Pure & Appl. Chem., Vol. 62, No. 7, pp. 1231–1240, 1990.
Printed in Great Britain.

Recent advances in avermectin research

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Abstract - In 1976 scientists at Merck & Co. Inc. discovered a complex of eight closely related natural products, subsequently named avermectins, in a culture of Streptomyces avermitilis MA-4680 (NRRL8165) originating from an isolate by the Kitasato Institute from a soil sample collected at Kawana, Ito City, Shizuoka Prefecture, Japan. They are among the most potent anthelmintic, insecticidal and acaricidal compounds known.

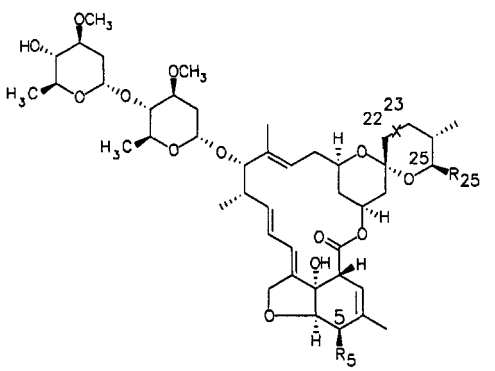
The avermectins are closely related to another group of pesticidal natural products, the milbemycins, the first examples described by Japanese workers, but later found to be more abundant in nature than the avermectins. Both the avermectins and milbemycins are sixteen-membered lactones, with a spiroketal system containing two six-membered rings. The principal difference is that the avermectins have an α-L-oleandrosyl-α-L-oleandrosyl disaccharide attached at the 13-position whereas the milbemycins have no 13-substitutent.

Two avermectins have been commercialized to date. Selective reduction of the 22,23-olefin of avermectin B_1 yields the 22,23-dihydro derivative assigned the non-proprietary name ivermectin. Ivermectin is widely used as an antiparasitic drug in animals and in man. Avermectin B_1 is the most effective of the avermectin family of natural products against agriculturally important insects and mites. It has been commercialized for agricultural use under the non-proprietary name abamectin.

Recent progress in the chemistry of the avermectins has focused on improved insecticidal activity and photostability.

INTRODUCTION

In 1976 scientists at Merck & Co. Inc. discovered a complex of eight closely related natural products, subsequently named avermectins, in a culture of Streptomyces avermitilis MA-4680 (NRRL8165) originating from an isolate by the Kitasato Institute from a soil sample collected at Kawana, Ito City, Shizuoka Prefecture, Japan. Their structures are shown in Fig. 1 (ref.1). They are among the most potent anthelmintic, insecticidal and acaricidal compounds known.



AVERMECTIN A : $R_5 = OCH_3$ B : $R_5 = OH$

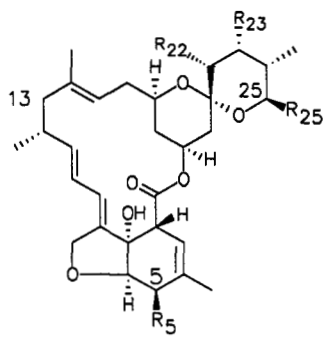
1 : X = −CH=CH− 2 : X = $-CH_2-\overset{\overset{OH}{|}}{C}H-$

a : R_{25} =  b : R_{25} = 

IVERMECTIN : $R_5 = OH$ X = $-CH_2-CH_2-$ R_{25} = 

Fig. 1. Avermectin Structures

The avermectins are closely related to another group of pesticidal natural products, the milbemycins, the first examples described by Japanese workers, but later found to be more abundant in nature than the avermectins (ref. 2-6). Both the avermectins and milbemycins are sixteen-membered lactones, with a spiroketal system containing two six-membered rings. The principal difference is that the avermectins have an α-L-oleandrosyl-α-L-oleandrosyl disaccharide attached at the 13-position whereas the milbemycins have no 13-substitutent. Milbemycin structures are shown in Fig. 2.



MILBEMYCIN ALFA SERIES

R_5 = OH , OCH_3

R_{22} = H , OH

R_{23} = H , $OCOCH(CH_3)(CH_2)_3CH_3$

R_{25} = CH_3 , C_2H_5

ANTHELMINTIC F-28249

ANTIBIOTICS S 541

R_5 = OH , OCH_3

R_{22} = H R_{23} = OH

R_{25} =

MILBEMYCIN ALFA$_1$ (A$_3$)	R_5 = OH	R_{22} = R_{23} = H	R_{25} = CH_3
MILBEMYCIN ALFA$_3$ (A$_4$)	R_5 = OH	R_{22} = R_{23} = H	R_{25} = CH_2CH_3
MILBEMYCIN D	R_5 = OH	R_{22} = R_{23} = H	R_{25} = $CH(CH_3)_2$
ANTHELMINTIC F-28249-ALPHA	R_5 = OH	R_{22} = R_{23} = H	R_{25} = $C(CH_3)CHCH(CH_3)_2$

Fig. 2. Milbemycin Structures

Two avermectins have been commercialized to date. Selective reduction of the 22,23-olefin of avermectin B_1 yields the 22,23-dihydro derivative assigned the non-proprietary name ivermectin Fig. 3. Although this structure, for the sake of simplicity, depicts the 25-secbutyl derivative it should be noted that both commercial products contain up to 20% of the 25-isopropyl analog.

