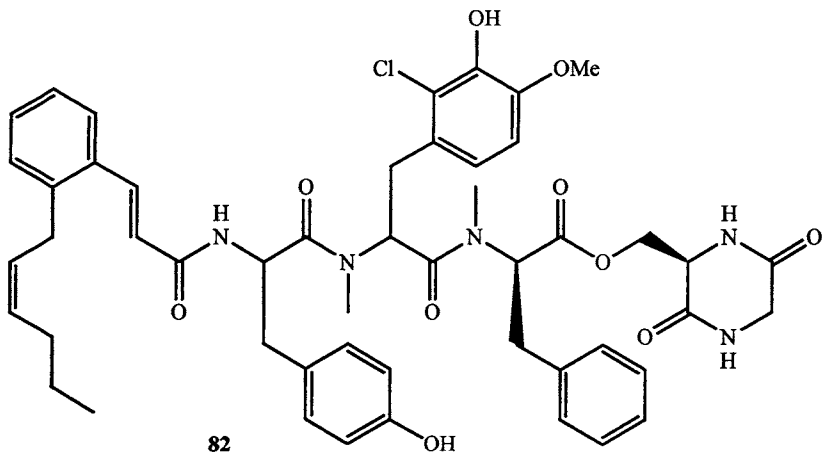


Fig. (15). Other oligopeptides.

Vancomycins were first marketed more than 35 years ago. It was found that they exhibit an excellent activity against Gram-positive bacteria. They still remain the antibiotic of choice for the treatment of infections caused by *Staphylococcus aureus*, of methicillin-resistant strains in particular. Vancomycin is not absorbed from the digestive tract and is hence preferentially used for the treatment of intestinal infections. The whole group of glycopeptide antibiotics includes more than 200 different chemical structures including antibiotics such as A42867, A82846, A8350, chloroorienticin, decaplanin, eremomycin, MM 45289, MM 477611, OA-7653, orienticin and UK72051.

As a matter of fact, vancomycin (**82**) is produced by *Amycolatopsis orientalis* [80], it is only mentioned here since the strain was previously called *S. orientalis*.

Avoparcin, a mixture of highly similar glycopeptides avoparcin α (**83**) and β (**84**) belongs to commercially available antibiotics used mainly in veterinary medicine. The aglycone called avoparcin ε (**85**) was later isolated from the cultivation broth of *Streptomycete*.

S. fungicidus [81] produces the antibiotic enduracidin including again two compounds, *viz.* enduracidins A (**86**) and B (**87**). Both enduracidins exhibit a high *in vivo* and *in vitro* antibacterial activity against Gram-positive bacteria, including bacteria resistant to other known antibiotics. Additional compounds structurally related with vancomycin (OA7653A=**88** and OA7653B=**89**) were isolated [82] from *S. hygroscopicus*. Their chemical structure was determined mainly by MS and NMR. The compounds in question contain 7 amino acid residues.

Other complex glycopeptide antibiotics (**90-96**) produced by *S. virginiae* [83] contain galactose in their chemical structure.

Two neuroprotectins (A=**97**, B=**98**) including the previously described complestatin were isolated from the fermentation broth of *Streptomyces* Q27107. Neuroprotectins protect primary cultured chick telencephalic neurons from glutamate- and kainate-induced excitotoxicities in a dose-dependent fashion

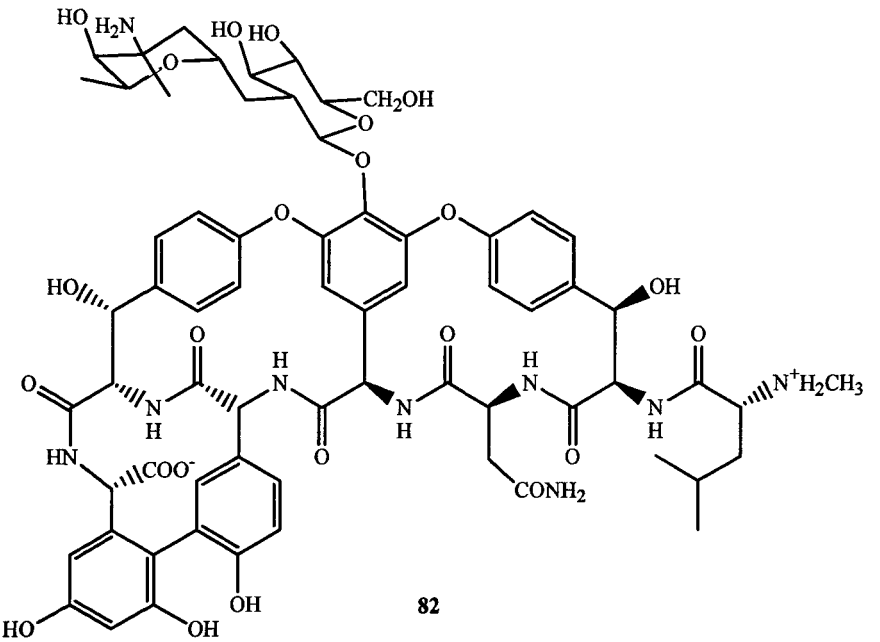
Complestatin (**99**) [84,85] was isolated from the mycelium of *S. lavendulae* and its structure was determined by NMR. It contains two unusual amino acids as shown in Fig. 16. The compound inhibits hemolysis of sensitized sheep erythrocytes mediated by guinea pig and human complement at concentrations of 0.4 and 0.7 μg/ml, respectively.

Chlorpeptin I (**100**) [86,87] was isolated from *Streptomyces* sp. It was found that it mainly contains aromatic amino acids and exhibits strong anti-HIV effects. Another glycopeptide antibiotic (**101**) [88] designated LY264826, structurally related with vancomycin but containing a C-methylated sugar in its chemical structure, is effective against enterococci. Other glycopeptides (**102-110**) derived from teicoplanin were prepared [89] and their activity against Gram-positive bacteria was investigated.

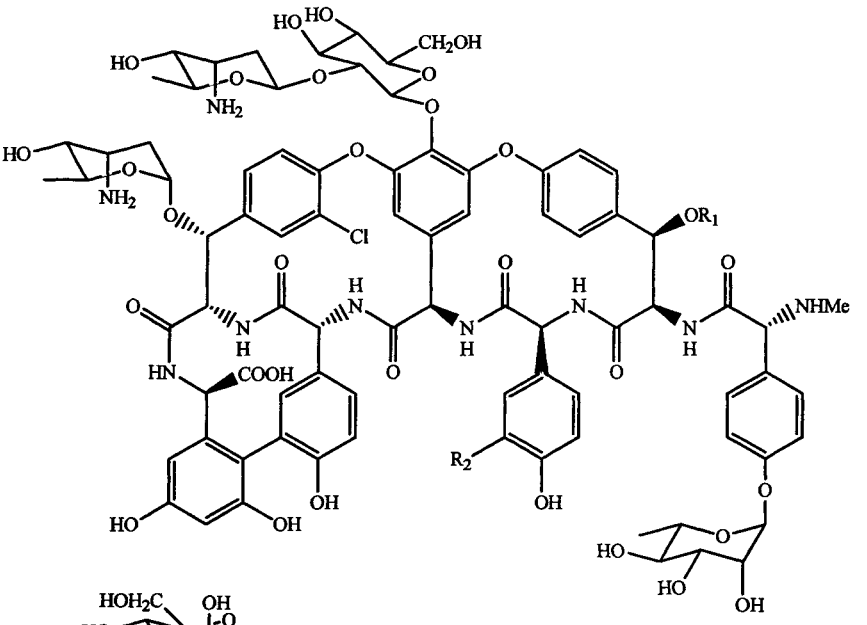
The aglycone was obtained by enzymatic hydrolysis of the side chain containing fatty acids and its additional reductive alkylation yielded products exhibiting a much higher activity against both staphylococci and enterococci (the most efficient compounds were active at 0.25 – 2 μg/ml).

