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# PRODUCTION OF AVERMECTINS: BIOTECHNOLOGIES AND ORGANIC SYNTHESIS

(review)

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Abstract

The proposed review analyzes the results of research on various aspects of improving the technology of obtaining avermectins, the 16-membered macrocyclic lactones which have a wide spectrum of antiparasitic action with a high therapeutic index and harmlessness for mammals (W.C. Campbell, 2012). According to published data, the unique ability of avermectins to suppress the development of insects, nematodes and ticks is associated with the ability to block the transmission of nerve impulses in the neuromuscular synapse. The essence of this mechanism of action, leading to paralysis and death of parasites, is to stimulate the release of chlorine ions, depolarization of the cell membrane and pathological disorders of its functions (A.J. Wolstenholme et al., 2016). Of the known 8 components (A1a, A1b, A2a, A2b, B1a, B1b, B2a and B2b) of the avermectin complex produced by the microorganism *Streptomyces avermitilis*, the avermectin B1 is the most active against parasite pathogens (S. Omura, 2002; W.C. Campbell, 2012). Therefore, the main studies on the production of avermectins are associated with the selection of highly productive strains which predominantly synthesize avermectins B1 (S.S. Ki et al., 2005; H. Gao et al., 2010; W. Liu et al., 2015; L. Meng et al., 2016), and the preparation of semi-synthetic analogs of avermectins B1 with improved physicochemical and pharmacological properties (J. Vercruysse et al., 2001; A. Awasthi et al., 2012). Attempts to develop a technology for the complete chemical synthesis of avermectins have not yet yielded significant results due to the low yield of the target product and the complexity of the synthesis scheme (S. Yamashita et al., 2016). Considerable attention has been paid to the biochemical aspects of the diversity of 16-membered macrocyclic lactones and their producers, as well as to semisynthetic analogues, and prospects for searching for new highly efficient and environmentally friendly semisynthetic analogues of avermectin B1 have been defined. Main streams of researches on genetics, biochemistry and physiology of the producer of avermectins, ways of regulated culture of *S. avermitilis* strains and biosynthesis of required components of avermectin complex are discussed (S. Kitani et al., 2009; J. Guo et al., 2018). The data on the problem of emerging resistance in some species of parasites to long-used avermectin-containing drugs are analyzed. This phenomenon is shown to have a multifactor nature, including mutation of genes determining GluCl subunits and increased P-glycoprotein expression (J.H. Gill et al, 1998; R.K.Prichard, 2007; F.D. Guerrero et al., 2012; P.C. Pohl et al., 2014; P. Godoy et al, 2016). For the successful control of nematodes, insects and mites of agricultural, sanitary and medical importance, it seems appropriate to create drugs based on natural avermectins and their new semi-synthetic derivatives, for example, 5-O-succinylavermectin B1 and C2017 compounds.

Keywords: avermectins, milbemycins, nemadectins, doramectin, abamectin, moxidectin, ivermectin, moxidectin, milbemycin oxime, 5-O-succinylavermectin B1, compound C2017, avermectin oximes, *Streptomyces avermitilis*, organic synthesis, antiparasitic drugs, nematicides, insectoacaricides

Avermectins (16-membered macrolides produced by *Streptomyces avermitilis*) [1, 2] have extensive nematicide and insectoacaricide effects; for over 35

years, they have been effectively used to treat and prevent parasitic diseases in humans, animals, and plants [3-7]. Annual sales of avermectins exceed $850 million [8, 9]. The integral antiparasitic effect of this class of drugs is pertaining to their ability to affect glutamate-dependent (the main target) $Cl^-$-ion channels specific to invertebrates [10], as well as $GABA_A$ (γ-aminobutyric acid)-dependent receptors [11]. Besides, avermectins have an affinity to various ion channels and receptors of the Cys-loop superfamily, P2X4 and farnesoid receptors, G protein-coupled inwardly rectifying potassium channels, GIRK receptors) and sundry channels, making this class pharmacologically promising [12, 13]. Ivermectin, which is an avermectin, has been found capable of blocking PAK1-dependent growth of benign and malignant tumor cells [14, 15]. Antitumor effects have been described for other 16-membered antiparasitic macrolides, too [16-19]. Ivermectin has recently been found to inhibit the replication of yellow fever virus [20] and the sporogony of *Plasmodium falciparum* in *Anopheles gambiae* [21]; avermectins have been found to curb tuberculosis [22] and to reduce the cellular absorption of ethanol [23]; ivermectin has demonstrated a curative effect in experimental pathologies, e.g. remyelination in autoimmune encephalitis resulting from allosteric activation and restoration of the disordered functions of ATP-dependent (purinergic) P2X4Rs ionophore receptors [24-27].

This review mainly covers the methods for the production of natural avermectins and their semisynthetic derivatives.

