Formosan Entomologist
Journal Homepage: entsocjournal.yabee.com.tw

Spinosyn Insecticides: Part I. Blockbuster Products from a Remarkable Discovery 【Review article】

簡介賜諾司類殺蟲劑 (Spinosyn Insecticides) (一)：來自奇特發現的明星產物【綜合論述】

Eddie Hang Chio*
招衡*

*通訊作者E-mail : EHC13029@Gmail.com

Received: 2011/02/28 Accepted: 2011/04/06 Available online: 2010/03/01

Abstract

The discovery of spinosyn insecticide is a remarkable story about how to find useful natural products from fermentation. The key factor that contributed to its discovery is dereplication. Spinosyn is a mixture of metabolites produced by aerobic fermentation of the actinomycete species Saccharopolyspora spinosa. Their chemical structures are mainly a polyketide derived tetracyclic macrolide appended with two amino sugars. Their insecticidal activities were first observed in the mosquito larvicide screen and its first product launched was in the USA in 1997 for pests on cotton plants. Since then, hundreds of products containing spinosad have been launched for crop protection as well as for the protection of food-animals and companion-animals in many countries. Most recently, spinosad was approved to treat head-lice on human in the USA. Extensive structure-activity relationships studies demonstrated that the mixture of modified factor L and factor J of spinosyn, later named spinetoram, has even broader spectrum than spinosad. The outstanding selectivity, low mammalian toxicity, environmentally friendly properties, plus the unique mode of action make spinosyn stands out as one of the most preferable and commercial successful insecticides globally.

摘要

Spinosyn為一種好氧性放射菌 (Saccharopolyspora spinosa) 的天然代謝物質，在殺蟲劑的應用上，因具有高度選擇性、低哺乳動物毒性、低環境毒性與特殊的作用機制，而富有高度商業價值並在全世界被廣泛使用。Spinosyn之化學結構為多烯酮所衍生之四環大環內酯，且具有兩個胺基醣取代基，其開發過程堪稱天然產物開發的典範，透過孑孓實驗，可大量快速篩檢出待測物的毒性效果，而在篩選過程中，最重要的觀念為去除重複 (dereplication)。Spinosyn為一類化學物質之統稱，在商品化後，賜諾殺 (Spinosad) 含Spinosyn A與D最先在1997年推出，應用於棉花之蟲害防治上，自此之後，大量的產品被推出，應用層面除了植物保護外，也可用於食用動物與伴侶動物，甚至是頭蝨的防治，足見其高度選擇性之優點。近來在化學結構上的研究發現，置換取代基之Spinosyn L與J，合稱為Spinetoram (賜諾特)，具有更廣泛之殺蟲效力，同樣深具開發潛力。

Key words: Spinosyn insecticides and product sales, natural product research, dereplication, Saccharopolyspora spinosa

關鍵詞: 賜諾司類殺蟲劑、天然產物研究、去重複、Saccharopolyspora spinosa。

Full Text: PDF(1.54 MB)

台灣昆蟲 31: 1-13 (2011)
Formosan Entomol. 31: 1-13 (2011)

Spinosyn Insecticides: Part I. Blockbuster Products from a Remarkable Discovery

Eddie Hang Chio*

Visiting Specialist, Department of Entomology, National Taiwan University, 27, L. 113, Sec. 4, Roosevelt Rd., Taipei City, Taiwan

ABSTRACT

The discovery of spinosyn insecticide is a remarkable story about how to find useful natural products from fermentation. The key factor that contributed to its discovery is dereplication. Spinosyn is a mixture of metabolites produced by aerobic fermentation of the actinomycete species *Saccharopolyspora spinosa.* Their chemical structures are mainly a polyketide derived tetracyclic macrolide appended with two amino sugars. Their insecticidal activities were first observed in the mosquito larvicide screen and its first product launched was in the USA in 1997 for pests on cotton plants. Since then, hundreds of products containing spinosad have been launched for crop protection as well as for the protection of food-animals and companion-animals in many countries. Most recently, spinosad was approved to treat head-lice on human in the USA. Extensive structure-activity relationships studies demonstrated that the mixture of modified factor L and factor J of spinosyn, later named spinetoram, has even broader spectrum than spinosad. The outstanding selectivity, low mammalian toxicity, environmentally friendly properties, plus the unique mode of action make spinosyn stands out as one of the most preferable and commercial successful insecticides globally.

Key words: Spinosyn insecticides and product sales, natural product research, dereplication, *Saccharopolyspora spinosa*

Introduction

Natural products (NP) have been used for the benefit of humankind for thousands of years including poisons for game and fish, medicines, and crop protection. The best NP example as a crop protection agent may be the pyrethrins from certain species of chrysanthemum (Anonymous, 2003). Since the introduction of DDT after World War II, many NP were replaced by their more potent and cheaper

*Corresponding email: EHC13029@Gmail.com

synthetic counterparts. The introduction of these new synthetic pesticides has been typically driven by the development of resistance in insect pests to previous insecticides. More frequent applications of the same type of insecticides only facilitated the speed of resistance (Broadhurst, 1998). In addition, the emergence of the secondary insect pests became an increasing problem as a result of the elimination of beneficial insects that keep the secondary pests in check (Dutcher, 2007). Over time, environmental and health risks associated with these broad spectrum insecticides became increasingly apparent (Carson, 2002). This has spurred the search for more environmentally sustainable insect control options.

Eli Lilly and Company (Lilly) was fortunate enough to play a role in the discovery of a new class of NP insecticide called spinosyns (Boeck *et al*., 1989; Boeck *et al*., 1994; Boeck *et al*., 1996). Spinosyns as a group are selective, effective and environmentally friendly. The discovery of spinosyns validates the fact that NP can still provide a unique opportunity for finding new, useful and financially rewarding molecules.