Chloptosin (**111**) [90] also containing unusual amino acids was found to exhibit apoptotic activity against human adenocarcinoma.

Glycopeptide antibiotic A35512B (**112**) [91] was isolated from a soil streptomycete.

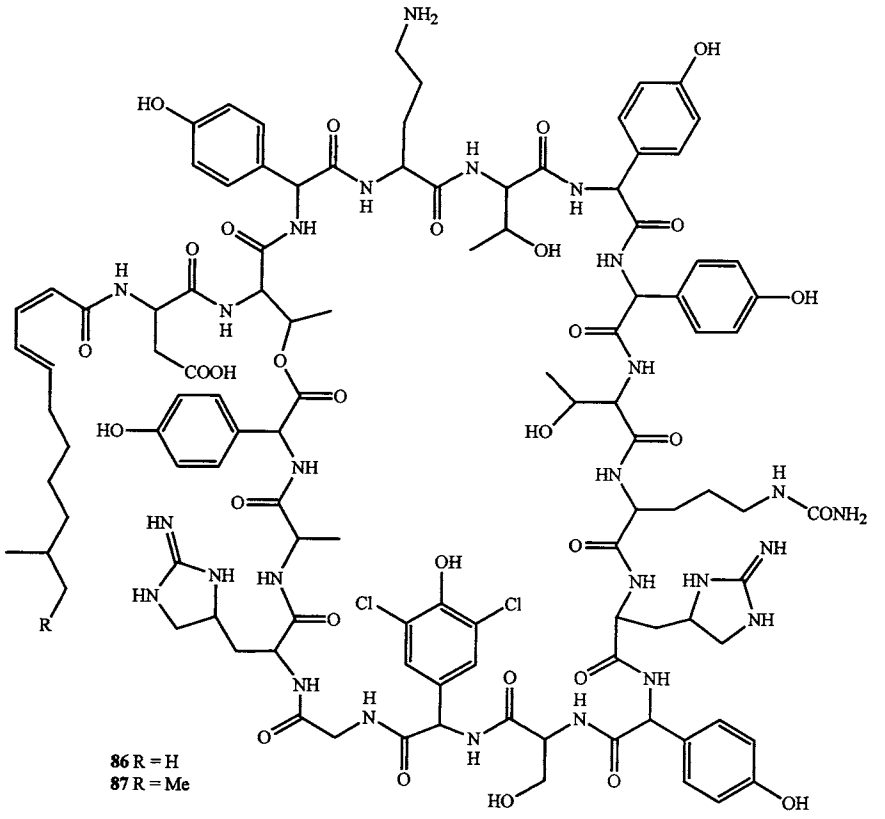


82

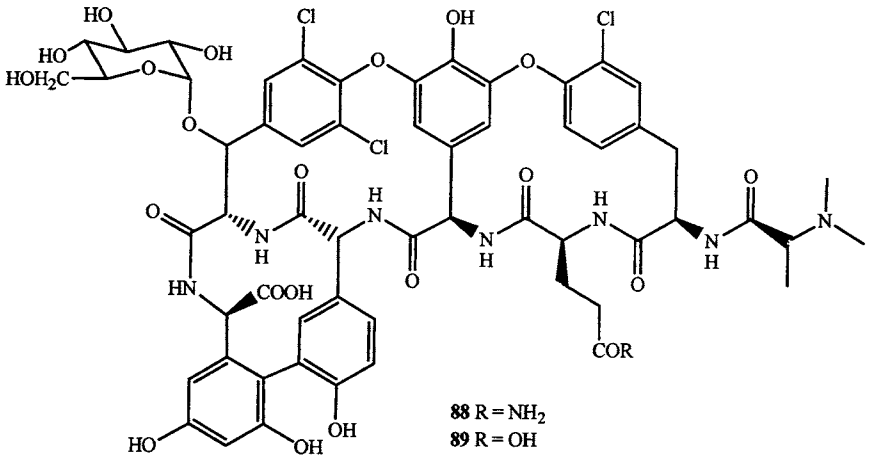


α–D-Man

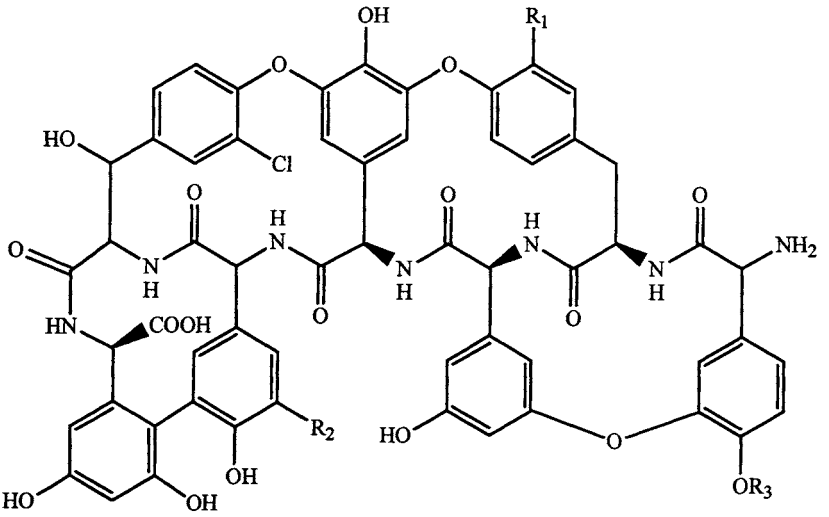
83 R_1 = α-D-Man, R_2 = H
84 R_1 = α-D-Man, R_2 = Cl
85 R_1 = H, R_2 = Cl



86 R = H
87 R = Me

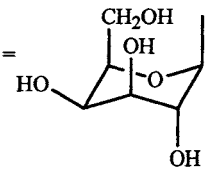


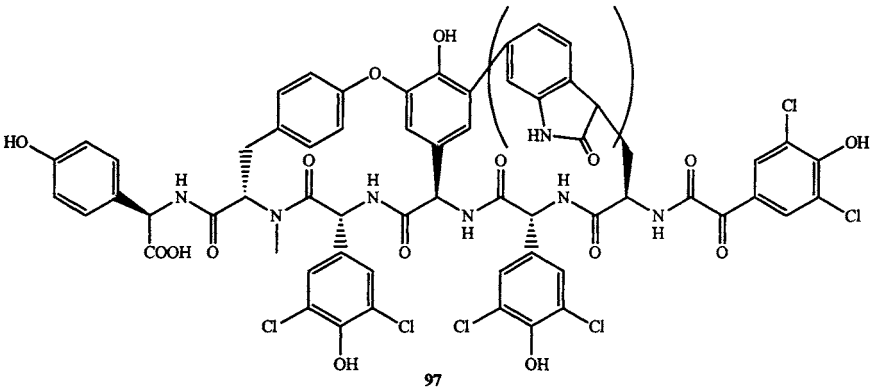
88 R = NH_2
89 R = OH



	R1	R2	R3		R1	R2	R3
90	Cl	Cl	H	**94**	H	H	H
91	Cl	H	H	**95**	Cl	Cl	Gal-Gal
92	Cl	Cl	Gal	**96**	Cl	Cl	Gal-Gal
93	H	Cl	H				

Gal =





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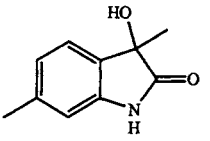

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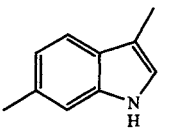