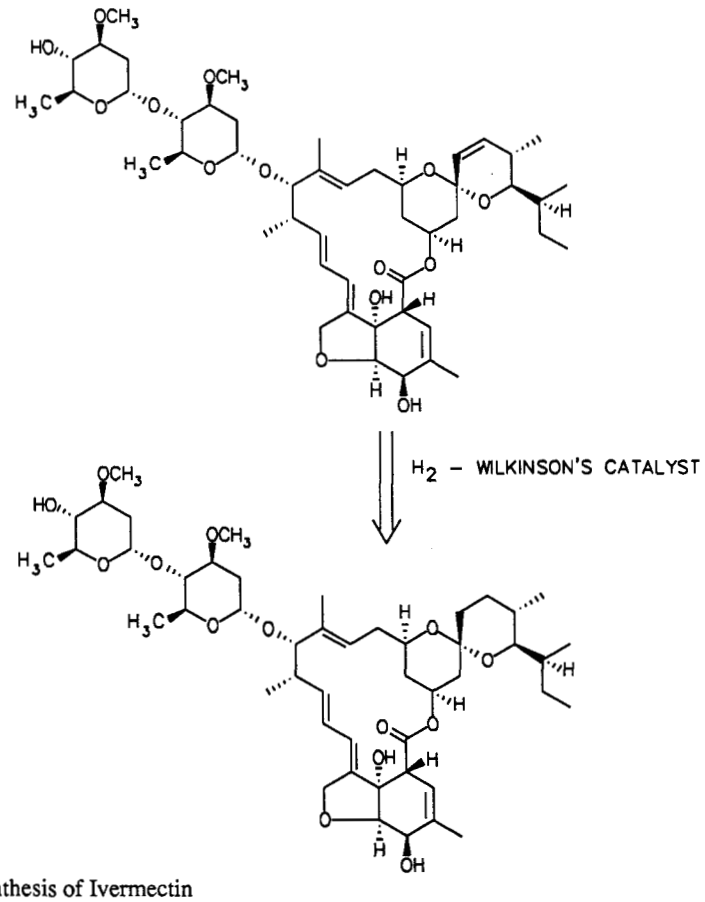


Fig. 3. Synthesis of Ivermectin

A summary of the biological properties of ivermectin is shown in TABLE 1.

TABLE 1. Ivermectin

USED IN CATTLE AT	**0.2 MG/KG**
SHEEP	0.2
SWINE	0.3
HORSES	0.2
DOGS	0.006
MAN	0.05 to 0.2

EFFECTIVE AGAINST PARASITIC NEMATODES
GRUBS
LICE
MITES
TICKS
BOTS

NOT ACTIVE AGAINST TAPEWORMS
FLATWORMS
BACTERIA
FUNGI

The activity of ivermectin against the filarial parasite Dirofilaria immitis in dogs (Table 2) suggested a possible role for the control of filarial parasites of humans. It has been extensiviely tested in human onchocerciasis and is now considered to be the drug of choice. In a single yearly dose it suppresses microfilariae in the skin and eyes and in most cases prevents the progession of the disease to blindness. TABLES 3 and 4 show the results of a 30 patient study recorded over 1 year.

TABLE 2. Efficacy of Ivermectin on developing larvae of Dirofilaria immitis in experimentally infected dogs.

Dose µg/kg	Treatment Date Days	Number of Dogs	% Efficacy
0.3	30	7	0
1.0	30	7	53.2
2.0	30	7	97.2
2.0	45	7	63.8
3.3	30	7	98.1
0		7	0

A.J. Paul, K.S. Todd, J.P. Sundberg, J.A. Dipietro, J.W. McCall, Am. J. Vet., 47, 883 (1986)

TABLE 3. Double-Blind Study of Ivermectin and Diethylcarbamazine in patients with Onchocerca volvulus infections.