Numerous articles about spinosyn have been published but seldom mention any detail about its discovery and why spinosyn won the US-EPA Presidential Green Chemistry Challenge Award three times. How spinosyn was discovered and the commercial products from this discovery will be discussed in Part I of this article. Why it keeps winning the Green Chemistry Challenge Award will be discussed in Part II.

The Remarkable Discovery of Spinosad

The insecticidal activity of spinosad was discovered in the early 80s at the Lilly Research Laboratories (LRL), a Division of Eli Lilly and Company in Greenfield, Indiana. Hard work, team work, innovative thinking and a certain degree of serendipity are always involved in great discovery. Discovering spinosad from fermentation was no exception. The pivotal factor contributing to the spinosad discovery was dereplication. Dereplication is a technical term mainly used in natural product research. It is a way to avoid rediscovering the known secondary metabolites over and over again (Anonymous, 1913).

Natural products research

In the early 80s, Lilly was one of the global leaders in natural product research which mainly was for human antibiotic discovery. In general, the antibiotic discovery program starts by isolating microorganisms from diverse soil samples. The microbiologists let such microbes grow under defined conditions (also known as fermentation). The antimicrobial activities from the resultant fermentation broths were then evaluated (also known as screen). Finally, if the fermentation broth demonstrated desirable antibiotic activities, the chemist would try to isolate, purify and identify the active components from the fermentation broths. Almost all of the antibiotics on the market today are directly or indirectly discovered by following the above-mentioned process (Tanaka and Omura, 1993; Harvey, 2007).

Agricultural antibiotics

Lilly had a health portfolio of antibiotics on the market at that time. Besides killing human pathogens, many antibiotics are also effective against animal pathogens. In addition, antibiotics are well-known growth promoters for food-animals (Anonymous, 2002a) which made it logical for Lilly to sell antibiotics for food-animal use. Lilly did this through Elanco, a Division of Lilly.

The LRL in Greenfield was the research center supporting Elanco's Animal Sciences and Plant Sciences subdivision. From time to time, promising antibiotic candidates discovered by the antibiotic research

group were sent to the Greenfield laboratory for further evaluation as potential products for food animals or for crop protection.

Dereplication in Entomology research

The Entomology Research Committee was worried about antibiotics insecticides could lead to cross-antibiotic resistance in human, proposed to test fermentation broths with the mosquito larvicide screen and only followed-up with those that killed mosquitoes but were not antimicrobial. The logic behind this proposal was simple. It would not only avoid the cross-resistance issue in humans but also fit the principle of dereplication. In fermentation screen, the antimicrobial activities only act as an indicator but cannot tell us the structure of the active components in the fermentation. It takes months before the active components can be isolated, purified, and identified. There are approximately 16,500 known metabolites which have been isolated and characterized from Actinomycetes (Demain and Sanchez, 2009). It is extremely frustrating to find out the active components are one of the known structures that had been discovered earlier. Therefore, an effective dereplication strategy at the early stage of the screen program is critical. With only few exceptions, almost all known antibiotics showed insecticidal activity (Tanaka and Omura, 1993; Demain and Sanchez, 2009). Selecting those metabolites that are insecticidal but not antimicrobial should provide a better opportunity to discover something new. Another benefit of this exercise was to cut down the number of active leads coming out from the screen operation. The short list of active leads enabled the scientists focusing on the really promising candidates. The final protocol was that cultures that were insecticidal against the mosquitoes must be cross-checked with their antimicrobial data generated by the microbiology department. Only those that were insecticidal but showed no sign of antibacterial or antifungal activity were selected for re-fermentation; this was a necessary step to validate the previous activity as reproducible. Amazingly, more than 60% of cultures that showed biological activities previously could not be repeated in the re-fermentation process (Baltz, 2005; Pelaez, 2006; VonNussbaum *et al*., 2006). It's no wonder people refer to fermentation research as art rather than science!

Lucky foreseen

The decision to pick only non-antimicrobial cultures turns out to be a correct one. A link between agriculture antibiotics and resistant bugs in humans was finally confirmed in the late 90s. Since then, many antibiotics for farm animals have been banned (Das *et al*., 1997; Anonymous, 1999; Thorp and Cargill, 1999; Anonymous, 2000).

Innovative approach

As far as innovation goes, a mosquito larvicide screen was modified so thousands of broth samples could be evaluated per day in a 96-microtiter plate format (Chio, 2007a). The established WHO assay protocol just could not keep up with the high volume of sample submission (Christophers, 1961). A couple of years after the modified mosquito larvicide screen was fully operational, four cultures demonstrated excellent potency against mosquitoes but showed no sign of activity against bacteria or fungus. Out of these four cultures, A83543 was the most consistent performer and got top priority for further development. Culture A83543 was isolated from soil collected by another Lilly employee on vacation in the Virgin Islands. The culture was later identified as a new species of an uncommon genus of soil Actinomycetes and was named *Saccharopolyspora spinosa* sp. nov (Mertz andYao, 1990).

Dereplication in Fermentation research

Starting the fermentation process with

a rare species of Actinomycetes from soil is another excellent dereplication approach. One strategy in place for finding novel secondary metabolites was to investigate non-traditional microorganisms (not Streptomyces nor Micromonospora). A rare species of Actinomycetes could increase the odds in producing metabolites with new structures (Stewart *et al*., 2005).

