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Contemporary trends in development of active substances possessing the pesticidal properties: spinosyn insecticides

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Abstract: A new group of insecticides belonging to the category of bio-pesticides, namely – spinosyns, is discussed. The spinosyns are the products of bacterial fermentation by microorganisms *Saccharopolyspora spinosa* (Actinomycetes group) in a nutrient media. The chemical structures of spinosyns, also those developed by the semi-synthetic and biochemical modifications, as well as their insecticidal properties and mechanism of biological activity, the range of their application in crop protection and the commercially available products based on them, are reviewed.

Keywords: biopesticides, spinosyns, macrocyclic lactones, forozoamine, 2',3'4'-tri-O-metyloramnose, *Saccharopolyspora spinosa,* spinosyn A and D, spinosad, *Saccharopolyspora pogona*, butenylspinosyns, spinetoram, aglicones and pseudo aglicones

INTRODUCTION

The spinosyn insecticides are the secondary metabolites of bacterial fermentation by microorganisms *Saccharopolyspora spinosa* in a nutrient media, and are known as bio-pesticides. Structurally they belong to the chemical group of macrocyclic lactones, which involves also avermectin and milbemecin insecticides [1]. The natural products give a great opportunity to prepare many new products, among the others – also useful as insecticides. Introduction of Spinosad to the agricultural practice was a milestone for the use of natural products in crop protection.

In the 80-ties of the yester-century in Lilly Research Laboratories (US) was

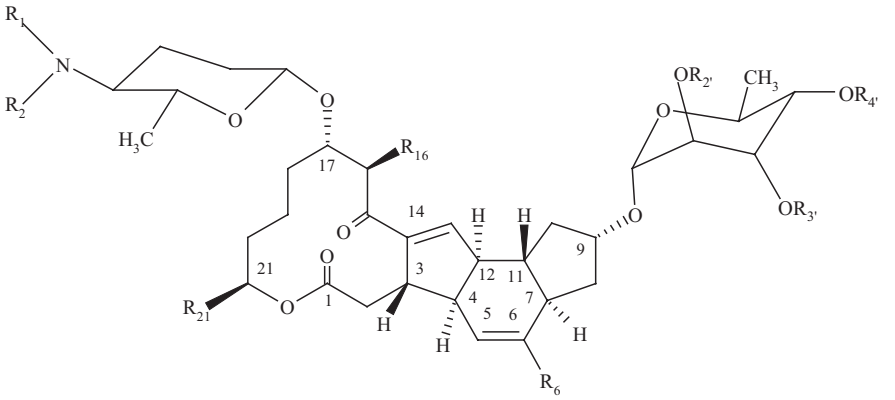
pursued a research project aimed to find the new natural products, for their future application in pharmaceutical and agrochemical industries. The course of research work was developed by the fermentation processes of the soil samples, collected from the different grounds. The resulted extracts were then undertaken the screening tests. The researchers found a new species of actinomycetes, *Saccharopolyspora spinosa,* in the soil samples collected in 1982 from the area surrounding the deserted rum distillery on a Carribean Island. The fermentation wort extracts when tested, exhibited as well contact as stomach insecticidal action against the nymph of southern armyworm, *Spodoptera eridana*. The seldomness to demonstrate by natural products a contact insecticidal activity against the Lepidoptera species, encouraged scientists to further studies, which resulted in the identification of the series of new macrocyclic structures, at present called spinosyns, which were produced by *Saccharopolyspora spinosa* during the fermentation.

As it was mentioned above, a history of spinosyn insecticides has its beginning in Eli Lilly, where Plant Sciences Business and Dow Chemical Company found in 1989 a joint venture under the name of DowElanco. In 1997 a Dow Chemical bought up shares of Lilly in Dow Elanco and changed a name of the joint venture on Dow Agro Sciences. And the last was established as the manufacturer of the first spinosyn insecticide, namely – Spinosad.

SPINOSYNS

A product of fermentation (i.e. extract from the fermentation broth), defined initially as the A83543 product, has proved to consist quite a large group of the specific substances produced by *Saccharopolyspora spinosa*, which were marked by the consecutive alphabet letters A, B, C, D, etc. (A83543A, A83543B, A83543C, A83543D, ..). Currently they are specified as spinosyn A, spinosyn B, spinosyn C, etc.