	Skin Density of Microfilariae		
Study Day	Placebo	Ivermectin	Dec
-1	99.4	130.4	100.3
2	108.2	38.8	27.0
4	99.7	14.1	14.4
8	105.1	6.6	4.1
14	125.9	2.2	6.8
28	102.6	0.6	9.2
90	84.5	1.0	18.0
180	65.3	2.9	21.8
270	80.8	5.0	27.5
360	93.0	11.8	45.1

10 Patients received a single oral dose of ivermectin 12 mg
10 Patients received DEC daily for 8 days - Total dose 1.3 g
10 Patients received Placebo

M. Lariviere, M. Aziz, D. Weimann, J. Ginoux, P. Gaxotte, P. Vingtain, B. Beauvais, F. Derouin, H. Schulz-Key, D. Basset, C. Sarfati, Lancet, 2, 174 (1985)

TABLE 4. Double-Blind Study of Ivermectin and Diethylcarbamazine in patients with Onchocerca Volvulus Infections

Patients with Punctate Keratitis

Study Day

	-1	4	14	28	90	180	270	360
PLACEBO	5	5	4	4	6	8	8	7
DEC	7	6	5	6	5	4	4	4
IVERMECTIN	7	5	5	5	2	1	3	2

Patients with Onchocerca Volvulus Microfilariae In The Anterior Chamber

Study Day

	-1	4	14	28	90	180	270	360
PLACEBO	9	8	9	5	7	7	7	6
DEC	5	2	4	2	4	6	5	4
IVERMECTIN	8	9	7	7	5	1	1	2

10 patients received a single oral dose of Ivermectin 12 mg
10 patients received DEC daily for 8 days - total dose 1.3 g
10 patients received Placebo

M. Lariviere, M. Aziz, D. Weimann, J. Ginoux, P. Gaxotte, P. Vingtain, B. Beauvais, F. Derouin, H. Schulz-Key, D. Basset, C. Sarfati, Lancet, 2, 174 (1985)

Both forms of lymphatic filariasis are found in India. The Bancroftian form is the commonest and accounts for more than 90% of the disease whereas Brugian filariasis accounts for the rest. In a study carried out in India (ref. 6) in 40 patients with Wuchereria bancrofi filariasis treated with single oral doses, all of the dose levels chosen (25, 50, 100, 200 µg/Kg) were efficacaous in clearing microflarial from the blood of all patients treated. After 3 months some microfilaria recurred in the blood of most patients (Table 5). Further studies are planned and underway using different doses and regimens. Nevertheless ivermectin appears to hold promise as a new treatment for lymphatic filariasis.

TABLE 5. Ivermectin in the Treatment of Wuchereria Bancrofti Filariasis

Efficacy

Geometric Mean Microfilariae/mL

Single Oral Dose	DAY						
	0	1.5	5	12	30	90	180
25 µg/kg	761	2.9	< 1	< 1	5.2	42.9	98
50 µg/kg	1154	3.3	< 1	< 1	3.5	103.6	92.3
100 µg/kg	610	3.0	< 1	< 1	< 1	19.9	95.9
200 µg/kg	478	< 1	< 1	< 1	1.5	43.7	70.8

V. Kumaraswami, E.A. Ottesen, V. Vijayassekaran, S. Uman-Devi, M. Swaminathan, M.A. Aziz, G.R. Sarma, R. Prabhakar and S.P. Tripathy, JAMA, 259, 3150 (1988).

Avermectin B_1 is the most effective of the avermectin family of natural products against agriculturally important insects and mites. It has been commercialized for agricultural use under the non-proprietary name abamectin. A summary its biological activity is shown in TABLE 6.

TABLE 6. Activity of Avermectin B1 Against Mites and Insects

Mite Species (Contact effect against adult mites)	LC_{90} (ppm)
Phyllocoptruta oleivora (citrus rust mite)	0.02
Tetranychus urticae (two-spotted spider mite)	0.03
Tetranychus turkestani (strawberry mite)	0.08
Panonychus ulmi (European red mite)	0.04
Panonychus citri (citrus red mite)	0.24
Polyphagotarsonemus latus (broad mite)	0.03
Insect Species (Foliar Residue Bioassay)	**LC_{90} (ppm)**
Leptinotarsa decemlineata (Colorado potato beetle)	0.03
Manduca secta (tomato hornworm)	0.02
Epilachna varivestes (Mexican bean beetle)	0.20
Acyrthosiphon pisum (pea aphid)	0.40
Trichoplusia ni (cabbage looper)	1.0
Heliothis zea (corn earworm)	1.5
Spodoptera eridania (southern armyworm)	6.0

R. A. Dybas, A. St. J. Green (1984) Avermectins: Their Chemistry and Pesticidal Activity. Proceedings, 1984 British Crop Protection Conference-Pests and Diseases, Brighton, England, 31, 947-954.