Serendipity aspect

In fermentation products research, another long-recognized concern has been the potential existence of an extremely potent new compound in the fermentation broth at only barely detectable amounts that could easily be overlooked and prematurely discarded. In the spinosad discovery, this was especially true. A certain degree of serendipity was involved here leading to the spinosad discovery. Like many other laboratories, Lilly follows the World Health Organization guidelines and used the Yellow-Fever mosquito larvae (*Aedes aegypti*) as an insecticidal indicator (Christophers, 1961). The guideline calls for using the 4th instar larvae as an indicator. Twenty-five 4th instar larvae are countered and put in a large mouth Mason jar with 300 mL of water containing a known amount of insecticides. Their LC_{50} values are calculated 24 hours post-treatment. However, this WHO standard procedure was not suitable for the high through-put screen operation. To fit the automated format, larvae were pipetted into the 96-well microtiter plate containing fermentation samples and the Minimum Inhibition Concentration measurement was used to evaluate the insecticidal effects of the fermentation samples. The head of the 4th instar larva was too big for the special pipette; therefore, the 3rd instar larvae were used instead. This simple change happened to increase the assay detection limit from approximately 5 PPM to 0.25 PPM, a 20 fold increase in sensitivity. Such improved sensitivity led to the discovery of spinosad at less than 1 part per million levels in the initial broth preparation (Chio, 2007b). If the WHO guideline was followed exactly with the 4th instar larvae as the indicator, spinosad activity could never been discovered. Other traditional antimicrobial assays such as the zone inhibition assay were not able to detect spinosad due to its non-antimicrobial nature, and the HPLC method was not viable either because of the low titer in the initial fermentation preparations. Only mosquito activity could be used to guide the separation and isolation studies for spinosad until its titer was significantly improved to a point where HPLC could locate their active peaks (Kirst *et al*., 1992, 1993).

Side story

A minor, but critical, side story about the discovery of spinosad involved a bad case of contamination. It is not uncommon to see contamination where the culture media is so rich and the bioassay is conducted in a non-sterile environment. During the mosquito larvicide screen development, some serious contamination problems occurred; cross culture contamination occurred either in the 96-well microtiter plate or was caused by an air-borne microorganism. After several trial-and-error attempts, adding a 0.05% Keflin in the reconstitution solution was able to solve this problem. At this concentration, Keflin was strong enough to stop the microbial grow without affecting the outcome of the mosquito assay (Chio, 2007a). The ectoparasite and endoparasite screen operations at the animal sciences subdivision were finally shut down for good due to the pesky contamination problem.

Third parties

It is worthwhile to mention that while spinosad was discovered at Lilly, its development involved 2 other companies; DowElanco and Dow AgroSciences Company. DowElanco was a joint venture

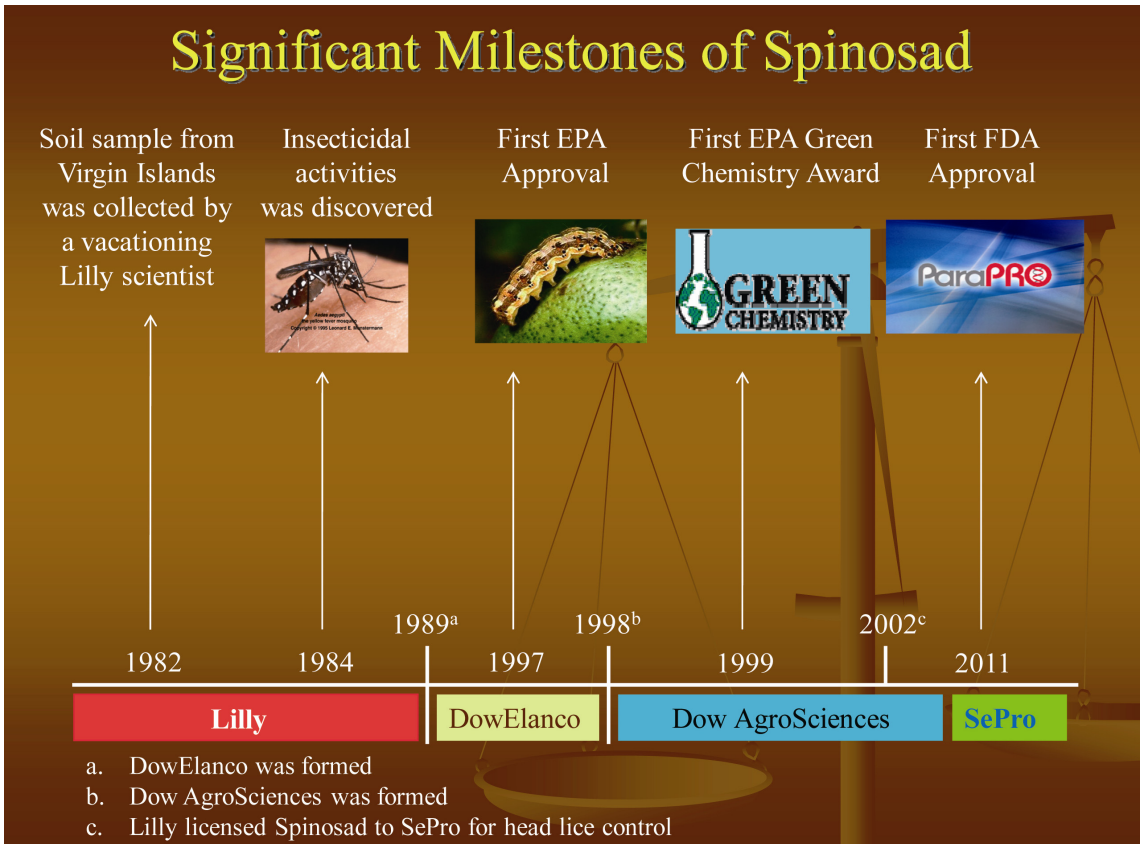

Fig. 1. Significant milestones in spinosad discovery and development.

company established in 1989 by Midland, Michigan based Dow Chemical Company and Lilly, with Lilly holding a 40 percent interest and Dow owning the remaining 60 percent. In 1998, Dow acquired Lilly's 40 percent stake in DowElanco and renamed DowElanco as Dow AgroSciences Company that owned all the rights to develop spinosad for crop protection purposes while Lilly retained the rights to develop spinosad for animal health protection (Anonymous, 1998). This agreement enabled both companies to leverage their special marketing and sales expertise to promote spinosad for crop protection and for animal health. In 2002, Lilly licensed spinosad to SePro for head lice control (Anonymous, 2002b). Figure 1 shows the significant milestones for spinosad discovery and development.