Avermectin synthesis technologies conventionally [2] imply obtaining highly productive strains that preferably synthesize B1 avermectins; the nutrient media must be optimized to grow the producer; the final step is to produce semisynthetic analogs of avermectins B1 with improved physicochemical and pharmacological properties [28-30]. In recent years, there has emerged another area of focus, which is to use synthetic biology methods to synthesize the required products, e.g. ivermectin or milbemycins [31-34]. In the 1980s and 1990s, researchers attempted a fully chemical synthesis of some avermectins, B1a and A1a [35]; however, they proposed multistage technologies that had limited output of 0.08% at max, making the microbiological method clearly advantageous. Research into more efficient fully chemical synthesis of avermectins is a work in progress [36-39].

Selection of producers, microbiological synthesis, and biotechnologies. The main focus in upgrading the producer of avermectins, *Streptomyces avermitilis* (ex Burg et al. 1979) Kim and Goodfellow, 2002 [40] is to obtain productive strains generating the avermectin complex or one of its components, mainly B1, while suppressing the synthesis of oligomycins that adversely affect the growth and development of the producer. State-of-the-art industrial strains genealogically trace back to wild-type *S. avermitilis* MA-4680 (strains NRRL 8165; NCIMB 12804; http://gcm.wfcc.info), which is a Japanese soil isolate that has deworming effects. The strain is deposited in the microbiological collections of many countries, although labeled differently (ATCC 31267, VKM Ac-1301, etc.) [41]. VKM Ac-1301 from the All-Russian Collection of Microorganisms is the ancestor of all Russian avermectin producers (http://www.vkm.ru/contact.htm) [42, 43]. The further step was to select mutants, both spontaneous and induced by physical (UV and X-ray irradiation) and chemical (nitric mustard, methyl-methane sulfonate, etc.) agents, as well as to genetically improve the producer [44, 45]. One of such strains, an *S. avermitilis* MA-4848 derivative, produces eight known avermectins; it was first obtained in the United States by UV mutagenesis using a lyophilized suspension of the MA-4680 (ATCC 31267) parent strain coupled with optimized nutrient medium and growth environment. As a result, the avermectin complex output rose from 9 to

500 rg/ml with a relative B1 content of about 35%. This composition, named C-076, has nematicide, acaricide, and insecticide effects. The lyophilized and frozen MA-4848 strain is deposited by the names of ATSS 31271 and ATP 31272, respectively (Patent US 4285963; 1981), its productivity was further raised to > 9,000 g/ml at B1 content of 95% and more [33, 46, 47]. Russia has also obtained *S. avermitilis* strains that produce a full 8-component avermectin complex (A1a, A1b, A2a, A2b, B1a, B1b, B2a, and B2b) with an intensive biocide effect [48, 49]. The productivity of the *S. avermitilis* VNIISKhM 56 strain is 500 rg/ml in terms of the avermectin complex, where avermectins B (B1 + B2) account for up to 50-70% (Patents RU No. 2087535, No. 2125609). The producers obtained by selection did not synthesize toxic oligomycin that the mycelium extract of the original VKM Ac-1301 strain was rich in. The first Russian drug, Aversect-1 (MGP Bifidum, part of Biotekhnologiya R&D, Moscow), registered by the Directorate General for Veterinary, Ministry of Agriculture of the Russian Federation, in 1992, contained avermectins produced by *S. avermitilis* 198 (VNIISKhM 50) and VNIISKhM 51 strains step-selected from *S. avermitilis* VKM Ac 1301 (Patent RU No. 2087535). Selection from these strains produced the VNIISKhM 54 (Patent RU No. 2054483) and VNIISKhM 56 (Patent RU No. 2087535) strains with a productivity of 400 to 500 μg/ml. These strains became fundamental to selecting even more active producers; they are still in use. In particular, directed selection of *S. avermitilis* VNIISKhM 54 via a series of intermediate variants produced *S. avermitilis* CCM 4697, which has an avermectin production of up to 2,300 μg/ml and a relative B1 content of about 50%; see Patent RU No. 2156301. NITsB 132 (Patent RU No. 2147320) features a biosynthesis of avermectins at a minimum of 3,500 μg/ml, including 1,500 μg/ml of B1, with a B1a content of about 80%. Ukrainian and Belorussian researchers have isolated avermectin producers: *S. avermitilis* UKM Ac-2179 and *S. avermitilis* X-1 [50, 51]. Reports have been published on *S. avermitilis* that produce natural (C-076) and artificial avermectins based on recombinant strains (Patent RU No. 2096462). Biosynthesis of avermectins by *S. avermitilis* UKM Ac-2179 was found to rise drastically when exposed to pyruvate, L-threonine or L-methionine, whereby the cultural fluid also accumulated amino acids, lipids, and phytohormones [52], which is consistent with the earlier data [53] and can be used to develop a waste-free avermectin biosynthesis technology.