Avermectin B_1, in thin films, is rapidly degraded on exposure to air and to ultraviolet light. In fact its utility against certain crops is limited by this rapid degradation. Fig. 4 shows the degradation of avermectin B_1 as a thin film on a glass petri dish cover held in the dark or exposed to a Kratos model LH 153 Solar Simulator. In the dark the degradative, processes are probably oxidative.

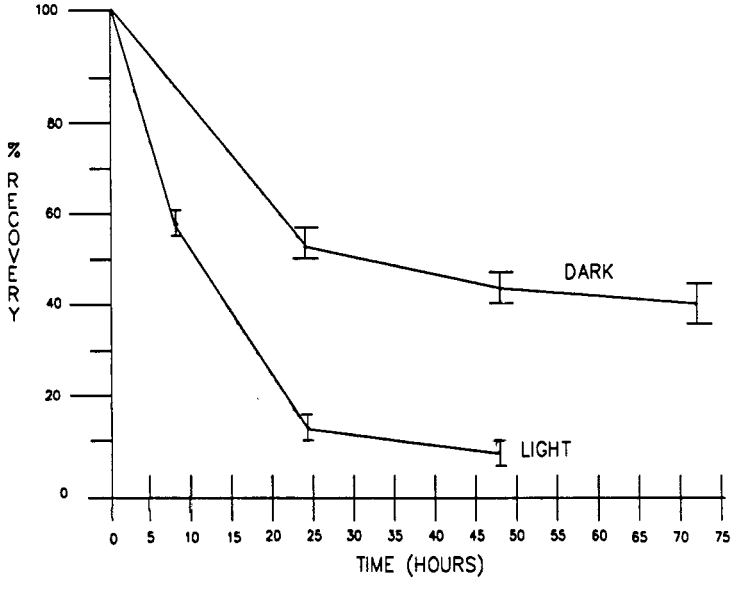


Fig. 4. Photodecomposition of Avermectin B_1

It has been shown for example, in our laboratories, that the 8a position is readily converted into a hydroperoxide. This reactivity is not unreasonable since the 8a position it both allylic and adjacent to an oxygen. When avermectin B_1 is dissolved in methanol or cyclohexane in a quartz tube and exposed to 300 nM ultraviolet light an equilibrium to the 8,9-Z and 10,11-Z isomers is achieved in 30 - 60 minutes Fig. 5 and complete loss of 254 nM absorption occurs in less than 24 hours. Mass spectral analysis of the products indicated up to four additional oxygen atoms.Since the early events in photodecomposition are related to ultraviolet absorption at the diene portion of the molecule it was decided to undertake chemistry at that site to

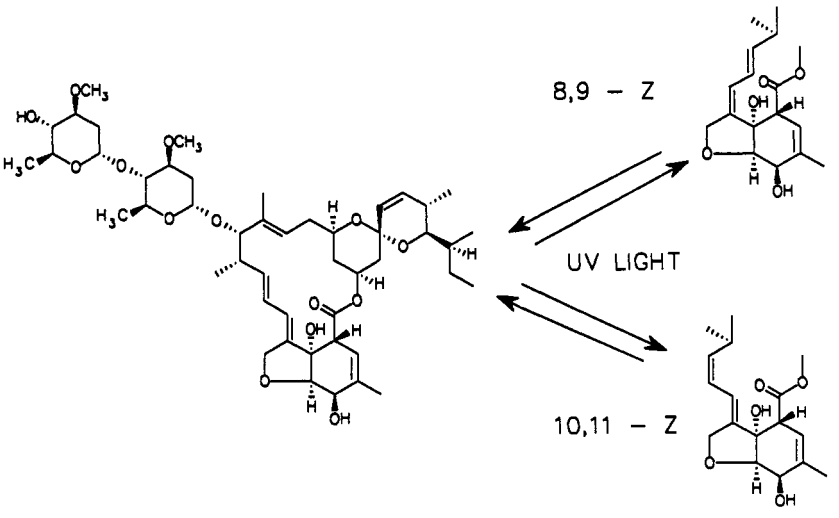


Fig. 5. Photoisomerization of Avermectin B_1

Hydrogenation of avermectin B_1 with hydrogen and a palladium catalyst gave 10,11,22,23-dihydro avermectin B_1 as shown in Fig. 6. Direct hydrogenation was studied with many catalysts and in no case could reduction of the diene be accomplished without prior or concomitant reduction at the 22,23-olefin.