Chemical Structure of Spinosyns

Spinosyn is a mixture of secondary metabolites produced by aerobic fermentation of the actinomycete species *Saccharopolysora spinosa*. Most of the factors in this mix are insecticidal (Boeck *et al*., 1989; Boeck *et al*., 1994, 1996). In nature, the combined amount of factor A and factor D dominate the other factors in the spinosyns complex by a significant margin, therefore the naturally-occurring spinosyn mix is named spinosAD (Thompson *et al*., 1995; Dripps *et al*., 2008). Within spinosad, factor A is the major factor. Their chemical structures are mainly a polyketide derived tetracyclic

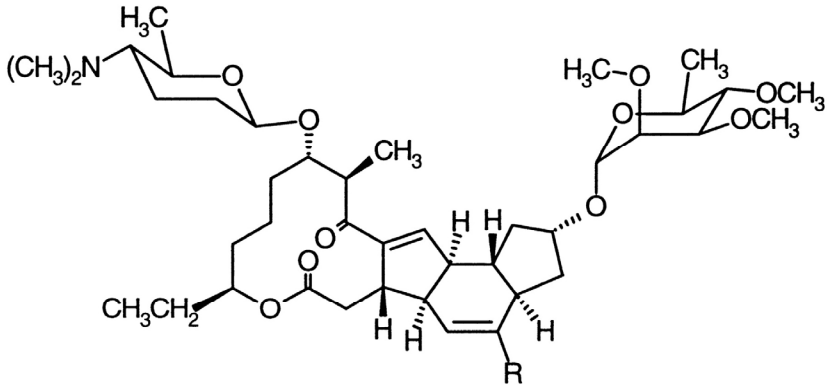

Fig. 2. Structure of spinosad.

macrolide with forosamine on the C17 hydroxyl group and rhamnose on the C9 hydroxyl group (Kirst *et al*., 1992, 1993; Dripps *et al*., 2008). The chemical structure of spinosad can be found in Figure 2.

Semisynthetic Spinosyns

Following the discovery of spinosad, over 400 spinosyns analogs were made by means of biotransformation, genetic engineering on the culture and structural modification on the spinosyns. None of the analogs, however, showed significant superior insecticidal activity to spinosad (Dripps *et al*., 2008). Earlier studies demonstrated that the amino sugars played a critical role in the insecticidal activities of spinosyns. Both the pseudo aglycone (detached one sugar) and the aglycone (detached both sugars) of spinosad were inactive against mosquitoes (Boeck *et al*., 1989; Tanaka and Omura, 1993; Boeck *et al*., 1994, 1996). Attempts at quantitative structure-activity relationship (QSAR) modeling suggested that hydrogenation of the 5, 6 double bond in spinosad would improve photostability, but such QSAR modeling provided no further active leads until an artificial neural network (ANN) software was implemented (Dripps *et al*., 2008). The ANN analysis determined that the 3′-*O*-ethyl group at the rhamnose sugar was the most potent in altering nicotinic function in the insect nervous system, showing improved activity against corn earworm (*Helicoverpa zea*) and beet armyworm (*Spodoptera exigua*) (Sparks *et al*., 1999; Sparks *et al*., 2000; Sparks *et al*., 2008). The combination of a reduced 5, 6 double bond and the 3′-*O*-ethyl group on spinosyn J showed a greater level of activity against corn earworm (*Helicoverpa zea*) and sweetpotato whitefly (*Bemisia tabaci*) than either the 3′-*O*-ethyl modifycation alone or the 5, 6 double bond reduction alone. Further testing confirmed that this combination of synthetic modifycations increased activity and residual control across a wide range of insect pest species. Spinetoram is a mixture of modified spinosyn factor J and spinosyn factor L. The conceptual path that led to spinetoram is outlined in Figure 3 (Dripps *et al*., 2008).