Chemical structure of spinosyns comprise a tricyclic ring system fused to a 12-membered macrocyclic lactone ring, to which are attached the two different sugars: an amino sugar (D-forosamine) in the position C-17 and a neutral sugar (2',3',4'- tri-*O*-metylo-L-ramnose) in a position C-9 (I) [2]:



(I)

The first spinosyn was isolated from the fermentation medium and chemically and structurally was defined as spinosyn A (A83543A). The consecutive studies demonstrated that the original primary strain of *Saccharopolyspora spinosa* (wild type, WT) produces some other spinosyns as well, namely spinosyns B, C, D, E, F, G, H, J. The mixture of the lasts was a subject of first patents (spinosyns as the new type of insecticides, methods for their preparation) [3-7].

It was proved that the main constituents of the above mentioned spinosyns mixture are spinosyn A and spinosyn D. The highest insecticidal activity against larvae of *Heliothis virescens* (tobacco budworm) was displayed by spinosyn A, followed by spinosyn D, which means that the most active are the spinosyns produced by *Saccharopolyspora spinosa* in the highest concentrations. The fermentation broth extract containing a mixture of these two spinosyns was named spinosad and was a first commercial product belonging to a group of spinosyn insecticides of Dow Agrosciences (firstly it was registered under the name Tracer).

Besides the spinosyns produced by fermentation of the wild types of *S. spinosa* were also identified several others produced by the mutated strains. As the primary strain was able to produce spinosyns only in quite a slight amount, the research works were undertaken to improve its quality in the respect to the yield of spinosyns production (taking account also for a possibility to obtain new spinosyns). Not going into particulars of these studies, it can be stated that the results of them gave an opportunity to obtain a series of succeeding spinosyns, in the overall majority not produced by a wild type *S. spinosa*. These spinosyns obtained by fermentation process of mutated strains included spinosyns L, M,

N, Q, R, S, T [8-9], and spinosyns K, O, P, U, V, W, Y [10].

The structural differences between spinosyns rely on: the degree of forosamine *N*-metylation, the presence or absence of *O*-methylated groups in ramnose, and the presence or absence of the methyl group(s) in the positions C-6, C-16 and C-21 of their tetracyclic ring system. (Table 1) [11].

Table 1. Structure of spinosyns

SPINOSYN	$\mathbf{R_1}$	$\mathbf{R_2}$	$\mathbf{R_{21}}$	$\mathbf{R_{16}}$	$\mathbf{R_6}$	$\mathbf{R_{2'}}$	$\mathbf{R_{3'}}$	$\mathbf{R_{4'}}$
A	Me	Me	Et	Me	H	Me	Me	Me
B	H	Me	Et	Me	H	Me	Me	Me
C	H	H	Et	Me	H	Me	Me	Me
D	Me	Me	Et	Me	Me	Me	Me	Me
E	Me	Me	Me	Me	H	Me	Me	Me
F	Me	Me	Et	H	H	Me	Me	Me
G	Me	Me	Et	Me	H	Me	Me	Me
H	Me	Me	Et	Me	H	H	Me	Me
J	Me	Me	Et	Me	H	Me	H	Me
Q	Me	Me	Et	Me	Me	H	Me	Me
R	H	Me	Et	Me	H	H	Me	Me
K	Me	Me	Et	Me	H	Me	Me	H
L	Me	Me	Et	Me	Me	Me	H	Me
M	H	Me	Et	Me	H	Me	H	Me
N	H	Me	Et	Me	Me	Me	H	Me
O	Me	Me	Et	Me	Me	Me	Me	H
S	Me	Me	Me	Me	H	H	Me	Me
T	Me	Me	Et	Me	H	H	H	Me
U	Me	Me	Et	Me	H	H	Me	H
P	Me	Me	Et	Me	H	Me	H	H
V	Me	Me	Et	Me	Me	H	Me	H
W	Me	Me	Et	Me	Me	Me	H	H
Y	Me	Me	Me	Me	H	Me	Me	H

The one of the selection ways was directed towards the creation of such mutation strains which do not possess the ability to ramnose hydroxyl groups methylation (mutants with unfunctional 2'-, 3'- or 4'-methyltransferases). The further variants in ramnose methylation were reached after sinefungin (a natural nucleoside isolated from *Streptomyces griseolus* and *Streptomyces incamatus* [12]) addition, what resulted in a specific blocking of the 4'-*O*-methyltrasferaze

during a fermentation process of *S. spinosa* wild type strain [11].