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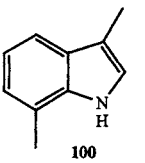
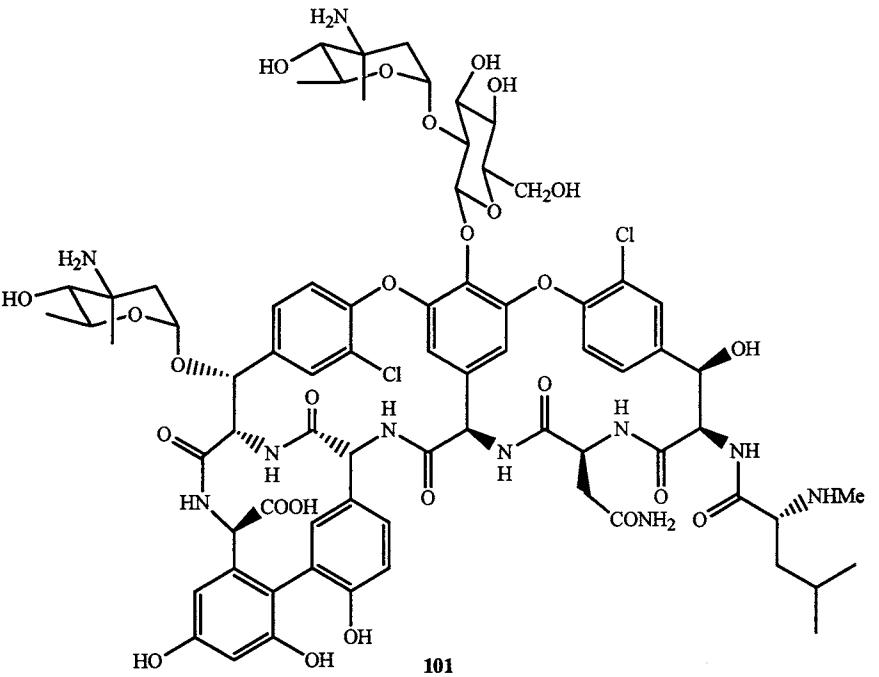


100



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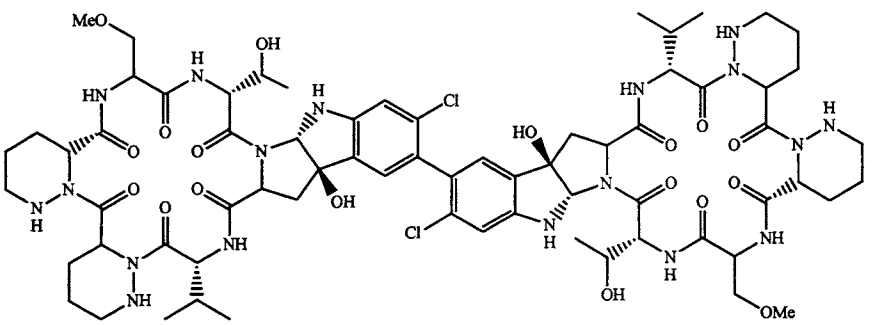
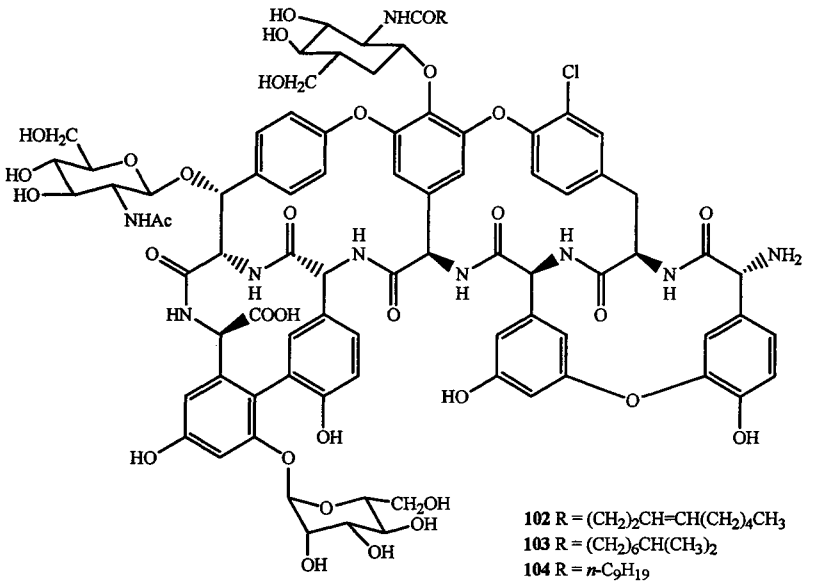
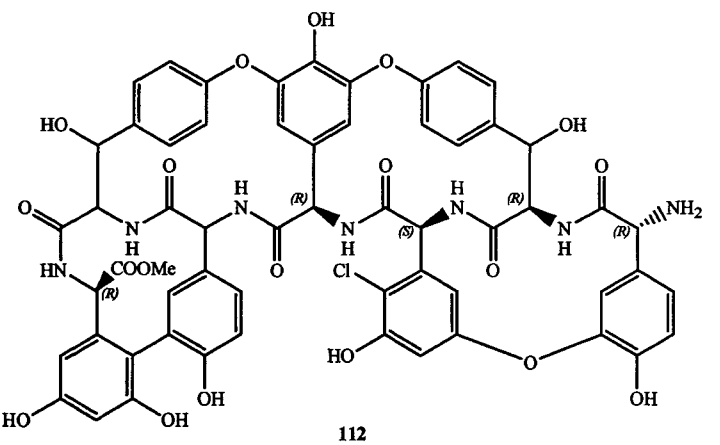


111



102 R = $(CH_2)_2CH{=}CH(CH_2)_4CH_3$
103 R = $(CH_2)_6CH(CH_3)_2$
104 R = *n*-C_9H_{19}
105 R = $(CH_2)_6CH(CH_3)CH_2CH_3$
106 R = $(CH_2)_7CH(CH_3)_2$
107 R = $(CH_2)_8CH(CH_3)_2$
108 R = *n*-$C_{11}H_{23}$
109 R = $(CH_2)_4CH(CH_3)CH_2CH_3$
110 R = *n*-C_8H_{17}

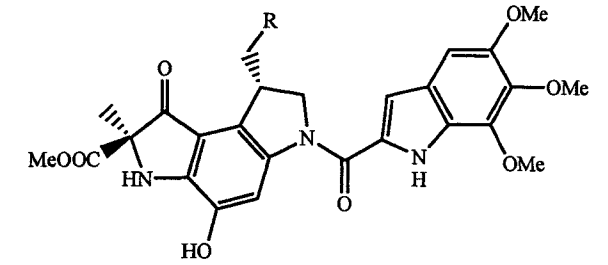


112

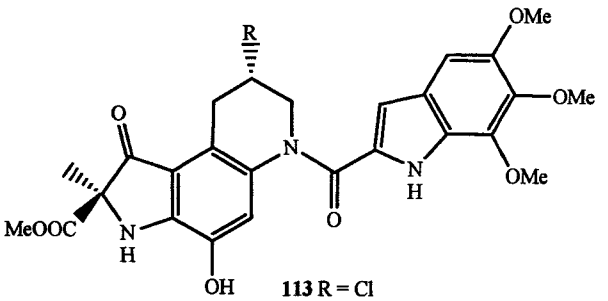
Fig. (16). Glycopeptide antibiotics

The chemical structure of new anticancer antibiotics duocarmycin C1 (**113**) and C2 (**114**), isolated from the cultivation broth of *Streptomyces* sp. [92,93] was elucidated. The antibiotic was found to exhibit a high antibacterial activity against Gram-positive bacteria (0.01μg/ml). Both duocarmycins are active against lymphocyte leukemia and sarcoma in mice [94-96]. Duocarmycins B1 (**115**) and B2 (**116**) [97] were isolated after addition of bromine to the cultivation medium. Additional seven duocarmycins were isolated, however, only duocarmycins C and B contained a halogen atom [98]. Pyrindamycins A and B, found later to be identical with duocarmycins C1 and C2, were isolated from a streptomycete designated SF2582 [99]. Pyrroindomycin A, which does not contain chlorine and pyrroindomycin B with chlorine in its structure (**117**) are the main components of the antibiotic complex isolated from the cultivation broth of *S. rugosporus* [100]. When clarifying their structure by means of physico-chemical methods it was found that they contain a trisaccharide and exhibit an excellent activity against Gram-positive bacteria [101]. A semisynthetic derivative of pyrroindomycin B (pyrroindomycin B-AC-2) (**118**) has an outstanding activity against the exponential phase cells, however, it does not affect the stationary phase cells. The antibiotic is very efficient against *Staphylococcus aureus*. As the compound really exhibits significant antibiotic effects, optimization of the cultivation process was investigated [102]. Thus for instance at a glucose concentration higher than 7.5 g/l its yield decreases, however, the concentration of glucose must not decrease below 5 g/l as the antibiotic is not produced any more under these conditions. The effect can be reversed by increasing the content of nitrogenous compounds such as ammonium chloride, arginine or glutamine. The effect of other carbon compounds was also investigated, however, it was found that some of them, *e.g.* sucrose or starch, are poorly metabolized. Other compounds, such as biotin or L-tryptophan, increase the production of pyrroindomycin.