To select highly active producers, Streptomyces spores were exposed to the mutagenic effect of a short-pulse X-ray with a quantum energy of 80 to 160 keV (Patent RU No. 2074256), UV radiation, nitrous acid, N-methyl-N″-nitro-N-nitrosoguanidine, ethyl methanesulfonate, sundry conventional or novel mutagens [54, 55].

Industrial production of avermectin-based drugs currently relies upon *S. avermitilis* strains: the abamectin-producing G8-17, SA-01, AV-LP, A-144, A-178, NA-108 (China); VKPM S-1440, VNIISKhM 56 (Russia) that synthesize the known avermectin complex, etc. Thus, intensive selection of avermectin producers basically cut the range of strains down to just a few.

Advancements in the Russian avermectin biosynthesis technology resulted in the invention of Aversect-1 (TU 10.07090-92 Aversect-1) based on the avermectin complex (*S. avermitilis* VNIISKhM 51 strain, Patent RU No. 2048520). One peculiar feature of avermectin biosynthesis is that avermectins are accumulated in the Streptomyces biomass rather than released into the environment. Leaving the process uncontrolled, especially when mycelium lysis begins, may result in the loss of the target product [56].

Standard avermectin-complex production technology elaborated for the Russian strains of *S. avermitilis*: VNIISKhM 50, VNIISKhM 51, VNIISKhM 56,

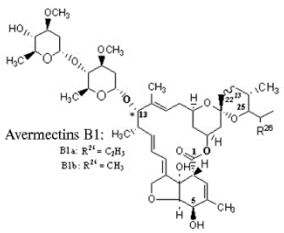
implies growth in shake flasks (250 and 750 ml) as well as in bioreactors (250 l) [56]. The conventional technology is being improved by target changes in the strain genome [45-47] or by adding metabolism-affecting components to the medium [52, 57-59]. Thus, in the presence of sinefungin, which inhibits the conversion of avermectins B into avermectins A, the proportion of avermectins B in the output of *S. avermitilis* NRRL 8165 reached 77% [60, 61]. The regulatory role of amino acids in the biosynthesis of avermectins and adjusting the B/A ratio of the avermectin complex has been demonstrated when growing a number of *S. avermitilis* strains [62, 63]. Some papers describe the biosynthesis, release, and purification of avermectins: the product concentrate is extracted by a water-immiscible organic solvent, e.g. ethyl acetate, or a mixture of solvents containing water and water-miscible low- (ethanol or propanol) or high-boiling (e.g. PEG-200) solvents [64, 65]. The basic principles behind the biosynthesis of avermectins have been researched by isotope methods and mutagenesis [64].

Cloning the genes of the avermectin complex and sequencing the genome of *S. avermitilis* enabled researchers to predict and experimentally verify the biosynthesis of sundry secondary metabolites, e.g. the polyene macrolide filipin III [66]. *S. avermitilis* has a genome sized 9,025,608 base pairs that contains at least 7,582 possible open reading frames and 38 clusters of secondary metabolite biosynthesis genes [67]. Avermectin biosynthesis is determined by 17 genes. Four of them (*aveA1 to aveA4*) encode multifunctional protein subunits (AveA1 to AveA4) comprising 3,973, 6,239, 5,532 or 4681 amino-acid residues, respectively, and forming the avermectin polyketide synthase complex [48]. AveA is a Type I, 12-module polyketide synthase [48]. The enzymes AveBI to AveBVIII (glycosyltransferase, thymidylyl transferase, TDF-4-keto-6-desoxy-L-hexose-3-ketoreductase, TDF-4-ketohexulose-reductase, TDF-TDF-4-keto-6-deoxyglucose-3-epimerase, TDF-4-keto-6-deoxy-glucose-2,3-dehydratase, TDF-6-deoxy-L-hexose-3-O-methyltransferase, TDF-4-keto-6-dexosy-L-hexose-3-ketoreductase, respectively) [33] synthesize the disaccharide of L-oleandrose from D-glucose-6-phosphate and bind to aglycone, AveE and AveF for the furan cycle, while the rest are involved in forming the spiroketal fragment (AveC), 5-O-methylation (AveD); AveR serves as the factor of positive biosynthesis regulation [64, 67].