Regarding their complicated chemical structure, spinosyns are effectively obtained in a way of fermentative process, using the respective strains. The development of this group is bi-directed and go ahead through:

– a chemical modifications of natural spinosyns (preparation of semi synthetic analogs),
– an antibiotic genetic engineering of the biosynthetic genes.

Spinosyns proved to be a very strong insecticides, with the stomach and contact mode of action, what is unique among the natural products [14].

The unique is also the mechanism of their biological activity, which relies on their influence of the nicotinic acetylcholine receptor (nAChR), as well as of the chloride channels controlled by GABA acid. This action induces the general paralysis of insect. However, it differs from the mode of action of avermectins, fipronils or cyclodienes (in a case of GABA-receptors), as well as from neonicotinoids or nicotine (considering the nicotinic acetylcholine receptors) [15]. In the last case spinosyns affect the nAChRs' located in the post synaptic cells, in detail they affect the receptor Dm alpha6 [16]. Until now, there is no other chemical substances class with this type of action on the insects nervous system.

SPINOSAD [15]

Spinosad is a first spinosyn insecticide introduced to the market by Dow Agrosciences in 1997, in the form of Tracer preparate, with the use for cotton protection against insect from Lepidoptera's group. Spinosad is a mixture of spinosyns A and D, and its nominal contents is as follow: spinosyn A (m.p. 84-99.5 °C) – 85%, spinosyn D (m.p. 161.5- 170 °C) – 15%.

Spinosad effectively controls the insects of the groups of Lepidoptera (moths and butterflies), Diptera (flies and midges), Thysanoptera (thrips). It also displays a toxicity against some species of Coleoptera (beetles), Orthoptera, Hymenoptera, Isoptera (termites), Dermaptera (earwigs), Psycoptera (psocids, booklouses) and Siphonaptera (flees).

Spinosad was registered in over sixty countries; in Europe – among the others, in Benelux (2002), Cyprus (1998, Tracer 48SC), France (2001), Spain (2001), Turkey (1998) [17]. In Poland spinosad was introduced to the list of chemicals admitted of market use in 2008 – it is applied to protect a wide range (over 200 species) of agriculture crops. In the US the crop protection by spinosad use contains over 180 of different crops. Spinosad possesses an important place also in the protection of the stored products, and in the overcoming of sanitary insects

(mosquitoes: e.g. Asiatic mosquito (aedes albopticus), tropical mosquito (Aedes aegypti) which transmits a yellow febra, tse-tse fly).

The applied doses of spinosad for agricultural crop protection falter between 25 – 100 g s.a./ha, for the stored products protection – between 1-50 mg s.a./kg, for sanitary harmful insects overcoming – LC_{50} equal or lesser 0.025 ppm of value.

The toxicity of spinosad against mammals is low: its LD_{50} p.o. value against rat is 3780 mg/kg (males), > 5000 mg/kg (females). Spinosad is practically no toxic against birds, in the case of aquatic organisms it exhibits a very slight toxicity (more details in [17]).

It does not show any cancerogenic, teratogenic, mutagenic and neurotoxic activity [18], has a very good profile of its environmental behavior and is recommended to use in the integrated programs of crop protection. It is worth to say that spinosad also measures up the requires of IFOAM (International Federation of Organic Agriculture Movements).

Spinosad is applied in the formulates of SC (suspension concentrate) and WG (water dispersible granules), but these two forms do not deplete the future possibilities in this area to produce its spray [19] and fumigant [20] based formulates. The examples of spinosad commercially available forms are, among the others: Conserve, Spintor, Success, Tracer, Entrust, Naturylate of Dow Agrosciences, veterinarian formulate Extinosad (Elanco).