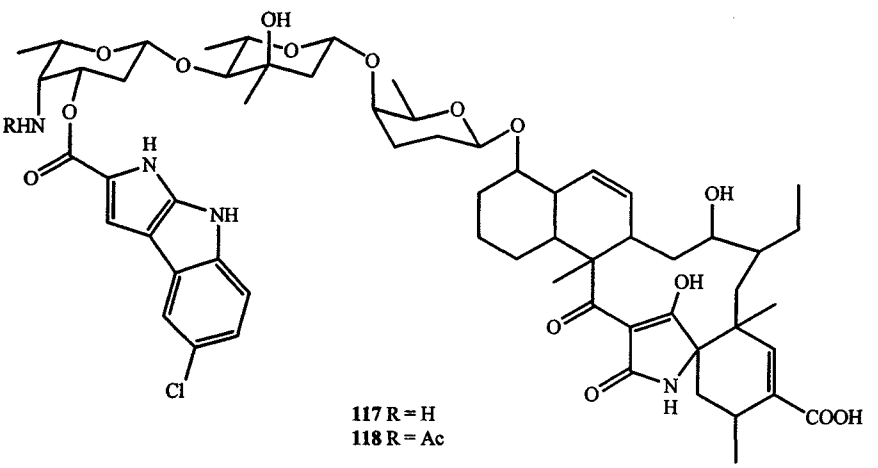
Virantmycin (**119**) isolated from *S. nitrosporeus* [103] exhibits an antiviral activity [104] and a weak antifungal activity. It prevents peroxidation of lipids in rat liver microsomes. In the cell assay, benzastatins C and D inhibited glutamate toxicity in N18-RE-105 cells with EC_{50} values of 2.0 and 5.4 μM, respectively [105]. Pyrroxamycin (98) isolated from an unidentified streptomycete [106] was found to inhibit Gram-positive bacteria and dermatophytes.



114 R = Cl
116 R = Br

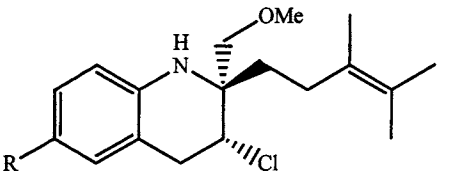


113 R = Cl
115 R = Br

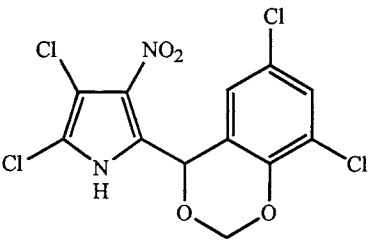


117 R = H
118 R = Ac

Fig. (17). Indol antibiotics.



119 R = COOH
120 R = $CONH_2$



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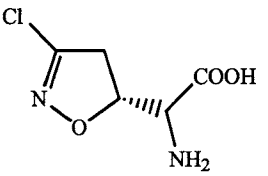

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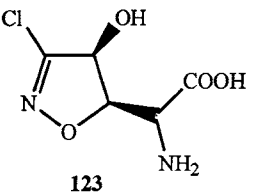

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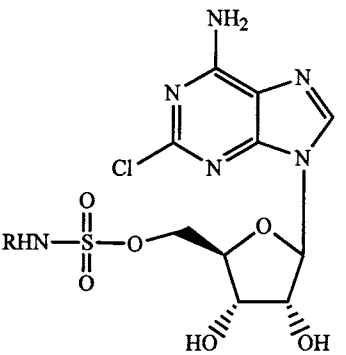
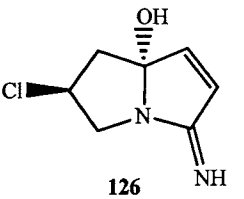
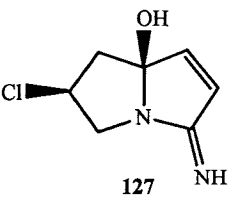


124 R = H
125 R = $COCH(CH_3)NH_2$



126



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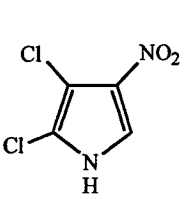
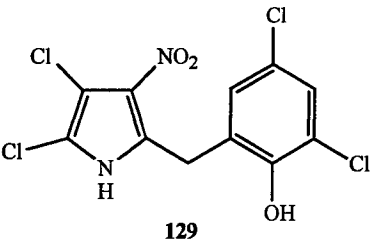


128



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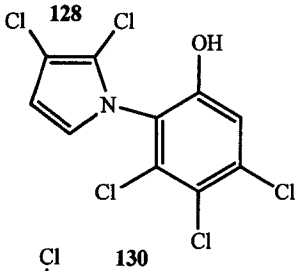

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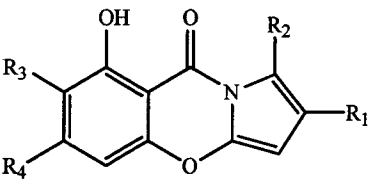
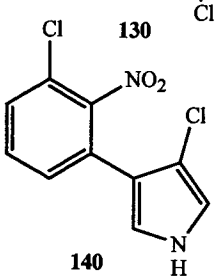
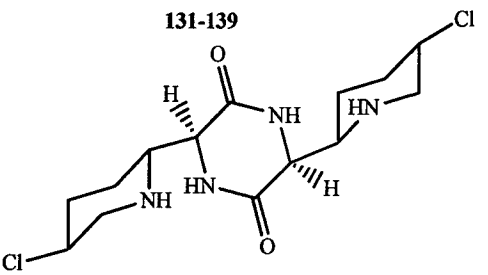


131-139



140



141

	R_1	R_2	R_3	R_4
131	Cl	H	Pr	OH
132	Cl	H	Et	OH
133	Cl	Cl	Pr	OH
134	Cl	H	Pr	OMe
135	Br	H	Pr	OH
136	Cl	Cl	Et	OH
137	Cl	H	Bu	OH
138	Cl	H	Et	OMe
139	Cl	Cl	Pr	OMe

Fig. (18). Other N-heterocycles

From a cultivation of *S. sviceus* in a 250 l fermentor almost 0.25 kg of the antibiotic U42126 (**122**) was isolated [107]. The antimetabolite antibiotic U-42126 significantly increased the life span of tumor-bearing L1210 leukemia mice at low drug levels with no demonstrable signs of toxicity to the hosts.

Even higher quantities of an antibiotic (**123**) exhibiting anticancer effects were isolated [108] with a total of 150 g of pure compound from 16 000 l.

S. rishirensis produces [109] a nucleoside antibiotic designated AT265 (**124**). Its structure was determined to be a 5′-O-sulphamoyl derivative stimulating phosphate groups [110]. Because of its unionized nature, the molecule can cross cell membranes. A derivative of this compound (**125**) designated ascamycin exhibits similar biological effects.

Clazamycins A and B (**126,127**) were isolated [111] from *S. ponicerus* (similar to *S. cinereoruber*) [112].

Two wide-spectrum antifungal compounds, *viz.* pyrrolomycin A and B (**128,129**) were isolated from a streptomycete [113,114]. Their structure was clarified on the basis of physico-chemical [114] and spectroscopic (X-ray) [115] properties.