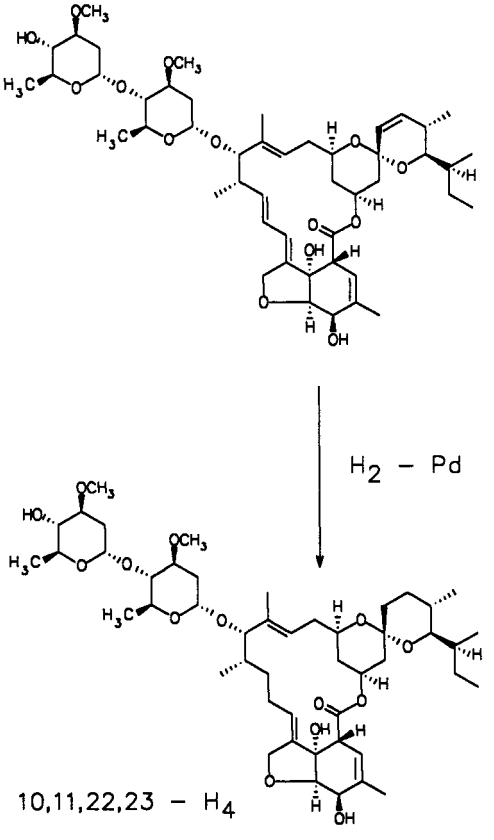


Fig. 6. Hydrogenation of Avermectin B_1

Selective reduction at the 10,11 position of the diene was accomplished by an indirect method shown in Fig. 7

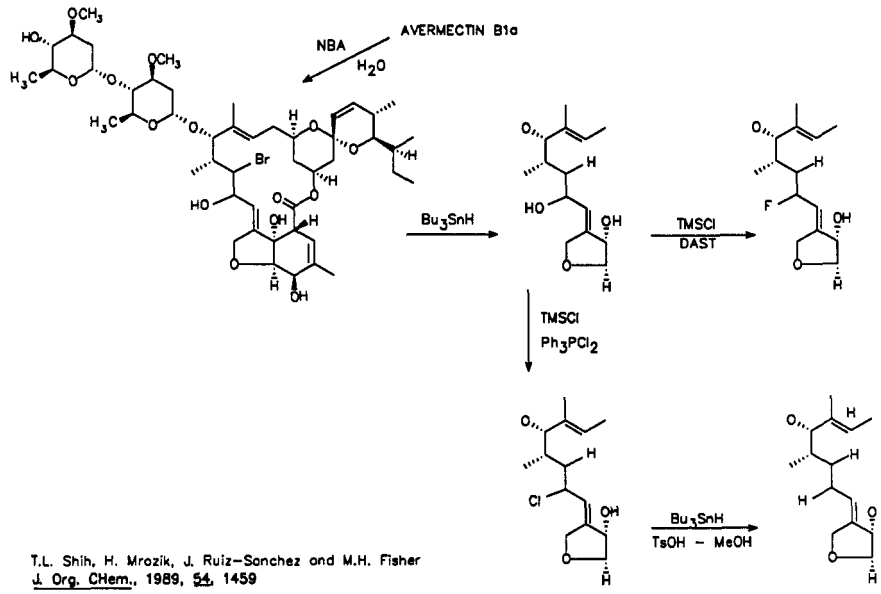


Fig. 7. Addition of N-Bromoacetamide to the Avermectin B1a - Diene

Reaction of avermectin B_1 with N-bromoacetamide afforded a 10,11-bromohydrin which was reduced with tributyltin hydride to a 10-hydroxy derivative. This alcohol was protected at the 5-position and converted into the 10-chloro and 10-fluoro analogs. Reduction of the 10-chloro derivative with tributyltin hydride and deprotection gave the desired 10,11-dihydro avermectin B_1.

Epoxidation of avermectin B_1 with MCPBA gave predominantly the 8,9-oxide as shown in Fig. 8, together with a small amount fo the 3,4-oxide. Presumably both reactions are assisted by the 7-α-hydroxy group.