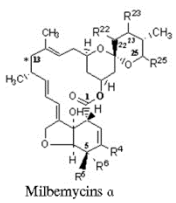
To synthesize avermectins, the producer first biosynthesizes the monomer structural units used in the polyketide avermectin synthesis, then assembles the precursor of the pentacyclic structural avermectin frame, tridecaketide, which involves the polyketide mechanism, then invokes post-polyketide transformations [1]. The former include converting tridecaketide into the avermectin intermediate with a 16-membered lactone ring: 6,8a-seco-6,8a-deoxy-5-oxoavermectin; converting 6,8a-seco-6,8a-deoxy-5-oxoavermectin into avermectin aglycone (at oxidative cycling, reduction and/or methylation); synthesizing modified L-oleandrose; glycosylation of aglycone with deoxythymidine-diphosphate-L-oleandrose (dTDP-L-Ole), whereby avermectins are synthesized [64].

When initiating the whole process, the first step is to biochemically configure (i.e. charge or recharge with the starting unit) the loading module (Module 0) for polyketide synthesis: the substrate center of acyltransferase activity (domain $AT_0$) of the polyfunctional synthase captures the available residue of the monocarbonic acid from the pool of acyl~S-CoA by acylating the thiol group of the cysteine in this enzyme domain [68-72]. The captured $AT_0$ acyl (2-methylbutyryl or isobutyryl) residue is transferred to the thiol group (substituting hydrogen in –SH) of the phosphopantetheinyl (Ppant) fragment bound to the serine residue of this domain, which carries the properties of acyl carrier proteins ($ACP_0$) and functions as the Ppant "sleeve" or the ACP manipulator. Thus, Module 1 prepares to receive the starting unit; similarly, AT1 activates dicarbon-

ic methylmalonic acid (methylmalonyl~S-Ppant-ACP) in the module. Upon condensation, the β-ketosyntase domain ($KS_1$) of Module 1 catalyzes the formation of a head-to-tail C-C-bond between the acyl residues of Modules 0 and 1, see the Claisen mechanism [1]. At the same time, the dicarbonic acid residue is decarboxylated [73], which produces diketide [68, 70] anchored at the $ACP_1$ domain, Module 1. This diketide is reduced by the ketoreductase domain ($KR_1$) to β-hydroxy-diketide ready for further condensation in Module 2. The structural diversity of the condensation products depends on the set of catalytically active domains in each module [74, 75]. The polyketide chain is lengthened step-by-step in 12 modules (one condensation per module) by 12 consecutive ester reactions of condensing 7 units of malonic acid and 5 units of methylmalonic acid activated as acyl~S-CoA, which produces the tridecaketide precursor of avermectin aglycone [1]. At each condensation cycle, the methylmalonic or malonic residue from methylmalonyl-CoA or malonyl-CoA is transferred to the phosphopantetheinyl group of the acyl carrier protein (ACP), which is stereochemically controlled by the acyltransferase (AT) of the next module [68, 76]. As the last chain growth cycle is over (Module 12, polyketide chain synthesis terminating), there occur biochemical reactions of unknown sequence that result in separating the acyclical aglycone from $ACP_{12}$ and forming 16-membered lactone (6,8a-seco-6,8a-deoxy-5-oxoavermectin) and aglycone. The papers [77, 78] show that the spiroketalization occurs after closing the cyclohexene cycle and the 16-membered macrolide cycle BEFORE the hexahydrobenzofuran fragment is formed. A number of further transformations produce the A and B components of the avermectin complex [64].




| Avermectins | $R^5$ | $R^{25}$ | 22~23 |
|---|---|---|---|
| A1a | $CH_3$ | $C_2H_5$ | -CH=CH- |
| A1b | $CH_3$ | $CH_3$ | -CH=CH- |
| B1a | H | $C_2H_5$ | -CH=CH- |
| B1b | H | $CH_3$ | -CH=CH- |
| A2a | $CH_3$ | $C_2H_5$ | $-CH_2-CH(OH)-$ |
| A2b | $CH_3$ | $CH_3$ | $-CH_2-CH(OH)-$ |
| B2a | H | $C_2H_5$ | $-CH_2-CH(OH)-$ |
| B2b | H | $CH_3$ | $-CH_2-CH(OH)-$ |




| Milbemycins | $R^5$ | $R^{25}$ | $R^{22}$ | $R^{23}$ | $R^4$ | $R^6$ |
|---|---|---|---|---|---|---|
| α1 | OH | $CH_3$ | H | H | $CH_3$ | H |
| α2 | $OCH_3$ | $CH_3$ | H | H | $CH_3$ | H |
| α3 | OH | $C_2H_5$ | H | H | $CH_3$ | H |
| α4 | $OCH_3$ | $C_2H_5$ | H | H | $CH_3$ | H |
| α5 | OH | $CH_3$ | OH | X | $CH_3$ | H |
| α6 | $OCH_3$ | $CH_3$ | OH | X | $CH_3$ | H |
| α7 | OH | $C_2H_5$ | OH | X | $CH_3$ | H |
| α8 | $OCH_3$ | $C_2H_5$ | OH | X | $CH_3$ | H |
| α9 | OH | $CH_3$ | H | H | Y | H |
| α10 | OH | $C_2H_5$ | H | H | Y | H |
| D | OH | $CH(CH_3)_2$ | H | H | $CH_3$ | H |
| F | OH | $CH(CH_3)_2$ | H | H | Y | H |
| G | $OCH_3$ | $CH(CH_3)_2$ | H | H | $CH_3$ | H |
| J | $R^5 = R^6 = O$ | $CH_3$ | H | H | $CH_3$ | $R^5 = R^6 = O$ |