Insecticidal Spectrum and Products

Since its insecticidal effect was ob-

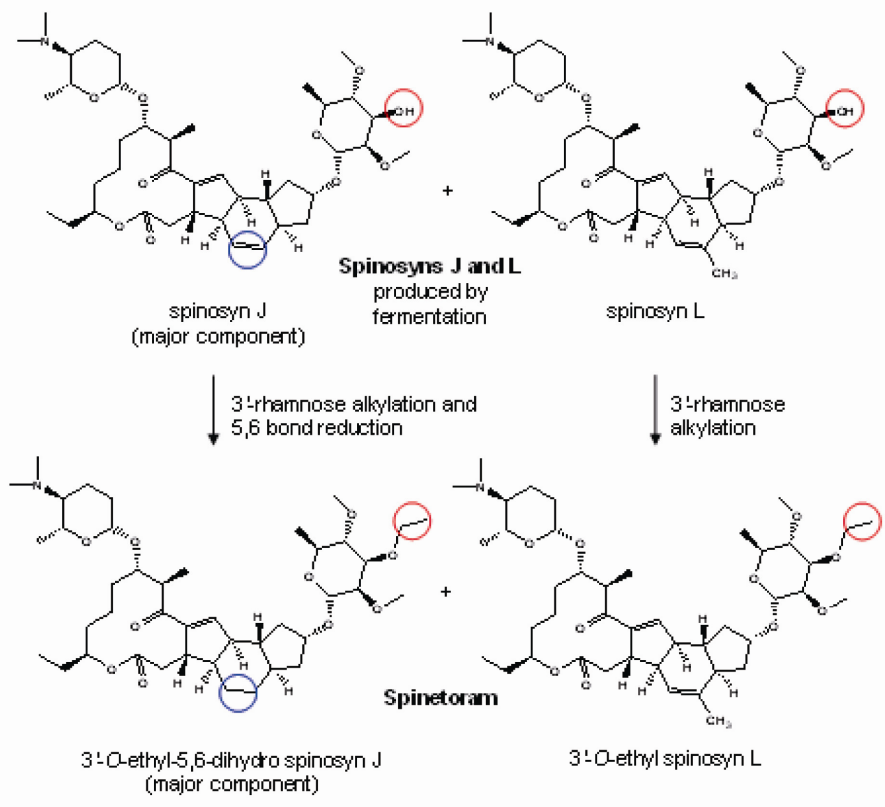

Fig. 3. Construction of spinetoram from spinosyn J and spinosyn L.

served in the laboratory against the mosquito larvae, spinosad has been tested extensively against other insects in laboratories and under field conditions (Thompson *et al*., 1995; DeAmicis *et al*., 1997; Thompson *et al*., 2000; Anonymous, 2009d; Anonymous, 2011b). Spinosyns as a group have a "moderate" spectrum of activity. They are most effective on chewing insects particularly Chrysomelidae, caterpillars and sawfly larvae. Almost all Dipteran larvae such as leaf mining flies, fungus gnats, shore fly and mosquitoes are susceptible to spinosad. It appears to have good activity on thrips, fleas and sporadic activity against mite species. Spinosad has also exhibited good activity against several species of tick in the laboratory and has been investigated as potential insecticide against ticks (*Boophilus sp*.) on cattle (Davey *et al*., 2005). Sucking insects in general are not affected unless very high concentrations are applied (Sparks *et al*., 1999; Salgado and Sparks, 2005). Spinetoram has a similar but broader spectrum of activities than spinosad especially for those pests on fruit trees (Anonymous, 2008b; Anonymous, 2009d). For some undiscovered reasons, spinosad exhibits wider margins of safety to many beneficial insects such as whitefly parasitoid (*Encarsia formosa*), minute pirate bug (*Orius insidiosus*), lady beetle (*Hippodamia convergens*), and lacewing (*Chrysopa rufilabris*) (Schoonover and Larson, 1995). For crop protection, the first registered spinosad product was launched in 1997 in the USA for cotton pests. Since then, over 250 products containing spinosad have been registered in 85 countries (Anonymous,

2011b; Anonymous, 2011c). For crop protection purposes, almost all spinosad products are registered under Indianapolis, Indiana-based Dow AgroSciences. These products include Tracer®, SpinTor® and Success® for Lepidopteran on a wide variety of field crops, Conserve® for turf and ornamental crops pests, Entrust® for pests on organic crops, GF-120® as a bait for fruit fly and Safer® as a bait for fire ants (Stark *et al*., 2004; Anonymous, 2011b; Anonymous, 2011c). The semi-synthetic spinosyn or spinetoram is also marketed by Dow AgroSciences, under brand names Delegate™ WG and Radiant™ SC insecti- cides and in some countries as Exalt™ and Endure™ insecticides. Delegate™ received its first registration in New Zealand in August 2007. Currently, products of spinetoram are registered in the United States, Canada, Mexico, Korea, Malaysia, Pakistan and New Zealand (Anonymous, 2008b; Anonymous, 2009d).

For mosquito larvae control, the Clarke Biotech Company has developed a slow-release formulation for spinosad. This unique product has been registered as Natular™ by Clarke in the USA and abroad since 2009 (Anonymous, 2010a; Anonymous, 2010c).

As mentioned earlier, for animal protection, spinosad products are registered under Elanco. Elanco first marketed Extinosad®, used in the Australian sheep and wool industry in 2001. Extinosad® has 3 different formulations; Eliminator, Aerosol for Wounds and Pour-on. Eliminator is registered for treatment and prevention of blowfly strike and for control of lice (*Bovicola ovis*) on sheep. Aerosol for Wounds is registered for the treatment and prevention of blowfly strike in mulesing, marking and other wounds of sheep. And Pour-On is registered for the control of lice (*Bovicola ovis*) in sheep (Kirst *et al*., 2002; Anonymous, 2009a; Anonymous, 2009b). Another product for animal health is Elector PSP®, which is registered for the control of house flies (*Musca domestica*), stable flies (*Stomoxys calcitrans*) and darkling beetles (*Alphitobius diaperinus*) in and around agricultural animal premises in Australia (Davey *et al*., 2005; Anonymous, 2009a). Elector® was recently approved in the U.S. for control of hornflies *(Haematobia irritans)* and both sucking and chewing lice on cattle (Davey *et al*., 2005; White *et al*., 2007a; White *et al*., 2007b). For animal health, spinosad has also been evaluated as control agents against screwworm and tsetse flies with encouraging results. Registrations for their usage are pending (De Deken *et al*., 2004; Coronado and Kowalski, 2009). In 2007, Lilly launched Comfortis® as a flea control agent for canines through its Elanco Companion Animal Health Division. This spinosad product is being formulated as a chewable tablet. It provides month-long protection against fleas on dogs (Anonymous, 2009b). In early 2011, Elanco launched two more spinosyn products for companion animals. Assurity™ for cats and Trifexis™ for dogs were launched on January 4 and January 17 respectively. Assurity™ contains spinetoram, is a topical flea treatment (Anonymous, 2011f) while Trifexis™ is a combination of spinosad and milbemycin in chewable tablet, for control of fleas and nematodes (Anonymous, 2011e). In 2002, Lilly signed an agreement with SePRO to allow them to develop spinosad as a head lice control agent for humans (Anonymous, 2002b). ParaPRO, a division of SePRO is currently developing this spinosad product named Natroba™ formerly known as Natrova, for the treatment of human head lice (Anonymous, 2002b). Clinical trial data for Natroba™ indicated that it was very effective against even the permethrin- resistant head lice (Mougabure *et al*., 2006). Natroba™ won its first FDA approval for head lice control on January 18, 2011 (Anonymous, 2011a).