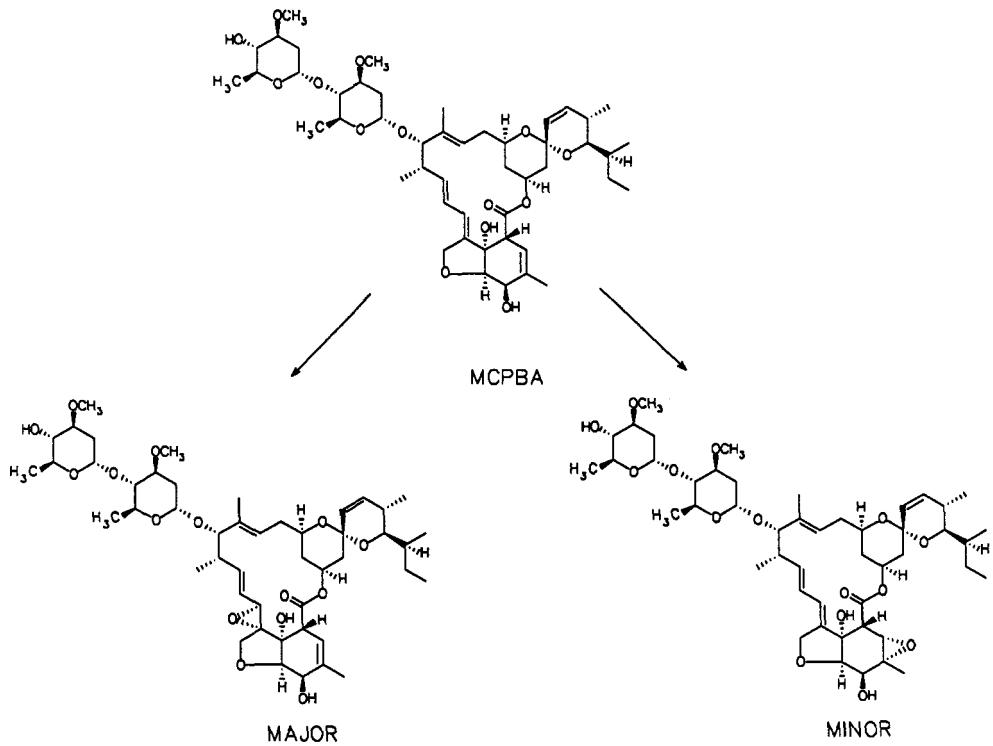


Fig. 8. Epoxidation of Avermectin B1a.

Reaction of 5-0-TBDMS ivermectin with a zinc/copper couple and methylene iodide in ether gave a mixture of three compounds shown in Fig. 9. Two monocyclopropanes were isolated in which reaction occurred at the α-face. Interestingly when the reaction was carried out with the unblocked 5-alcohol, the same products were formed. Presumably the stereochemistry is entirely controlled by the 7-hydroxy group as was expoxidation.

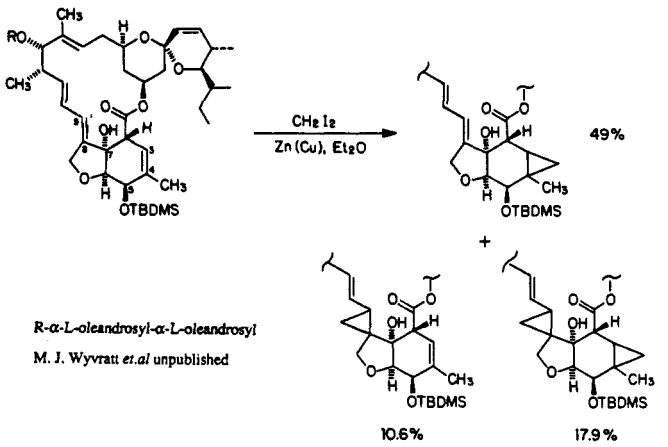


Fig. 9. Cyclopropanation Reactions

The miticidal activity of these derivatives is shown in TABLE 7.

Several of the reduced diene derivatives were found to be highly active. Interestingly avermectin B_1-8,9-oxide was highly active whereas the analogous cyclopropane was virtually inactive. Photodecomposition studies in a photoreactor (Table 8) and as thin films on petri dishes Fig. 10 showed both the 8,9-oxide and the 8,9-cyclopropane to be considerably more stable than the parent.

TABLE 7. Contact Activity of Avermectin Derivatives against Two-Spotted Spider Mite Adult Females.

Compound	Percent mortality at 96 hours 0.05 ppm
Avermectin (AVM B_1)	100
AVM B_1 8,9-oxide	100
AVM B_1 8,9-cyclopropane	15
AVM B_1 3,4-cyclopropane	20
10,11-dihydro AVM B_1	100
22,23-dihydro AVM B_1 (Ivermectin)	92
10,11,22,23-tetrahydro AVM B_1	100
3,4,10,11,22,23-hexahydro AVM B_1	11
3,4,8,9,10,11,22,23-octahydro AVM B_1	18
10-fluoro-10,11-dihydro AVM B_1	100
10-hydroxy-10,11-dihydro AVM B_1	72
Milbemycin (25-sec-butyl) 8,9-oxide	20

TABLE 8. Photo Decomposition Studies in Solution

300 NM UV LIGHT	
QUARTZ TUBES	
MeOH or CYCLOHEXANE SOLUTIONS	
	ISOMERIZATION EQUILIBRIUM
	30 - 60 MINUTES
AVM - B_1	
	COMPLETE LOSS OF 254 NM UV
	ABSORPTION IN LESS THAN 24 HOURS
8,9-METHYLENE-B_1	50% LEFT AFTER 24 HOURS
B_1-8,9-OXIDE	30 TO 50% LEFT AFTER 24 HOURS