X: $OCOCH(CH_3)C_4H_9$ Y: $CH_2OCO-$

**Fig. 1. Diversity of avermectin and milbemycin α molecular structures** (milbemycins β with an open 5-membered cycle are not shown).

There is a significant similarity in the assembly of linear tridecaketide avermectin precursors and similarly structured milmemycins that have similar antiparasitic properties [79]. However, the acyltransferase of the milbemycin

synthase loading module (MilA, also consists of 12 modules), *S. hygroscopicus* ssp. *aureolacrimosus*, ssp. *noncyanogenus* is different from AveA in the sense that it is specific to acetyl~S-CoA, propionyl~S-CoA and isobutyryl~S-CoA [73]. There are also some differences in the set of catalytic activities of avermectin synthase and milbemycin synthase: unlike MilA, AveA does not have the enoyl reductase (ER) domain in Modules 2 and 7; the dehydratase (DH) domain it has is inactive, which is what determines the somewhat different structure of aglycones in avermectins and milbemycins. Milbemycins feature an open 5-membered tetrahydrofuran cycle at C22, C23, and C25 [1, 80] (see Figure 1 and Appendix on http://www.agrobiology.ru).

Avermectin synthesis is regulated consistently with the general patterns of polyketide biosynthesis [69] in Streptomyces [81, 82]. Avermectin biosynthesis regulation factors are classified as general and specific [83-87]. In the cluster of genes synthesizing this class of macrocyclic lactones, *aveR,* which determines the generation of the specific regulatory protein AveR, functions as a specific positive regulator and controls the expression of genes for both polyketide condensation and post-polyketide modification [85]: a mutant with an *aveR* deletion will not synthesize avermectins, but will produce oligomycins in larger amounts than a wild strain. *aveR* is believed to encode a specific activator that is necessary for avermectin biosynthesis [86]. *aveI* is a gene identified as a negative regulator of the biosynthesis of these macrolides, as inactivating it results in a 16-fold production of avermectin B1a in *S. avermitilis* NRRL 8165 [33]. It has been found out that the increased expression of *aveT and sav_4189* that encode the regulatory factors SAV3619 (AveT) from the TetR (Tet Repressor Protein) family of repressor protein, and SAV4189, which is homologous to the MarR (multiple antibiotic resistance regulator) family, will increase the avermectin output [87, 88]. Among the general polyketide biosynthesis regulators found in other actinomycetes of the genus *Streptomyces* (e.g. in the actinorodine-synthesizing *S. coelicolor* M145), factors SAV3818 and AvaR3 are positive regulators, whereas AvaR1 is a negative regulator of avermectin biosynthesis [33].

Melingmycin produced by *S. nanchangensis* (structurally similar to milbemycin $\alpha$11) [89], the tetracyclic milbemycin-like compound in *S. microflavus,* neau3 Y-3 [90], and avermectin B1 homologs in *Anthogorgia caerulea* (Beibu Bay, China), have been added to the group of the described avermectin-like natural substances; like any other compound of this group, these new members are nematicides and insectoacaricides [91].

The diversity of organisms producing avermectin-like compounds (avermectins, milbemycins, and other similar substances) indicates the commonness of the combinatorial synthesis that uses the polyketide mechanism [89-91]. The aglycone structure of these natural lactones is based on the same tridecaketide. What makes natural avermectin-like compounds so diverse is the fact that biosynthesis involves various source units for tailing the carbon chain (2-R-derivatives of malonic acid [73] and a set of catalytically active polyketide synthase domains in various streptomycetes [92, 93].

Application of synthetic biology [32, 34, 73] and organic synthesis [93, 94] is an important trend in expanding the range and improving the production of avermectin-like substances [32]. Most commercial substances of this class are known to be semisynthetic derivatives of native abamectin [1, 2]. The basic approach to improving the production of abamectin and other avermectins consists in optimizing the growth conditions combined with directed biosynthesis in selected productive strains [32]. Thus, one research team attempted substituting the *ave*DH2-KR2 site in the cluster of avermectin biosynthesis genes in the