Neopyrrolomycin (**130**) inhibiting growth of Gram-positive and Gram-negative bacteria and of various fungi was isolated from a streptomycete [116].

A series of benzoxazines (**131-139**) were isolated [117] from fermentation broth of *S. rimosus*. These compounds were found to inhibit bacterial histidine kinase and were produced by a controlled cultivation with added NaBr and NaI. As mentioned above, streptopyrol (**131**) inhibits the nitrogen regulator II histidine kinase from *Escherichia coli* with IC_{50} of 20 μM and exhibits antimicrobial activity against a wide range of bacteria and fungi.

S. pyrocinia produces [118] an antifungal antibiotic pyrrolnitrin (**140**) active against mycobacteria. The antimycobacterial activity can be well correlated with the presence of the halogen and nitro group in the aromatic ring.

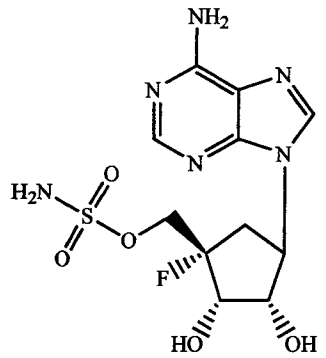
A compound designated 593A (**141**) was isolated [119] from *S. griseoluteus*. It was found that this compound exhibits high anticancer, antileukemic effects.

Fluorinated compounds

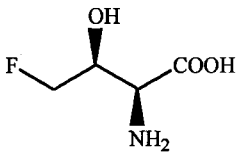
Practically only three fluorinated derivatives were described. As early as in 1957 nucleocidin (**142**) was isolated from the cultivation broth of *S. calvus* producing [120]. It is frustrating that the recent attempts at the isolation of this antibiotic from *S. calvus* were unsuccessful [120-123]. *S. cattleya* produces mM concentrations of 4-fluorothreonine (**143**) and fluoroacetate (**144**) when cultivated in a medium with fluorine ions.

All other fluorinated antibiotics were either prepared by a partial chemical synthesis or by cultivations to which fluorine ions were added. All metabolites prepared in this way and initially considered very promising with respect to their increased biological activity were an absolute failure, in spite of the fact that just the contrary was often described in the patent literature.

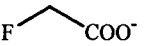
Several synthetic compounds can be presented as examples: 5-fluoro-N_b-acetyltryptamine (**145**) and 6-fluoro-N_b-acetyltryptamine (**146**) and the novel metabolites 5-fluoro- and 6-fluoro-substituted alkaloids (**147**) and (**148**) [124], 5-fluoro-β-hydroxy-N_b-acetyltryptamine (**149**) and 6-fluoro-β-hydroxy-N_b-acetyltryptamine (**150**) from the ethyl acetate extract of *S. staurosporeus* cultures after addition of tryptamine, 5-fluorotryptamine and 6-fluorotryptamine [125]. 5′′-Fluoropactamycin (**151**) and 5′′-fluoropactamycate (**152**) were also prepared by *S. pactum*, after supplementation of the medium with 3-amino-5-fluorobenzoic acid [126]. The macrolides FK-506 and FK-520 were obtained [127] by fermentation of two streptomycetes. The parent compounds were obtained from *S. tsukubaensis* No 9993 and from *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891. The following derivatives (**153**) [127] were prepared by a partial chemical synthesis, where the sugar is fluoro substituted β-D-galactopyranose or α-D-arabinofuranose.



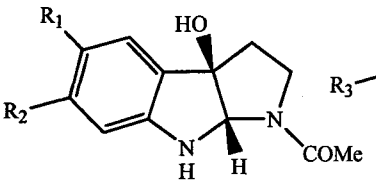
142



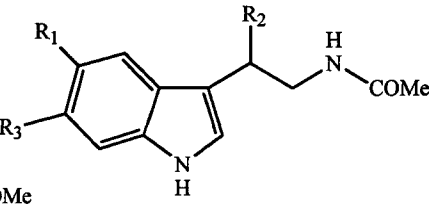
143



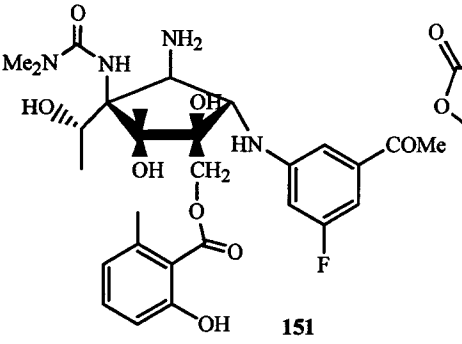
144



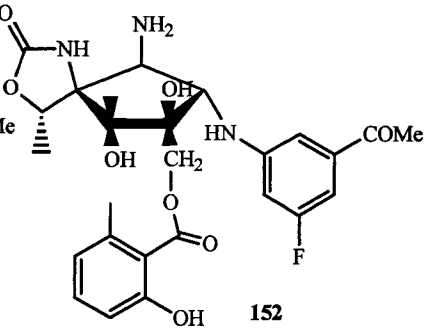
147 $R_1 = F, R_2 = H$
148 $R_1 = H, R_2 = F$



145 $R_1 = F, R_2 = R_3 = H$
146 $R_1 = R_2 = H, R_3 = F$
149 $R_1 = F, R_2 = OH, R_3 = H$
150 $R_1 = H, R_2 = OH, R_3 = F$



151



152

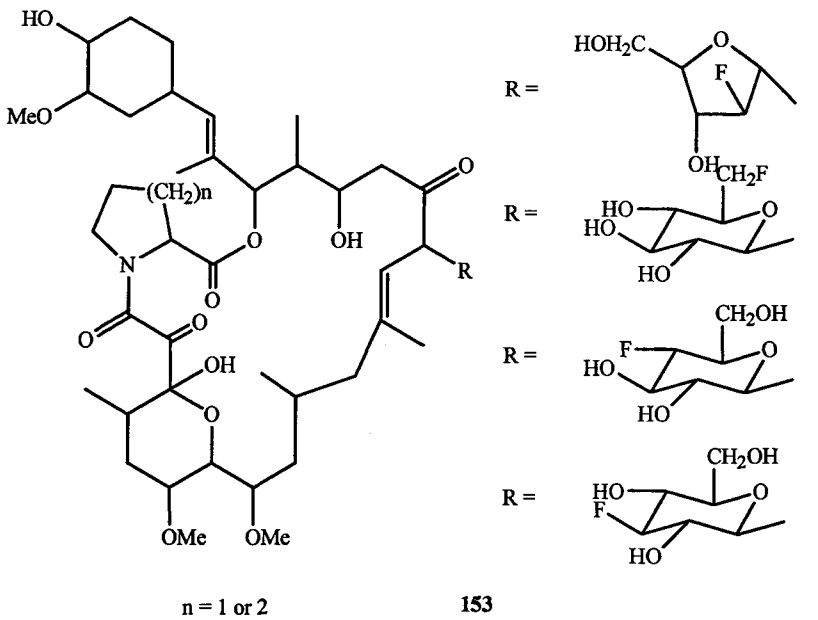


Fig. (19). F containing compounds

CONCLUSION

Halogen containing secondary metabolites exhibit interesting biological effects, such as antibiotic, anticancer and others. New producers of secondary metabolites such as streptomycetes isolated from unusual locations, *e.g.* from salty soils, lakes with increased salt concentrations, or marine streptomycetes or streptomycetes growing on surface or inside marine organisms are expected to produce new halogen containing secondary metabolites [128]. Molecular biological and genetic methods together with combinatorial biochemistry of streptomycetes also represent a promising approach towards the isolation of new halogen containing biologically active compounds [129].

REFERENCES

[1] Gottlieb, D.; Bhattacharyya, P.K.; Anderson, H.W.; Carter, H.E.; *J. Bacteriol.*, **1948**, *55*, 409-417.

[2] Bartz, Q.R.; *J. Biol. Chem.*, **1948**, *172*, 445-450.

[3] Malik V.S.; *Advances in Applied Microbiology*, **1972**, *15*, 297-336.