Blockbusters

Any pharmaceutical product that sells

over a US$ 1 billion per year can be called a blockbuster product (Anonymous, 2008a). For agrochemicals and products for animal health, an annual sale of US$ 100 million or more is considered a blockbuster. Spinosad has earned its blockbuster status as a crop protection agent, livestock protection agent and a companion animal protection agent. For crop protection purposes, spinosad has been registered in 80 countries to treat over 250 crops. Its annual sale as an agrochemical was estimated to be US$ 170 million back in 2007 (McDougall, 2007). Dow AgroSciences predicts spinetoram will join the blockbuster club in 2011 when Delegate™ and Radiant™ are fully registered (Anonymous, 2009d). For the livestock market, Extinosad®, Elector PSP® and Elector® are major products in Elanco Animal Health division that as a group, had sale reaching US$ 1.207 billion in 2009 (Anonymous, 2009c). Recently, Elanco announced that Comfortis®, a flea control agents for dogs, topped US$100 million in 2010 (Anonymous, 2010b). The most recent launch of Trifexis™ by Elanco has already spurred excitement in the animal health community. Its sale has been expected to be as good as Comfortis® if not better (Anonymous, 2011e). Two other special products that contain spinosad targeting niche markets are expected to be blockbusters as well. They are Natroba™ from ParaPRO and Natular™ from Clarke. The market for head lice treatment in the USA alone has been estimated to be US$ 65 million (Anonymous, 2011d). Natular™, the slow-release formulation of spinosad for mosquito larvae launched in 2009 with fantastic results and is expected to capture the lion's share of the global mosquito larvicide market in the near future (Anonymous, 2010a).

Conclusion

Back in the early 1980s, a goal was set at Eli Lilly and Company to find novel and useful insecticides from fermentation metabolites. With great effort from numerous devoted scientists with diversified expertise coupled with a little bit of luck, spinosad was discovered. Since then several spinosyn products have become blockbusters from this humble discovery. Natural product research used to be one of the most productive ways to discover useful molecules. Unfortunately new molecules are getting more and more difficult to uncover. Therefore many international companies with natural product research programs have been significantly reduced or completely shut down their fermentation research in recent years. The discovery of spinosad suggests that more useful molecules can be found from Mother Nature if smarter dereplications are chosen. In Taiwan, natural product research faces many challenges. The most devastating one happens to be the poor coordination between the academic and the industry. The academic research priority may or may not match with the market need. For instance, spinosyn insecticides are one of the most successful products on the market, there is practically no grant from the ROC government to support research in bio-insecticides derived from the secondary metabolites in fermentation. Hopefully the discovery story of spinosad and its associated financial success can rekindle the enthusiasm in natural product research in Taiwan in the near future.

References

Anonymous. 1913. Webster's Revised Unabridged Dictionary, published 1913 by C. & G. Merriam Co.

Anonymous. 1998. DowElanco website 1998: http://www.thefreelibrary.com/DowElanco+Changes+Name+to+Dow+AgroSciences-a019744148.

Anonymous. 1999. Opinion of the Scientific Steering Committee on Antimicrobial Resistance. Commission of the Euro-

pean Communities DGXXIV Scientific Steering Committee. http://europa.eu.int/comm/food/fs/sc/ssc/out50_en.pdf Last accessed 28 October 2001.

Anonymous. 2000. Centers for Disease Control. 2000. Issues in healthcare settings: laboratory detection of oxacillin/methicillin - resistant *Staphylococcus aureus* (MRSA). http://www.cdc.gov/ncidod/hip/Lab/FactSheet/mrsa.htm Last accessed 28 October 2001.

Anonymous. 2002a. Antibiotic as food animals growth promoter: http://www.fao.org/docrep/article/agrippa/555_en.htm.

Anonymous. 2002b. ParaPro Website: http://www.sepro.com/default.php?page=newsdetail&newsid=12002002

Anonymous. 2003. History of chrysanthemum http://www.mums.org/journal/articles/chrysanthemum_history.htm

Anonymous. 2008a. European Commission, Pharmaceutical Sector Inquiry, Preliminary Report (DG Competition Staff Working Paper), 28 November 2008, page 17.

Anonymous. 2008b. Spinetoram facts: Talking Point Document, June 17, 2008. Dow AgroSciences internal communication.

Anonymous. 2009a. Elanco website: http://www.elanco.com.au/product_range/index.html

Anonymous. 2009b. Elanco website: https://www.elancopet.com.au/products/comfortis.cfm

Anonymous. 2009c. Eli Lilly and Company annual 2009 report: http://newsroom.lilly.com/releasedetail.cfm?releaseid=441003

Anonymous. 2009d. Spinetoram facts sheet: http://www.epa.gov/opprd001/factsheets/spinetoram.pdf

Anonymous. 2010a. Clarke's Natular: http://www.clarke.com/index.php?option=com_content&view=category&layout=blog&id=64&Itemid=146

Anonymous. 2010b. Lilly News on December 17, 2010. Elanco's Comfortis is now a blockbuster. Eli Lilly internal communication.