*S. avermitilis* NA-108 industrial strain with the *mil*DH2-ER2-KR2 fragment from the milbemycin biosynthesis cluster of the *S. bingchenggensis* strain; this effectively created the highly productive *S. avermitilis* AVE-T27 strain that produces 3,450±65 μg of ivermectin per ml [95]. The well-known semisynthetic ivermectin is produced by hydrogenating the 22,23-double bond of abamectin in the presence of the Wilkinson catalyst $[(PH_3P)_3RCl]$ [1, 2]. Substituting *ave*LAT-ACP and *ave*DH2-KR2 with *mil*LAT-ACP and *mil*DH2-ER2-KR2, respectively, produced the *S. avermitilis* AVE-H39 strain that synthesizes two new ivermectin-like metabolites that contain methyl radical (output of 2,093±61 μg/ml) and ethyl radical (output of 951±46 μg/ml) at C25. Those kill *Caenorhabditis elegans* 2.5 times more efficiently than milbemectin [95]. The productive mutant milbemycin-synthesizing *S. avermitilis* SAMA1M7 strain was obtained by substituting the genes *aveA1* and *aveA3* (AveA3 Module 7) in the productive *S. avermitilis* SA-01 industrial strain with templates for the genes *milA1* and *milA3* (MilA3 Module 7) of the milbemycin producer *S. hygroscopicus* subsp. *aureolacrimosus* NRRL 5739 [79]. The *S. avermitilis* SAMA1M7 strain produced milbemycins α3, α4, D (in small amounts), as well as their 5-O-methyl derivatives (about 292 μg/ml) [79]. Subsequent inactivation of 5-O-methyltransferase (AveD) in *S. avermitilis* SAMA1M7 and introducing the *aveD* stop codon with plasmid pΔAveD produced the *S. avermitilis* SAMA1M7ΔD strain that synthesizes milbemycins α3 and α4 (the basic components of commercial milbemectin) at 377 μg/ml [79]. One research team has successfully performed heterologous expression of the cluster of avermectin biosynthesis genes, *ave*, in *S. lividans* 1326, which produced A2a, B1a, and A1a [96]. *S. avermitilis* or its mutant devoid of branched-chain α-ketoacid dehydrogenase (bkdF) are able to synthesize avermectin-like compounds with differently structured C25 radicals if carbonic acids (precursors of the starting units for the synthase loading module) are added to the nutrient medium; this is due to the inactivation of the gene *bdkF*. In the presence of cyclohexane carboxylic (CHC) acid, the producer synthesizes a compound similar to avermectin B1 that has a cyclohexyl radical at C25; this is known as doramectin [1, 96, 97]. Mutant *S. avermitilis* TG2002 was constructed by substituting the avermectin synthase loading module (aveATL-ACPL) of *S. avermitilis* M1 with the CHC-synthesizing module (pnATL-ACPL) of phoslactomycin synthase (Pn) from *S. platensis* SAM-0654 using plasmid pTG2002 [99]. The recombinant *S. avermitilis* TG2002 has a doramectin output of 58±2 μg/ml, which is six times that attained by fermenting the parent strain, *S. avermitilis* M1 (9±1 μg/ml), with a doramectin/avermectin ratio of 300 [99].

Avermectin structure, action, and resistance. All the known avermectins and similar milbemycins, as well as the recently discovered melingmycin [89], 28-homo-avermectin B1a, and 28-isopropyl-avermectin B1a [91], are efficient against parasites even at very low concentrations of about 1 nmole/l [100]. Nevertheless, these compounds are not identical; varying the substitutes at different sites of the pentacyclic nucleus (C4, C5, C13, C22 to C23, C25) will modulate their biological activity only to a certain degree. In avermectins, deleting one (the remote one) oleandrose residue will somewhat lower the antinematode effect; deleting the disaccharide residue (aglycones of avermectins with 13-OH groups) will considerably reduce this effect while preserving the insectoacaricide effect. Substituting the 13-OH group with hydrogen (which is observed in milbemycin) will restore the antiparasitic effect [76]; lepimectin, which is a derivative of milbemycin that has a polar structural fragment at C13, is an efficient parasiticide. In general, avermectins and milbemycins with lipophilic groups at C13 are more active, whereas polar substitutes make such compounds less active. Similar correlation of structure and insecticide/ixodicide activity is observed in

avermectin B1: substituting the 4″-OH group with a 4″-epi-methylamine group will greatly amplify the effect the substance has on various lepidopterans while reducing the ixodicide effects [101, 102].