SYNTHETIC MODIFICATIONS OF SPINOSYNS. SPINETORAM

A complicated chemical structure of spinosyns renders their synthetic preparation unpractical and the most important path to obtain them relies on the fermentative processess. However, Evans and Bluck [21-22] conducted a total synthesis of (+)-spinosyn A, which does not occur in the fermentation broth extract and does not possess any biological activity. The other authors [23-25] performed also the synthesis of (-)-spinosyn A. Nonetheless, these synthetic routes, so far, are not used in the industrial applications. In turn, quite a wide development was done within the scope of research on the synthetic modifications of naturally obtained spinosyns, with the goal to increase the capacity and spectrum of their insecticidal activity [13, 26-30].

Spinosyns exhibit a slight polarity, so they are practically insoluble in water. This property makes difficult their transport in the tissues. Therefore, the efforts to spread the bioactivity of spinosyns in a semi synthetic way were also directed to increase of their intrinsic activity and to increase their stability against the surface photo degradation. The important role in achieving the synthetically

modified spinosyns played pseudo aglicons (obtained by removal of one of the sugar ligands from the natural spinosyn structure) and aglicons (obtained by removal of both sugars from the natural spinosyn structure) [31-33].

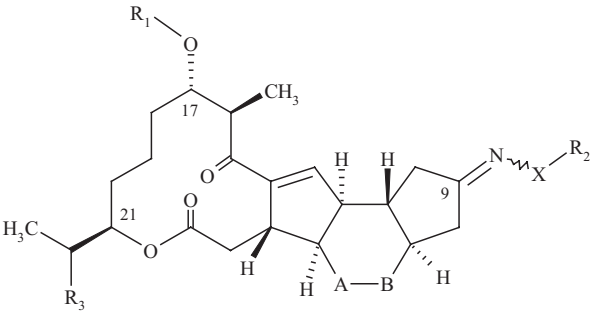
The preferable nomenclature for pseudo aglicons:

– in the case of absence of amino sugar moiety (forosamine): the term spinosyn A17-Psa, spinosyn B17-Psa, etc. is used,
– in the case of absence of neutral sugar moiety (ramnose): the term spinosyn C9-Psa, spinosyn D9-Psa, spinosyn J9-Psa, etc. is used.

As the particular spinosyns differs in their macrocyclic part, their aglicons also vary. It is used to name them as: aglicon of spinosyn A, aglicon of spinosyn B, etc, generally – the term of "spinosyns aglicons" is used.

Pseudo aglicons can be obtained by acidic hydrolysis, which – in some cases – is also the method to produce aglicons [5, 34]. As it was stated, some of pseudo aglicons, e.g. spinosyn A 17-Psa, are generated during the fermentation [5]. On the basis of the compounds isolated from the fermentation medium it was obtained over 1000 semi synthetic analogs of natural spinosyns [35]. The modifications concerned the ramnose and forosamine moieties; many derivatives with the substituents vary than sugar-type ligands, or sugar-type but vary than a typical ramnose or forosamine ligand were obtained, starting from 9- and 17- pseudo aglicons. The other derivatives were obtained by the modification of the 5,6,7-tricyclic part of spinosyns structure, as well as by changes in the 12-membered macrocylic lactone ring. In those last cases the modifications involved the hydrogenation, epoxidation, halogenation, addition of alkyl and nitrogen containing groups, also the elimination and addition of substituents in a lactone part.

The discussed above directions of the semi synthetic modifications of spinosyns were realised firstly in DowElanco, then in Dow AgroSciences. In the end of 90-ies of the past century a next large company – Bayer CropSciences, joined up to the research on the field of spinosyn insecticides, what resulted in many new spinosyn derivatives. For instance, in patents [36-37] of Bayer CropScience is described a method of preparation of a new group of derivatives, being the analogs of 9-ketospinosyns, with a general structure (II).