[4] Vining L.C.; Westlake D.W.S.; *Biotechnology of industrial antibiotics*, **1984**, 387-412.

[5] Simonsen, J.N.; Paramasigamani, K.; Vining, L.C.; McInnes, A.G.; Walter, J.A.; Wright, L.C.; *Can. J. Microbiol.*, **1978**, *24*, 136-142.

[6] Smith, C.G.; *J. Bacteriol.*, **1958**, *75*, 577-583.

[7] Labeda, D.P.; *Int. J. Syst. Bacteriol.*, **1995**, *45*, 124-127.

[8] Durckheimer, W.; *Angew. Chem.*, **1975**, *87*, 751-764.

[9] Podojil, M.; Blumauerova, M.; Cudlin, J.; Vanek, Z.; *Biotechnology of industrial antibiotics*, **1984**, 259-280.

[10] Hunter, I.S.; Hill, R.A.; *Biotechnology of antibiotics*, **1997**, 659-682

[11] Behal, V.; Hunter, I.S.; *Genetics Biochemistry of antibiotic production*, **1995**, 359-385.

[12] Behal, V.; *Critical Review Biotechnol.*, **1987**, *5*, 275-317.

[13] Vanek, Z.; Cudlin, M.; Blumauerova, M.; Hostalek, Z.; *Folia Microbiol.*, **1971**, *16*, 225-240.

[14] Abulnaja, K.O.; *Alexandria J. Pharm. Sci.*, **1997**, *11*, 17-21.

[15] Bertan, M.; Revallova, V.; Vrublova, K.; Martincer, L.; Timcova, J., *Czech Patent* No. 282681, **1997**.

[16] Ryan, M.J.; Lotvin, J.A.; Strathy, N.; Fantini, S.E.; *US Patent* No. 5589385, **1996**.

[17] Lepetit, S.P.A. ; *GB Patent* No. 772149, **1957**.

[18] McCormick, J.R.D.; Sjoler, N.O.; Hirsch, U.; Jensen, E.R.; Doerschuk, A.P.; *J. Am. Chem. Soc.*, **1957**, *79*, 4561-4563.

[19] McCormick, J.R.D.; Jensen, E.R.; *J. Am. Chem. Soc.*, **1969**, *91*, 206-206.

[20] Ryan, M.J.; US Patent No. 5965429, **1999**.

[21] Wells, J.S.; Osullivan, J.; Aklonis, C.; Ax, H.A.; Tymiak, A.A.; Kirsch, D.R.; Trejo, W.H.; Principe, P.; *J. Antibiot.*, **1992**, *45*, 1892-1898.

[22] Tymiak, A.A.; Ax, H.A.; Bolgar, M.S.; Kahle, A.D.; Porubcan, M.A.; Andersen, N.H.; *J. Antibiot.*, **1992**, *45*, 1899-1906.

[23] Devasthale, P.V.; Mitscher, L.A.; Telikepalli, H.; Vervelde, D.; Zou, J.Y.; Ax, H.A.; Tymiak, A.A.; *J. Antibiot.*, **1992**, *45*, 1907-1913.

[24] Tymiak, A.A.; Aklonis, C.; Bolgar, M.S.; Kahle, A.D.; Kirsch, D.R.; Osullivan, J.; Porubcan, M.A.; Principe, P.; Trejo, W.H.; Ax, H.A.; Wells,

J.S.; Andersen, N.H.; Devasthale, P.V.; Telikepalli, H.; Vervelde, D.; Zou, J.Y.; Mitscher, L.A.; *J. Org. Chem.*, **1993,** *58*, 535537.

[25] Patel, M.; Gullo, V.P.; Hegde, V.R.; Horan, A.C.; Gentile, F.; Marquez, J.A.; Miller, G.H.; Puar, M.S.; Waitz, J.A.; *J. Antibiot.*, **1987,** *40*, 1408-1413.

[26] Smith, E.B.; Munayyer, H.K.; Ryan, M.J.; Mayles, B.A.; Hegde, V.R.; Miller, G.H.; *J. Antibiot.*, **1987,** *40*, 1419-1425.

[27] Liu, C.M.; Hermann, T.E.; Prosser, B.L.T.; Palleroni, N.J.; Westley, J.W.; Miller, P.A.; *J. Antibiot.*, **1981,** *34,* 133-138.

[28] Westley, J.W.; Evans, R.H.; Sello, L.H.; Troupe, N.; Liu, C.M.; Blount, J.F.; Pitcher, R.G.; Williams, T.H.; Miller, P.A.; *J. Antibiot.*, **1981**, *34*, 139-147.

[29] Drautz, H.; Keller-Schierlein, W.; Zahner, H.; *Arch. Microbiol.*, **1975**, *106*, 175-190.

[30] Tsukamoto, M.; Nakajima, S.; Arakawa, H.; Sugiura, Y.; Suzuki, H.; Hirayama, M.; Kamiya, S.; Teshima, Y.; Kondo, H.; Kojiri, K.; Suda, H.; *J. Antibiot.*, **1998,** *51*, 908-914.

[31] Schimana, J.; Fiedler, H.P.; Groth, I.; Sussmuth, R.; Beil, W.; Walker, M.; Zeeck, A.; *J. Antibiot.*, **2000,** *53*, 779-787.

[32] Krone, B.; Zeeck, A.; *Liebigs Ann. Chem.*, **1983**, 471-502.

[33] Shomura, T.; Gomi, S.; Ito, M.; Yoshida, J.; Tanaka, E.; Amano, S.; Watabe, H.; Ohuchi, S.; Itoh, J.; Sezaki, M.; Takebe, H.; Uotani, K.; *J. Antibiot.*, **1987**, *40*, 732-39.

[34] Gomi, S.; Ohuchi, S.; Sasaki, T.; Itoh, J.; Sezaki, M.; *J. Antibiot.*, **1987**, *40*, 740-749.

[35] Omura, S.; Macrolide antibiotics: chemistry, biology, and practice (Ed. by Satoshi Omura), 2nd ed. Amsterdam, Elsevier Science, 2002.

[36] Suwanborirux, K.; Chang, C.J.; Spjut, R.W.; Cassady, J.M.; *Experientia* **1990**, *46*, 117-120.

[37] Hayakawa, Y.; Kawakami, K.; Seto, H.; Furihata, K.; *Tetrahedron Lett.*, **1992**, *33*, 2701-2704.

[38] Williams, T.H.; *J. Antibiot.*, **1975**, *28*, 85-86.

[39] Mukhopadhyay, T.; Franco, C.M.M.; Reddy, G.C.S.; Ganguli, B.N.; Fehlhaber, H.W.; *J. Antibiot.* **1985**, *38*, 948-951

[40] Kellerschierlein, W.; Meyer, M.; Zeeck, A.; Damberg, M.; Machinek, R.; Zahner, H.; Lazar, G.; *J. Antibiot.*, **1983**, *36*, 484-492.

[41] Hooper, A.M.; Rickards, R.W.; *J. Antibiot.*, **1998**, *51*, 845851.

[42] Brufani, M.; Cellai, L.; *J. Antibiot.*, **1979**, *32*, 167-168.