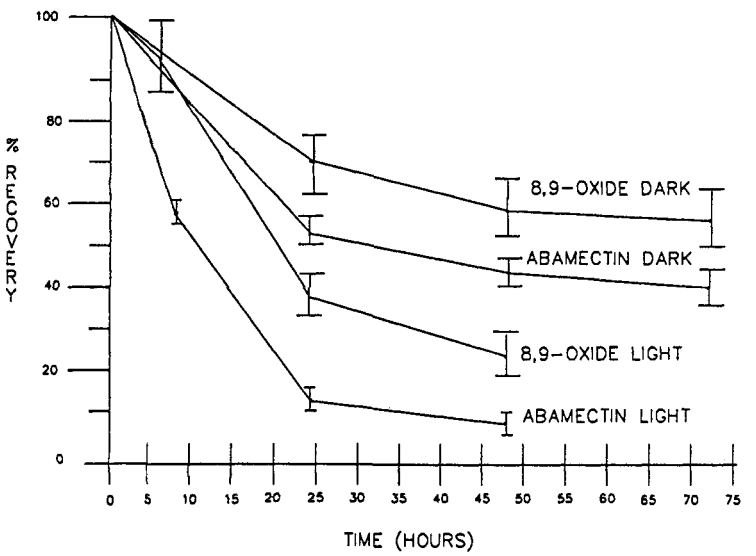


Fig. 10. Comparative Photodecomposition of Avermectin B1a & it's 8,9-Oxide as Thin Films.

TABLE 9. Foliar Residual Activity of Avermectin Derivatives against Two-Spotted Spider Mites Adult Females

Compound	Percent mortality at 0.1 ppm 0 DAT[a]	15 DAT[a]
Avermectin B_1 (AVM B_1)	96.2	16.9
AVM B_1 8,9-oxide	99.5	70.7
10,11-dihydro AVM B_1	98.0	67.0
10,11-22,23-tetrahydro AVM B_1	95.1	< 5
10-fluoro-10,11-dihydro AVM B_1	92.3	60.2

[a] 0 DAT and 15 DAT = 0 and 15 days after treatment spider mites placed onto foliage; mortality counts made 96 hours after infestation

TABLE 10. Activity of Avermectin Derivatives against Southern Armyworm Neonates on Sieva Beans

Compound	EC_{90} PPM
Avermectin B1	8.0
Ivermectin	8.0
Avermectin B1 Monosaccharide	8.0
Ivermectin Monosaccharide	0.5
Ivermectin Aglycone	> 0.5
13-Deoxy IVM Aglycone	0.5
13-β-Cl-13-deoxy IVM Aglycone	0.5
13-β-F-13-deoxy IVM Aglycone	0.5
13=NOCH3-13-deoxy IVM Aglycone	0.5

The most active derivatives were also for foliar residual activity against two-spotted spider mites. Three derivatives, avermectin B_1-8,9-oxide, 10-11-dihydroavermectin B_1 and 10-fluoro-10,11-dihydroavermectin B_1 showed much improved residual activity when compared to avermectin B_1, (Table 9).

Avermectin B_1-8,9-oxide has been selected for further study partially because it appeared to be the most effective compound but also because of its ease of synthesis.

Inspection of biodata shown in TABLE 6 indicates that whereas avermectin B_1 is extremely effective against a variety of mites, it is much less effective against insects, especially the cabbage looper, the corn earworm and the southern armyworm. The level of activity against these species is insufficient to justify commercial development for these uses. Thus, an extensive program of synthetic chemistry and biological testing was initiated in an attempt to find avermectin derivatives with improved insecticidal activity. The southern armyworm was selected as the target species.

Early in the program it was discovered that a variety of monosaccharide and aglycone derivatives showed a sixteen-fold improvement in activity against the southern armyworm compared to avermectin B_1 (Table 10). Interestingly the 22,23-dihydro analogs were more effective than their unsaturated counterparts. However, although a wide variety of monosaccharides and aglycones were synthesized and tested the EC_{90} could not be improved over 0.5 ppm.