(II)

where:
X – O, NH, NR_4; **R_1** – H, amino sugar; **R_2** – H, substituted alkyl, cycloalkyl, aryloalkyl, heteroalkyl, aryl, heteroaryl, COR', CSR' (when X is NH or NR_4), and where **R'** - amine, alkylamine, aryl, arylamine, heteroarylamine, arylalkyl, heteroaryl, heteroarylalkyl'; **R_3** – H, OH; **R_4** – substituted alkyl, or performes 3-, 4-, 5-, 6-, 7-, or 8-membered ring with R_2, which in turn can be separated by one or more heteroatoms: as O, S, SO, SO_2NH, NR_5 (**R_5** – alkyl, cycloalkyl, aryl, heteroaryl, etc. groups); **A – B** are the ones of the possible ligands: -CH=CH-, -CH=C(CH_3)-, -CH_2-CH_2-, -CH_2-CH(CH_3)-.

The starting 9-ketospinosyns were prepared by oxidation of 9-pseudo aglicons or aglicons of the respective natural spinosyns [26]. In the general structure of the 9-ketospinosyns derivatives (II) there is a possible variant, where position C-21 is substituted by 1-hydroxyethyl group, which does not occur in the hitherto identified natural spinosyns. A method to receive spinosyns of this characteristics, and their use for the preparation of the further new derivatives by a semi synthetic transformations, was a subject of another patent of Bayer CropScience [38]. The introduction of 1-hydroxyethyl substituent into C-21 position was performed by the biochemical method.

As the result of the intensively conducted synthetic modifications of the natural spinosyns skeleton, it was let in the market a second in turn spinosyn insecticide, under the common name of spinetoram.

Spinetoram (XDE-175, XDE-175-J, XDE-175-L) [39-43] was elaborated by Dow AgroSciences and is a mixture of two main components: 5, 5-dihydroxyspinosyn J (XDE-175-J) (50-90% wt) and 3'-*O*-ethylspinosyn L (XDE-175-L) (50-10% wt).

A production of spinetoram begins from the fermentation process what leads to obtain mixture of spinosyns J (major) and L(minor), which differs the substituent

in the position C-6 of the structure, means detaily – spinosyn J has a hydrogen atom at this position, whereas spinosyn L - a methyl group. Both of them in the position 3' of ramnose sugar have a free hydroxyl group.

A mixture of those both in a chemical modification is undertaken the etherification of this hydroxy group to form an ethoxy ligand, followed by a selective hydrogenation of the double bond C5-C6 in the ethoxylated spinosyn J. The introduction of ethyl group into ramnose sugar moiety significantly increases the insecticidal activity of the mixture, whereas the hydrogenation of spinosyn J C5-C6 double bond substantially reduces the problem of the residues in soil. In fact – spinetoram is very easily biodegradable in soil, its half-decay time accounts 3-5 days. Spinetoram is also easily degradable in the surface waters (half-decay time of less than 1 day). In comparison with spinosad, spinetoram displays almost 10-fold higher rate of effectiveness, and exhibits higher insecticidal activity. Its biological potential and the duration of its residual activity enables its application to control a wide spectrum of the harmful insects of many species: Lepidoptera, Diptera, Thysanoptera, Homoptera, Coleoptera, Orthoptera, Siphonaptera, Isoptera.

Spinetoram is characterised by a low acute toxicity against mammals, and does not exhibit mutagenic and teratogenic properties, also its ecological characteristics is very good.

It is envisaged to wide range control of fruits and crop plants. In 2007 EPA (the US Environmental Protection Agency) accepted the complete registration of the technical spinetoram, and of the formulates based on it, namely Delegate WG, GF-1640WG-NC (they both consist of 25% of spinetoram), Radiant SC and GF-1587SC-NC (consisted of 11.7% of spinetoram). In September 2008 the European Commission confirmed the completness (fulfilling) of the documentation, performed to the detailed research in the aim to include spinetoram to the Annex I of the EC Directive 91/414/EWG. Spinetoram is already distributed and used in many countries of South America, in Canada, Korea, Malasya, New Zealand, Pakistan.