[43] Kellerschierlein, W.; Meyer, M.; Cellai, L.; Cerrini, S.; Lamba, D.; Segre, A.; Fedeli, W.; Brufani, M.; *J. Antibiot.*, **1984**, *37*, 1357-1361.
[44] Hooper, A.M.; Rickards, R.W.; *J. Antibiot.*, **1998**, *51*, 958-962.
[45] Lee, J.J; Lee, J.P.; Keller, P.J.; Cottrell, C.E.; Chang, C.J.; Zahner, H.; Floss, H.G.; *J. Antibiot.*, **1986**, *39*, 1123-1134.
[46] Holzbach, R.; Pape, H.; Hook, D.; Kreutzer, E.F.; Chang, C.J.; Floss, H.G.; *Biochemistry*, **1978**, *17*, 556-560.
[47] Mascaretti, O.A.; Chang, C.J.; Hook, D.; Otsuka, H.; Kreutzer, E.F.; Floos, H.G.; *Biochemistry*, **1981**, *20*, 919-924.
[48] Keller-Schierlein, W.; Muntwyler, R.; Pache, W.; Zahner, H.; *Helv. Chim. Acta*, **1969**, *52*, 127-142.
[49] Yamamoto, I.; Nakagawa, M.; Hayakawa, Y.; Adachi, K.; Kobayashi, E.; *J. Antibiot.*, **1987**, *40*, 1452-1454.
[50] Wright, D.E.; *Tetrahedron* **1979**, *35*, 1207-1237.
[51] Ninet, L.; Benazet, F.; Charpentie, Y.; Dubost, M.; Florent, J.; Lunet, J.; Mancy, D.; Preud'Homme, J.; *Experientia* **1974**, *30*, 1270-1272.
[52] Ollis, W.D.; Smith, C.; Sutherl, I.O.; Wright, D.E.; J. *Chem. Soc. Chem. Comm.*, **1976**, 350.
[53] Ollis, W.D.; Smith, C.; Sutherl, I.O.; Wright, D.E.; *Tetrahedron* **1979**, *35*, 105-127.
[54] Galmarini, O.L.; Deulofeu, V.; *Tetrahedron* **1961**, *15*, 76-86.
[55] Keller-Schierlein, W.; Heilman, W.; Ollis, W.D.; Smith, C.; *Helv. Chim. Acta*, **1979**, *62*, 7-20.
[56] Lam, K.S.; Hesler, G.A.; Gustavson, D.R; Crosswell, A.R.; Veitch, J.M.; Forenza, S.; Tomita, K.; *J. Antibiot.*, **1991**, *44*, 472-478.
[57] Hofstead, S.J.; Matson, J.A.; Malacko, A.R.; Marquardt, H.; *J. Antibiot.*, **1992**, *45*, 1250-1254.
[58] Zein, N.; Casazza, A.M.; Doyle, T.W.; Leet, J.E.; Schroeder, D.R.; Solomon, W.; Nadler, S.G.; *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 8009-8012.
[59] Hu, J.; Xue, Y.C.; Xie, M.Y.; Zhang, R.; Otani, T.; Minami, Y.; Yamada, Y.; Marunaka, T.; *J. Antibiot.*, **1988**, *41*, 1575-1579.
[60] Otani, T.; Minami, Y.; Marunaka, T.; Zhang, R.; Xie, M.Y.; *J. Antibiot.*, **1988**, *41*, 1580-1585.
[61] Iida, K.; Ishii, T.; Hirama, M.; Otani, T.; Minami, Y.; Yoshida, K.; *Tetrahedron Lett.*, **1993**, *34*, 4079-4082.
[62] Minami, Y.; Yoshida, K.; Azuma, R.; Saeki, M.; Otani, T.; *Tetrahedron Lett.*, **1993**, *34*, 2633-2636.

[63] Zhen, Y.; Ming, X.; Yu, B.; Otani, T.; Saito, H.; Yamada, Y.; *J. Antibiot.*, **1989**, *42*, 1294-1298.
[64] Kondo, H.; Nakajima, S.; Yamamoto, N.; Okura, A.; Satoh, F.; Suda, H.; Okanishi, M.; Tanaka, N.; *J. Antibiot.*, **1990**, *43*, 1533-1542.
[65] Konig, W.A.; Krauss, C.; Zahner, H.; *Helv. Chim. Acta*, **1977**, *60*, 2071-2078.
[66] Anyanwutaku, I.O.; Zirbes, E.; Rosazza, J.P.N.; *J. Nat. Prod.*, **1992**, *55*, 1498-1504.
[67] Komiyama, K.; Funayama, S.; Anraku, Y.; Mita, A.; Takahashi, Y.; Omura, S.; Shimasaki, H.; *J. Antibiot.*, **1989**, *42*, 1344-1349.
[68] Aizawa, S.; Akutsu, H.; Satomi, T.; Kawabata, S.; Sasaki, K.; *J. Antibiot.*, **1978**, *31*, 729-731.
[69] Narayanan, S.; Iyengar, M.R.S.; Ganju, P.L.; Rengaraju, S.; *J. Antibiot.*, **1980**, *33*, 1249-1255.
[70] Kuroda, Y.; Okuhara, M.; Goto, T.; Yamashita, M.; Iguchi, E.; Kohsaka, M.; Aoki, H.; Imanaka, H.; *J. Antibiot.*, **1980**, *33*, 259-266.
[71] Yoshida, H.; Arai, N.; Sugoh, M.; Iwabuchi, J.; Shiomi, K.; Shinose, M.; Tanaka, Y.; Omura, S.; *J. Antibiot.*, **1994**, *47*, 1165-1166.
[72] Argoudelis, A.D.; Herr, R.R.; Mason, D.J.; Pyke, T.R.; Zieser, J.F.; *Biochemistry*, **1967**, *6*, 165-170.
[73] Scannell, J.P.; Pruess, D.L.; Blount, J.F.; Ax, H.A.; Kellett, M.; Weiss, F.; Demmy, T.C.; Williams, T.H.; Stempel, A.; *J. Antibiot.*, **1975**, *28*, 1-6.
[74] Nisbet, L.J.; Mehta, R.J.; Oh, Y.; Pan, C.H.; Phelen, C.G.; Polansky, M.J.; Shearer, M.C.; Giovenella, A.J.; Grappel, S.F.; *J. Antibiot.*, **1985**, *38*, 133-138.
[75] Igarashi, M.; Nakamura, H.; Naganawa, H.; Takeuchi, T.; *J. Antibiot.*, **1997**, *50*, 1026-1031.
[76] Lackner, H.; Bahner, I.; Shigematsu, N.; Pannell, L.K.; Mauger, A.B.; *J. Nat. Prod.*, **2000**, *63*, 352-356.
[77] Omura, S.; Verpyl, D.; Inokoshi, J.; Takahashi, Y.; Takeshima, H.; *J. Antibiot.*, **1993**, *46*, 222-228.
[78] Shiomi, K.; Yang, H.; Inokoshi, J.; Verpyl, D.; Nakagawa, A.; Takeshima, H.; Omura, S.; *J. Antibiot.*, **1993**, *46*, 229-234.
[79] Dolak, L.A.; *J. Antibiot.*, **1973**, *26*, 121-125.
[80] Nagarajan, R.; *J. Antibiot.*, **1993**, *46*, 1181-1195.
[81] Iwasaki, H.; Horii, S.; Asai, M.; Mizuno, K.; Ueyanagi, J.; Miyake, A.; *Chem. Pharm. Bull.*, **1973**, *21*, 1184-1191.