Anonymous. 2010c. Presidential Green Chemistry Challenge Award Winner, 2010. http://www.epa.gov/gcc/

Anonymous. 2011a. BusinessWire. January 18, 2011. ParaPRO announces FDA approval of Natroba™ for treatment of head lice.

Anonymous. 2011b. Dow AgroSciences products: http://www.dowagro.com/prod/index.htm

Anonymous. 2011c. Dow AgroSciences website: http://www.dowagro.com/turf/prod/spinosad.htm

Anonymous. 2011d. Indianapolis Star. January 19, 2011. Dreaded lice may be easier to kick now.

Anonymous. 2011e. PRNewswire. January 17, 2011. Elanco launches broad-spectrum canine parasiticide.

Anonymous. 2011f. Veterinary Practice News. January 1, 2011. Elanco launches flea control product just for cats.

Baltz RH. 2005. Antibiotic discovery from actinomycetes: will a renaissance follow the decline and fall? SIM News 55: 186-196.

Boeck LD, Chio H, Eaton TN, Godfrey OW, Michel KH, Nakatsukasa WN, Yao R. 1989. A83543 Compounds. Lilly Docket # X-7800, January 30, 1989. Indianapolis, Indiana.

Boeck LD, Chio H, Eaton TN, Godfrey OW, Michel KH, Nakatsukasa WN, Yao R. 1994. Process for producing A83543 compounds. US pat. 5362634.

Boeck LD, Chio H, Eaton TN, Godfrey OW, Michel KH, Nakatsukasa WN, Yao, R. 1996. Insecticide and miticide A83543 compounds and their method of producing by fermentation. US Pat. 5496931.

Broadhurst MD. 1998. The influence of the molecular basis of resistance on insecticide discovery. Philosophical transactions: Biol Sci 353: 1723-1728.

Carson R. 2002 [1st. Pub. Houghton

Mifflin, 1962]. *Silent Spring*. Mariner Books. ISBN 0-618-24906-0.

Chio EH. 2007a. A quick insecticide bioassay with mosquitoes. Formosan Entomol. 27: 183-188.

Chio EH. 2007b. Insecticides from fermentation secondary metabolites. Formosan Entomol. 27: 97-106.

Christophers RS. 1961. Book reviews: *Aedes aegypti* (L.), the Yellow Fever Mosquito. Its Life History, Bionomics, and Structure. Science. 133 (3463): 1473-1474

Coronado A, Kowalski A. 2009. Current status of the new world screwworm *Cochliomyia hominivorax* in Venezuela. Med. Vet. Entomol. 23 (Suppl. 1): 106-110.

Das I, Fraise A, Wise R. 1997. Are glycopeptide-resistant enterococci in animals a threat to human beings? The Lancet 9057: 997.

Davey RB, Miller JA, George JE, Snyder DE. 2005. Effect of repeated spinosad treatments on cattle against *Boophilus annulatus* under south Texas field conditions. Southwestern Entomol 30: 245-255.

DeAmicis CV, Dripps JE, Hatton CJ, Karr LL. 1997. Physical and biological properties of the spinosyns: novel macrolide pest-control agents from fermentation. In: Hedin PA, Hollingworth RM, Maser EP, Miyamoto J, Thompson GD (eds). Phytochemicals for pest control. Am. Chem. Soc. Symp. Series 658 144-154 (American Chemical Society, Washington DC).

De Deken R, Speybroeck N, Gillain G, Sigue H, Batawi K, van den Bossche P. 2004. The macrocyclic lactone "spinosad," a promising insecticide for tsetse fly control. J Med Entomol 41: 814-818.

Demain AL, Sanchez S. 2009. Microbial drug discovery: 80 years of progress. J. Antibiot. 62, 5-16.

Dripps J, Olson B, Sparks T, Crouse G. 2008. Spinetoram: how artificial intelligence combined natural fermentation with synthetic chemistry to produce a new Spinosyn insecticide. Plant Management Network. Published 22 August 2008.

Dutcher JD. 2007. A review of resurgence and replacement causing pest outbreaks in IPM. pp 27-43. In: General Concepts in Integrated Pest and Disease Management. Section 1. Springer Science+ Business.

Harvey AL. 2007. Natural products as a screening resource. Curr Opin Chem Biol 11: 480-484.

Kirst HA, Creemer LC, Naylor SA, Pugh PT, Snyder DE, Winkle JR, Lowe LB, Rothwell JT, Sparks TC, Worden TV. 2002. Evaluation and development of spinosyns to control ectoparasites on cattle and sheep. Curr Topics Med Chem 2: 675-699.

Kirst HA, Michel KH, Mynderse JS, Chio EH, Yao RC, Nakatsukasa WM, Boeck LD, Occlowitz J, Paschel JW, Deeter JB, and Thompson GD. 1992. Discovery, isolation and structure elucidation of a family of structurally unique fermentation-derived tetracyclic macrolides. pp. 214-225. In: Baker D R, Fenyes JG, Steffens JJ (eds). Synthesis and Chemistry of Agrochemicals III. Am Chem Soc, Washington, DC.

Kirst HA, Michel KH, Mynderse JS, Chio EH, Yao RC, Nakatsukasa WM, Boeck L, Occolowitz JL, Paschal JW, Deeter JB, Thompson GD. 1993. Discovery and Identification of a novel fermentation derived insecticide. pp 109-116. In: Brown WC (ed). Development in Industrial Microbiology Series: Microbial Metabolites, vol. 32. Society for Industrial Microbiology, Washington, DC.