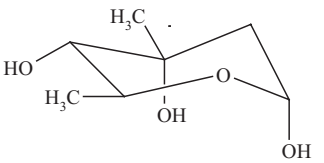
BIOCHEMICAL MODIFICATIONS OF SPINOSYNS. BUTENYLSPINOSYNS (POGONINS)

Although, the widely conducted research works on the field of semi synthetic modifications of spinosyns, still some of the regions of their structures remain inaccessible for chemical transformations. This concerns the macrocyclic ring in the area involving C-18 to C-21 carbon atoms. Carbon C-21 is known as a "western shore" of spinosyn skeleton and is substituted with methyl or ethyl

groups in the already identified natural spinosyns. The biosynthesis opens new possibilities to change this part of molecule.

The biosynthetic pathway, as it was already mentioned, was used, for instance, to introduce the hydroxyl group in a C-21 position. It was done by the strains of microorganisms of *Streptomyces djakartensis, Streptomyces giseofuscus, Streptomyces caelestis,* etc. [38].

Using the genetically modified strains of *Saccharopolyspora erythracea*, there were obtained spinosyns, in which a sugar of forosamine (C-17 position) was changed on α-L-mycarose sugar (III) [44].



(III)

The use of the hybrid spinosyn poliketide synthases [of the avermectinic PKS (Protein Kinase Substrate) *Streptomyces avermetidis* and erythromycinic PKS *Saccharopolyspora erythracea* origin], which display the abilities to function in *Saccharopolyspora spinosa* resulted in the preparation of the biologically active spinosyns, modified in the position of C-21 [45-46]. In this manner were obtained, among the others: 21-cyclopropylspinosyns A and D, 21-cyclobutylspinosyns A and D, 21-methylthiomethylspinosyns A and D, and also 21-isopropylspinosyns A and D. All of these compounds presented an insecticidal activity against one or more pests, relevant from the agronomical point of view. The highest activity was observed for the 5,6-dihydro-21-cyclobutylspinosyn A derivative, which was at least equal to the natural spinosyn activity, or – in some cases, even more active, as for instance against cucumber aphids (*Aphis gossypii* Kalt.) and against whiteflies (Aleyrodidae).

In the 90-ies of the past century there were performed studies toward the estimation of the insecticidal properties of the extracts from the fermentation process of the soil samples collected in the State of Indiana (US). The analyzed extracts showed to be insecticidally active, and presented an interesting contact activity, quite similar to the mode of action characteristic for this of spinosyns. The results of detailed analysis revealed that in the examined post fermentation extract were present the spinosyns with 1-butenyl group in the C-21 position. The microorganism producing these structures was identified as *Saccharopolyspora pogona* (NRRL 3014) [47-50]. The discovered this way butenylspinosyns are also

known as pogonins. Regardless of having butenyl substituent in C-21 position, the butenylspinosyns differs from the natural spinosyns also by some other features. Although these new compounds are produced by the strains different than *Saccharopolyspora spinosa*, their structural similarity to the "classical" spinosyns influenced to leave also for them the common name of "spinosyns". However, a new order of their nomenclature was taken, providing for their differentiation from the classical structures. In the chemical structures of butenylspinosyns there are distinguished three main parts, which are different when compare to the already known spinosyns, in detail the changes are observed:

– in a macrocyclic ring,
– in the sugar bounded with the C-17 position,
– in the side chain bounded with the C-21 position.

His new nomenclature system is a composition of these three elements. Some closer details can be found in [35].

From a fermentation broth there were isolated and identified 31 of compounds. All of them are substituted in C-21 position with 1-butenyl group - as it is, or as the more extended substituent (obtained by attaching to the C-3 or C-4 carbon atoms of butenyl chain the other substituents, e.g. -CH(OH)-, $-CH_3$, $-CH=CH_2$), in the position of C-8 of macrocyclic ring quite often hydroxyl or methyl group are present. The further differences can be observed in forosamine sugar ligand, including its replacement by the other sugars. There were identified also C-17 pseudo aglicons.

The structurally simplest 1-butenylspinosyns are the ones A and D. Their insecticidal activity is very much the same as that of spinetoram.

Butenylspinosyns give the opportunities to develop and extend their synthetic modifications, attending as the by-products in the synthetic research of the new connections effective as insecticides and acaricides [50-51].

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