Avermectins and sundry 16-membered macrocyclic lactones target glutamate-dependent chloride ion channels (GluCl channels) that are common in invertebrates (nematodes, arthropods such as insects or ticks) but not in vertebrates. These channels are activated by nanomolar lactone concentrations. The irreversible activation of GluCl channels results in hyperpolarization of membranes incompatible with neural conductivity in neuromuscular synapses, which causes a strong and stable paralysis of the muscles in the pharyngeal system, musculocutaneous sac, and ovipositors [103, 104]. Invertebrates commonly have GABA receptor proteins that are related to, but have evolved differently from, GluCl channel-forming proteins (subspecies A) (g-butyric acid; $GABA_A$) ($GABA_A$-dependent $Cl^-$ channels), which are also targeted by avermectins, as GABA is the most important inhibitory neurotransmitter in the central nervous systems of mammals, human included. However, avermectins are safe for mammals as they cannot penetrate the blood-brain barrier and reach the $GABA_A$-sensitive Cl channels of the CNS [105]. As known, GluCl channels and $GABA_A$ receptors both belong to Cys-loop receptors that also include glycine, nicotine, and serotonin (5-HT3) ionotropic receptors [101, 106, 107] also affected by avermectins and milbemycins, although to a lesser affinity. Ivermectin has been found to affect the P2X4 receptor [108].

Testing the sensitivity to and resistance to ivermectin shows that parasitic resistance to ivermectin is associated with mutations in genes that determine the synthesis of GluCl subunits (*glc-1*, *avr-14,* and *avr-15*) coupled with increased expression of P-glycoprotein genes [109]. The greater sensitivity of collie dogs to ivermectin and moxidectin is due to an *MDR1* mutation that affects the gene responsible for the synthesis of P-glycoprotein, a mandatory component of the blood-brain barrier that keeps the barrier intact and prevents harmful substances from entering the brain. This mutation enables lactones to penetrate the blood-brain barrier in mammals [110].

16-membered macrocyclic lactones have a certain specific correlation of resistance and the compound structure. Thus, cross-resistance to ivermectin and doramectin is possible; however, doramectin is often more efficient against parasites resistant to avermectins [101]. One interesting phenomenon has been discovered [111]: a higher concentration of GABA at feeding *Tetranychus cinnabarinus* with an exogenous GABA or suppressing the expression of the GABA transaminase gene (GABA-T) makes the experimental pest specimens resistant to abamectin. Another interesting phenomenon that was described recently is the direct interaction of avermectins and epidermal growth factor receptor (EGFR). This factor activates EGFR/AKT/ERK pathways and induces superexpression of P-glycoprotein in the thickened chitin layers in *Drosophila melanogaster* larvae in a resistant population [112].

Semisynthetic avermectins. Quite often, the secondary metabolites need to be modified for use as an agent to improve their bioavailability, quality, physical and chemical properties, adverse effects, etc. This process creates more efficient counterparts to the original natural substances, including 16-membered avermectins [2, 113-115].

From the chemical point of view, avermectins can be presented as the derivatives of the corresponding milbemycin complex components obtained by tailing the latter with a 4-α-L-oleandrosyl-L-oleandrosyloxy group at C13 lactone nucleus. Consider examples of creating crucial pharmaceuticals, as well as prospective areas of research, e.g. synthesis of 5-O derivatives that the team behind

this paper has been researching since the mid-1990s.

The chemical modification strategy depends on the biological activity data available for the components of the antiparasitic avermectin and milbemycin complex. Let us illustrate this by data on how avermectin B1 derivatives affect mature spider mite females in direct application [116]. 96-hour mortality rate was 100% at 0.05 ppm for avermectin B1 (abamectin), 8,9-epoxy-avermectin B1, 10,11-dihydroavermectin B1, and 10-fluoro-10,11-dihydroavermectin B1; 92% for 22,23-dihydroavermectin B1 (ivermectin); 72% for 10-hydroxy-10,11-dihydroavermectin B1; 20% for 3,4-cyclopro-pylavermectin B1 and 8,9-epoxy-milbemycin (25-sec-butyl); 18% for 3,4,8,9,10,11,22,23-octahydroavermectin B1; 15% for 8,9-cyclopropylavermectin B1 and 11% for 3,4,10,11,22,23-hexahydroavermectin B1 [116]. The production of avermectin and milbemycin derivatives, the efficiency, antiparasitic spectrum, and eco-friendliness of which make them crucial for agriculture, is a multistep process that includes microbiological synthesis and chemical modification [1], see Figure 2 and Appendix on http://www.agrobiology.ru. The team behind this research is studying the suitability of 5-O and 5-C derivatives of avermectin B1, ivermectin, and other avermectins and milbemycins as antiparasitic drugs; some of them have already been patented [117]:

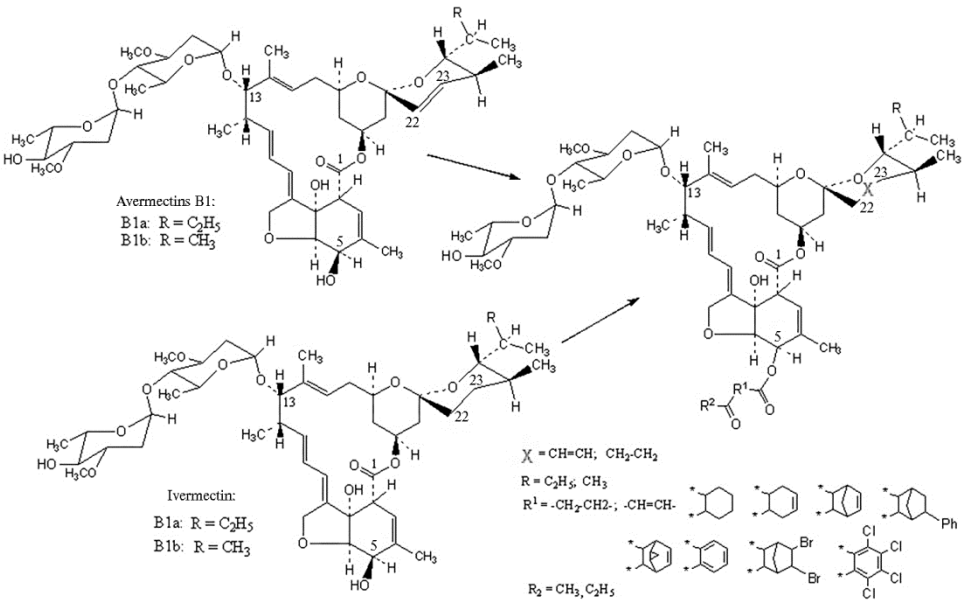



**Fig. 2. Production of 5-O derivatives of avermectin B1** (22 and 23 are, respectively, the structural components $-CH_2-$ or $=CH-$ for single or double bond for X).

We have also synthesized a series of 5-O, 5-O,4″-O, and 4″-O acyl derivatives and their esters, methyl carbamates, 5-O-sulfate sodium salt, etc. [118-122]. It has been found that 5-O-succinoylavermectin B1 (sumectin) and the compound code-named C2017 have pronounced antiparasitic effects; they have thus been used to develop liquid and solid drugs for topical and oral administration (Patents RU No. 2629600 and 2661615). Researchers from other countries are also trying to obtain similar compounds, in particular, 5-oxime derivatives and chitosan derivative [123, 124]. When testing the antiparasitic effects of sumectin, C2017, and abamectin on laboratory mice infested with *Aspiculuris tetraptera*, the authors found that an oral dose of 0.25 mg/kg had a 100% anti-helminthic effect regardless of the substance; however, unlike abamectin and

sumectin, C2017 also had a repellent effect (data unpublished). The authors have also found that C2017 far exceeds abamectin in affecting the binding of radioligand [G-$^3$H]SR 95531 with the membranes containing the $GABA_A$ receptors of cerebral cortex in rats, raising the maximum inhibition of specific binging $I_{max}$ by 86% (in-lab unpublished data).

To sum it up, one can state that natural and semisynthetic avermectins are broadly used to treat and prevent nematodiasis and arachnoentomosis in animals, humans, and plants. The most popular drugs are abamectin, ivermectin [1], doramectin [2, 3], selamectin [2], avermectin B1 benzoate [4], eprinomectin [1], as well as the similar milbemectin (a mixture of milmemycins α3 and α4) [1]. These substances are contained in various veterinary and medicinal preparations under various brand names; such preparations are used to treat onchocerciasis, dermatitis, etc. In recent years, ivermectin-based anti-rosacea creams [125], avermectin B1 hemisuccinate-based ointments and liquid against arachnoentomosis, etc. have been patented in Russia, including Russian oral drugs [126-129] as well as VEIS granules designed to combat synanthropic insects, see Certificate No. RU.77.99.88.002. E007964.09.14).

Thus, *Streptomyces avermitilis*-produced 16-membered macrolides (avermectins) and similar macrocyclic lactones are efficient against nematodes, insects, and ticks, as they affect the glutamate-dependent channels of Cl ions in invertebrates while also somewhat affecting the GABA-dependent Cys-loop receptors. Studies into fully chemical synthesis of avermectins have so far been futile due to too complex synthesis and low output. Chemical modification of natural macrolides: avermectin B1 (produced by *S. avermitilis*), milbemycin α3/α4 (*S. hygroscopicus* ssp. *aureolacrimosus*), nemadectin (*S. hygroscopicus* ssp. *noncyanogenus*), and similar biosynthetic drugs (e.g. doramectin produced by a mutant *S. avermitilis* strain with a defective gene of branched α-ketoacid dehydrogenase) has produced similar substances for use in veterinary medicine (ivermectin, eprinomectin, selamectin, and moxidectin), human medicine (ivermectin), botany (abamectin, emamectin benzoate, milbemycin oxime α3/α4); 5-O-succinoyl avermectin compounds B1 and C2017 are being researched as promising medications.

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