An important breakthrough came with the discovery of 4"-aminoavermectins. It was reasoned that since many macrolides contain amino sugars it could be interesting to devise a synthesis of avermectins also containing amino sugars. The synthetic scheme is shown in Fig. 11 (ref. 7). Avermectin B1 was protected at the 5-position as a TBDMS derivative and then oxidized under Swern oxidation conditions to provide the 4"-keto derivative. Reductive amination with ammonium acetate and sodium borohydride, followed by deprotection, gave the axial epiamino derivative as the major product, a smaller amount of the equatorial amino analog and smaller amount of 4"-epiavermectin B_1. N-alkylated derivatives were synthesized either by reductive amination using alkylamines or by alkylation of 4"-amino-4"-deoxyavermectins.

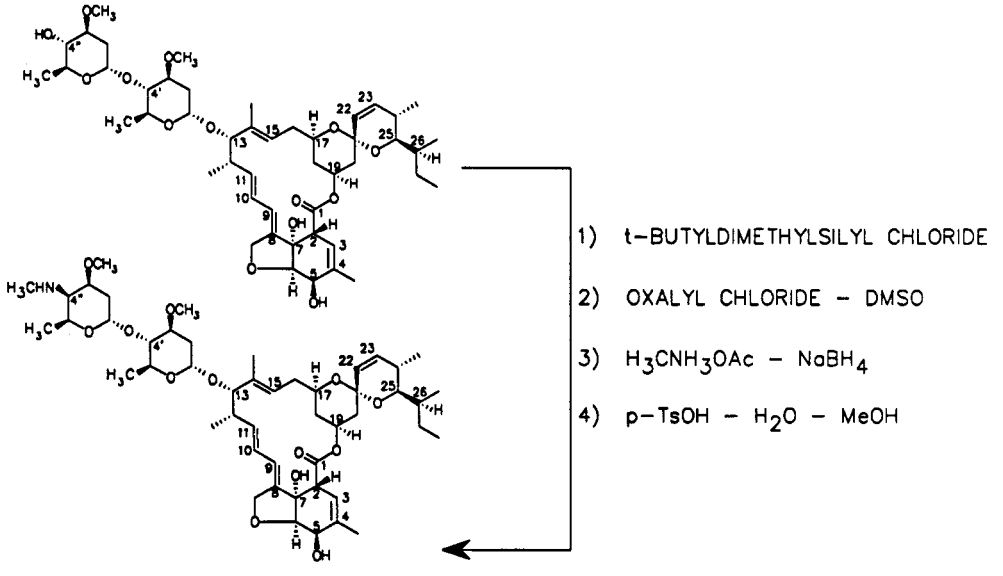


Fig. 11. Synthesis of 4"-Epiaminoavermecti.

The two epimeric 4"-amino-4"-deoxyavermectin B_1 derivatives had similar biological properties with the 4"-epiamino isomer being a somewhat more potent insecticide. Since the 4"-epiamino derivatives were also the major products of reductive amination, they were selected for further study.

The most active member of the series was 4"-deoxy-4"-epimethylamino avermectin B_1 which has been selected for development as an agricultural insecticide and assigned the code name MK-243 Fig. 12.

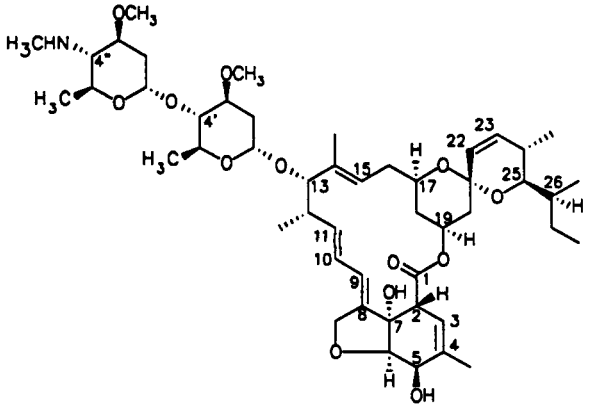


Fig. 12. MK-243

A summary of the foliar ingestion activity of MK-243 against a variety of insect larvae and adult spider mites and aphids is shown in TABLE 11.

TABLE 11. Foliar Ingestion Activity of 4"-Epi-Methylamino-4'-Deoxyavermectin B_1 against Insect Larvae and Adult Spider Mites and Aphids

SPECIES (Common Name)	LC_{90}(ppm) at 96 hours
Manduca sexta (L.) (tobacco hornworm)	0.003
Trichoplusia ni (Huebner) (cabbage looper)	0.014
Spodoptera exigua (Huebner) (beet armyworm)	0.005
Spodoptera frugiperda (J.E. Smith) (fall armyworm)	0.01
Leptinotarsa decemlineata (Say) (colorado potato beetle)	0.032
Epilachna varivestis (Mulsant) (Mexican bean beetle)	0.20
Tetranychus urticae (Koch) (two-spotted spider mite)	0.29
Aphis fabae (Scopoli) (bean aphid)	19.9

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