[82] Ang, S.G.; Williamson, M.P.; Williams, D.H.; *J. Chem. Soc. Perkin Trans. I*, **1988,** 1949-1956.
[83] Boeck, L.D.; Mertz, F.P.; Clem, G.M.; *J. Antibiot.*, **1985,** *38*, 1-8.
[84] Kaneko, I.; Kamoshida, K.; Takahashi, S.; *J. Antibiot.*, **1989,** *42,* 236-241.
[85] Seto, H.; Fujioka, T.; Furihata, K.; Kaneko, I.; Takahashi, S.; *Tetrahedron Lett.*, **1989**, *30*, 4987-4990.
[86] Matsuzaki, K.; Ikeda, H.; Ogino, T.; Matsumoto, A.; Woodruff, H.B.; Tanaka, H.; Omura, S.; *J. Antibiot.*, **1994,** *47*, 1173-1174.
[87] Tanaka, H.; Matsuzaki, K.; Nakashima, H.; Ogino, T.; Matsumoto, A.; Ikeda, H.; Woodruff, H.B.; Omura, S.; *J. Antibiot.*, **1997,** *50*, 58-65.
[88] Rodriguez, M.J.; Snyder, N.J.; Zweifel, M.J.; Wilkie, S.C.; Stack, D.R.; Cooper, R.D.G.; Nicas, T.I.; Mullen, D.L.; Butler, T.F., Thompson, R.C.; *J. Antibiot.*, **1998**, *51*, 560-569.
[89] Snyder, N.J.; Cooper, R.D.G.; Briggs, B.S.; Zmijewski, M.; Mullen, D.L.; Kaiser, R.E.; Nicas, T.I.; *J. Antibiot.*, **1998**, *51*, 945-951.
[90] Umezawa, K.; Ikeda, Y.; Uchihata, Y.; Naganawa, H.; Kondo, S.; *J. Org. Chem.*, **2000,** *65*, 459-463.
[91] Hunt, A.H.; *J. Am. Chem. Soc.*, **1983,** *105*, 4463-4468.
[92] Yasuzawa, T.; Iida, T.; Muroi, K.; Ichimura, M.; Takahashi, K.; Sano, H.; *Chem. Pharm. Bull.*, **1988**, *36,* 3728-3731.
[93] Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H.; *J. Antibiot.*, **1988,** *41*, 1915-1917.
[94] Nagamura, S.; Saito, H.; *Khimiya Geterotsiklicheskikh Soedinenii*, **1998**, 1636-1657.
[95] Cacciari, B.; Romagnoli, R.; Baraldi, P.G.; Da Ros, T., Spalluto, G.; *Expert Opinion Therapeutic Patents*, **2000**, *10,* 1853-1871.
[96] Ishii, S.; Nagasawa, M.; Kariya, Y.; Yamamoto, H.; Inouye, S.; Kondo, S.; *J. Antibiot.*, **1989,** *42*, 1713-1717.
[97] Ogawa, T.; Ichimura, M.; Katsumata, S.; Morimoto, M.; Takahashi, K.; *J. Antibiot.*, **1989**, *42*, 1299-1301.
[98] Yasuzawa, T.; Muroi, K.; Ichimura, M.; Takahashi, I.; Ogawa, T.; Takahashi, K.; Sano, H.; Saitoh, Y.; *Chem. Pharm. Bull.*, **1995**, *43*, 378-391.
[99] Ohba, K.; Watabe, H.; Sasaki, T.; Takeuchi, Y.; Kodama, Y.; Nakazawa, T.; Yamamoto, H.; Shomura, T.; Sezaki, M.; Kondo, S.; *J. Antibiot.*, **1988**, *41*, 1515-1519.

[100] Ding, W.D.; Williams, D.R.; Northcote, P.; Siegel, M.M.; Tsao, R.; Ashcroft, J.; Morton, G.O.; Alluri, M.; Abbanat, D.; Maiese, W.M.; Ellestad, G.A.; *J. Antibiot.*, **1994**, *47*, 1250-1257.
[101] Singh, M.P.; Petersen, P.J.; Jacobus, N.V.; Mroczenskiwildey, M.J.; Maiese, W.M.; Greenstein, M.; Steinberg, D.A.; *J. Antibiot.*, **1994**, *47*, 1258-1265.
[102] Abbanat, D.; Maiese, W.; Greenstein, M.; *J. Antibiot.*, **1999**, *52*, 117-126.
[103] Pearce, C.M.; Sers, J.K.M.; *J. Chem. Soc. Perkin Trans. I*, **1990**, 409-411.
[104] Omura, S.; Nakagawa, A.; *Tetrahedron Lett.*, **1981**, *22*, 2199-2202.
[105] Kim, W.G.; Kim, J.P.; Yoo, I.D.; *J. Antibiot.*, **1996**, *49*, 26-30.
[106] Yano, K.; Oono, J.; Mogi, K.; Asaoka, T.; Nakashima, T.; *J. Antibiot.*, **1987**, *40*, 961-969.
[107] Martin, D.G.; Duchamp, D.J.; Chidester, C.G.; *Tetrahedron Lett.*, **1973**, *27*, 2549-2552.
[108] Martin, D.G.; Chidester, C.G.; Mizsak, S.A.; Duchamp, D.J.; Baczynskyj, L.; Krueger, W.C.; Wnuk, R.J.; Meulman, P.A.; *J. Antibiot.*, **1975**, *28*, 91-93.
[109] Takahashi, E.; Beppu, T.; *J. Antibiot.*, **1982**, *35*, 939-947.
[110] Isono, K.; Uramoto, M.; Kusakabe, H.; Miyata, N.; Koyama, T.; Ubukata, M.; Sethi, S.K.; McCloskey, J.A.; *J. Antibiot.*, **1984**, *37*, 670-672.
[111] Miura, K.; Hamada, M.; Takeuchi, T.; Umezawa, H.; *J. Antibiot.*, **1979**, *32*, 762-765.
[112] Dolak, L.A.; DeBoer, C.; *J. Antibiot.*, **1980**, *33*, 83-84.
[113] Ezaki, N.; Shomura, T.; Koyama, M.; Niwa, T.; Kojima, M.; Inouye, S.; Ito, T.; Niida, T.; *J. Antibiot.*, **1981**, *34*, 1363-1365.
[114] Koyama, M.; Kodama, Y.; Tsuruoka, T.; Ezaki, N.; Niwa, T.; Inouye, S.; *J. Antibiot.*, **1981**, *34*, 1569-1576.
[115] Kaneda, M.; Nakamura, S.; Ezaki, N.; Iitaka, Y.; *J. Antibiot.*, **1981**, *34*, 1366-1368.
[116] Nogami, T.; Shigihara, Y.; Matsuda, N.; Takahashi, Y.; Naganawa, H.; Nakamura, H.; Hamada, M.; Muraoka, Y.; Takita, T.; Iitaka, Y.; Takeuchi, T.; *J. Antibiot.*, **1990**, *43*, 1192-1194.
[117] Trew, S.J.; Wrigley, S.K.; Pairet, L.; Sohal, J.; Shanu-Wilson, P.; Hayes, M.A.; Martin, S.M.; Manohar, R.N.; Chicarelli-Robinson, M.I.; Kau, D.A.; Byrne, C.V.; Wellington, E.M.H.; Moloney, J.M.; Howard, J.; Hupe, D.; Olson, E.R.; *J. Antibiot.*, **2000**, *53*, 1-11.
[118] Di Santo, R.; Costi, R.; Artico, M.; Massa, S.; Lampis, G.; Deidda, D.; Pompei, R.; *Biorg. Med. Chem. Lett.*, **1998**, *8*, 2931-2936.

[119] Gitterman, C.O.; Rickes, E.L.; Wolf, D.E.; Madas, J.; Zimmerman, S.B.; Stoudt, T.H.; Demny, T.C.; *J. Antibiot.*, **1970**, *23,* 305-310.
[120] O'Hagan, D.; Harper, D.B.; *J. Fluorine Chem.*, **1999**, *100,* 127-133.
[121] Morton, G.O.; Lancaster, J.E.; Van Lear, G.E.; Fulmor, W.; Meyer, W.E.; *J. Am. Chem. Soc.*, **1969**, *91*, 1535-1537.
[122] Jenkins, I.D.; Verheyden, J.P.H.; Moffatt, J.G.; *J. Am. Chem. Soc.*, **1976**, *98*, 3346-3357.
[123] Maguire, A.R.; Meng, W.D.; Roberts, S.M.; Willetts, A.J.; *J. Chem. Soc. Perkin Trans. I*, **1993**, 1795-1808.
[124] Yang, S.W.; Cordell, G.A.; *J. Nat. Prod.*, **1997**, *60*, 44-48.
[125] Yang, S.W.; Cordell, G.A.; *J. Nat. Prod.*, **1997**, *60*, 230-235.
[126] Adams, E.S.; Rinehart, K.L.; *J. Antibiot.*, **1994**, *47*, 1456-1465.
[127] Koch, K.; *US Patent* No. 5612316, **1997**.
[128] Fenical, W.; *Chem. Rev.* **1993**, *93*, 1673-1683.
[129] Hutchinson, C.R.; McDaniel, R.; *Curr Opin Investig Drugs.* **2001**, *2,* 1681-1690.