McDougall P. 2007. AgriService, Product section-2006 Market, November 2007.

Mertz FP, Yao RC. 1990. *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in sugar mill rum still. Int J

System Bacteriol 40: 34-39.

Mougabure Cueto G, Zerba EN, Picollo MI. 2006. Permethrin-resistant head lice (Anoplura: Pediculidae) in Argentina are susceptible to spinosad. J Med Entomol 43: 634-635.

Pelaez F. 2006. The historical delivery of antibiotics from microbial natural products—can history repeat? Biochem Pharmacol 71: 981-990.

Salgado VL, Sparks TC. 2005. The spinosyns: chemistry, biochemistry, mode of action, and resistance. pp 137-173. In: Gilbert LJ, Iatrou K, Gill SS (eds). Comprehensive Molecular Insect Science, vol. 6. Elsevier, Oxford, UK.

Schoonover JR, Larson LL. 1995. Laboratory activity of spinosad on non-target beneficial arthropods, 1994. Arthropod Management Tests 20: 357.

Sparks TC, Anzeveno PB, Martynow JG, Gifford JM, Hertlein MB, Worden TV, Kirst HA. 2000. The application of artificial neural networks to the identification of new spinosoids with improved biological activity toward larvae of *Heliothis virescens*. Pestic Biochem Physiol 67: 187-197.

Sparks TC, Crouse GD, Dripps JE, Anzeveno P, Martynow J, DeAmicis CV, Gifford J. 2008. Neural network-based QSAR and insecticide discovery: Spinetoram. J Comput.-Aided Mol Des 22: 393-401.

Sparks TC, Thompson GD, Kirst HA, Hertlein MB, Mynderse JS, Turner JR, Worden TV. 1999. Fermentation-derived insect control agents-the spinosyns. pp 171-188. In: Hall FR, Menn JJ (eds). Methods in Biotechnology, vol. 5, Biopesticides: Use and Delivery. Humana, Totowa, NJ.

Stark JD, Vargas R, Miller N. 2004. Toxicity of Spinosad in protein bait to three economically important Tephritid fruit fly species (Diptera: Tephritidae) and their parasitoids (Hymenoptera: Braconidae). J Econ Entomol 97: 911-195.

Stewart M, Capon RJ, Lacey E, Tennant S, Gill JH. 2005. Calbistrin E and Two Other New Metabolites from an Australian isolate of *Penicillium striatisporum,* J Nat Prod 68: 581-584.

Tanaka H, Omura S. 1993. Microbial products affecting receptor. Annu Rev Microbial 47: 57-87.

Thompson GD, Busacca JD, Jantz OK, Kirst HA, Larson LL, Sparks TC. 1995. Spinosyns: an overview of new natural insect management systems. Proc. Beltwide Cotton Production Conference 1039-43, San Antonio, TX.

Thompson GD, Button R, Sparks TS. 2000. Spinosad: a case study: an example from a natural products discovery programme. Pest Manag Sci 56: 696-702.

Thorp BH, Cargill PW. 1999. EU Ban on four antibiotic feed additives. Veterinary Record 144: 27-28.

VonNussbaum F, Brands M, Hinzen B, Weigand S, Habich D. 2006 Antibacterial natural products in medicinal chemistry exodus or revival? Angew Chem Int Ed 45: 5072-5129.

White WH, Hutchens DE, Jones C, Firkins LD, Paul AJ, Smith LL, Snyder DE. 2007a. Therapeutic and persistent efficacy of spinosad applied as a pour-on or a topical spray against natural infestations of chewing and sucking lice on cattle. Vet Parasitol 143: 329-336.

White WH, McCoy CM, Meyer JA, Winkle JR, Plummer PR, Kemper CJ, Starkey R, Snyder DE. 2007b. Knockdown and mortality comparisons among spinosad-, imidacloprid-, and methomyl-containing baits against susceptible *Musca domestica* (Diptera: Muscidae) under laboratory conditions. J Econ Entomol 100: 155-163.

Received: February 28, 2011
Accepted: April 6, 2011

簡介賜諾司類殺蟲劑 (Spinosyn Insecticides) (一)：來自奇特發現的明星產物

招衡*

訪問學者，國立臺灣大學昆蟲學系

摘　要

Spinosyn 為一種好氧性放射菌 (*Saccharopolyspora spinosa*) 的天然代謝物質，在殺蟲劑的應用上，因具有高度選擇性、低哺乳動物毒性、低環境毒性與特殊的作用機制，而富有高度商業價值並在全世界被廣泛使用。Spinosyn 之化學結構為多烯酮所衍生之四環大環內酯，且具有兩個胺基醣取代基，其開發過程堪稱天然產物開發的典範，透過孑孓實驗，可大量快速篩檢出待測物的毒性效果，而在篩選過程中，最重要的觀念為去除重複 (dereplication)。Spinosyn 為一類化學物質之統稱，在商品化後，賜諾殺 (Spinosad) 含 Spinosyn A 與 D 最先在 1997 年推出，應用於棉花之蟲害防治上，自此之後，大量的產品被推出，應用層面除了植物保護外，也可用於食用動物與伴侶動物，甚至是頭蝨的防治，足見其高度選擇性之優點。近來在化學結構上的研究發現，置換取代基之 Spinosyn L 與 J，合稱為 Spinetoram (賜諾特)，具有更廣泛之殺蟲效力，同樣深具開發潛力。

關鍵詞：賜諾司類殺蟲劑、天然產物研究、去重複、*Saccharopolyspora spinosa*。

*論文聯繫人
Corresponding email: EHC13029@Gmail.com