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Volume 12
Stereoselective Synthesis (Part H)

FOREWORD

The impressive series of *Studies in Natural Products Chemistry,* edited by Professor Atta-ur-Rahman, continues to appear in rapid succession. Indeed, the 12th volume has been published in a matter of only 5-6 years. During the past few years, natural products chemistry has undergone -- and continues to undergo -- an extremely encouraging and healthy renaissance. Until the mid-70's, structure determination and synthesis enjoyed its heyday. However, the explosive development of instrumentation and numerous new synthetic routes and reagents which appeared around that time seemed to make the study of natural products "routine" to many researchers. This era also coincided with the birth of modern biology, and many organic chemists drifted towards bioorganic chemistry and biology. The forefront of biology continues to tackle ever more challenging projects, but at the same time the scientific field has become far more multidisciplinary than before. With the exposure of organic chemists to more bioscience, natural products chemistry itself is becoming more dynamic. In collaboration with scientists in other disciplines, natural products chemistry now again plays a key role in understanding nature on a more precise structural basis. Both synthetic and structural organic chemists have started to address the question of the interaction between ligands and their receptors. Only organic chemists can accomplish challenging structure determination of noncrystalline compounds, or perform elegant transformations of molecules to fulfill their renewed goals. This statement of course applies to scientists in other disciplines as well. Through collaborative efforts between all disciplines at an advanced level, we start to better understand life processes and nature's mysteries. I regard natural products chemistry in this context. It is thus most welcome that researchers at the forefont have produced another extremely useful source of reference on a variety of timely topics, all of which prove extremely important. I congratulate the Editor, who has managed to gather so many expert contributions and superb review articles from leading scientists. This simply ranks as a most informative and interesting volume.

December 8, 1992

Koji Nakanishi
Columbia University

PREFACE

The plant kingdom offers a vast largely untapped source of a wide diversity of organic compounds, potentially useful in medicine. With the advent of powerful spectroscopic techniques and improved separation procedures it is now possible to elucidate the structures of compounds isolated in sub-milligram quantities. There has accordingly been a significant increase in the number of new compounds reported in the literature during the last decade. However efforts at bioassay-directed fractionation of plant extracts have lagged behind, largely due to the absence of pharmacological screens in most laboratories involved in phytochemical researches. This must change if compounds of significance are to be discovered from natural sources.

The present volume contains contributions from a number of eminent natural product chemists on the synthesis avermectin - milbemycin family of antiparasitic agents, macrolide antibiotics, β-lactam antibiotics, 1β- methylcarbapenem derivatives, taxane diterpenes and tumour - promoting diterpenes, indolizidines and indol [2,3-a] carbazole alkaloids, 2-amino alcohols and on the generation of cationic and radical intermediates for synthesis of natural products. It is hoped that the in - depth reviews by eminent exponents of the art of organic synthesis contained in this volume will generate the same interest and excitement as other previous volumes of this series.

I would like to thanks Miss Anis Fatima, Miss Farzana Akhtar and Mr. Ejaz Ahmed Soofi for their assistance in index preparation, Mr. Habib Alam and Mr. Asif Mehmood Raja for typing and Mr. Mahmood Alam for secretarial help.

November 1992   **Atta-ur-Rahman**, *Editor*
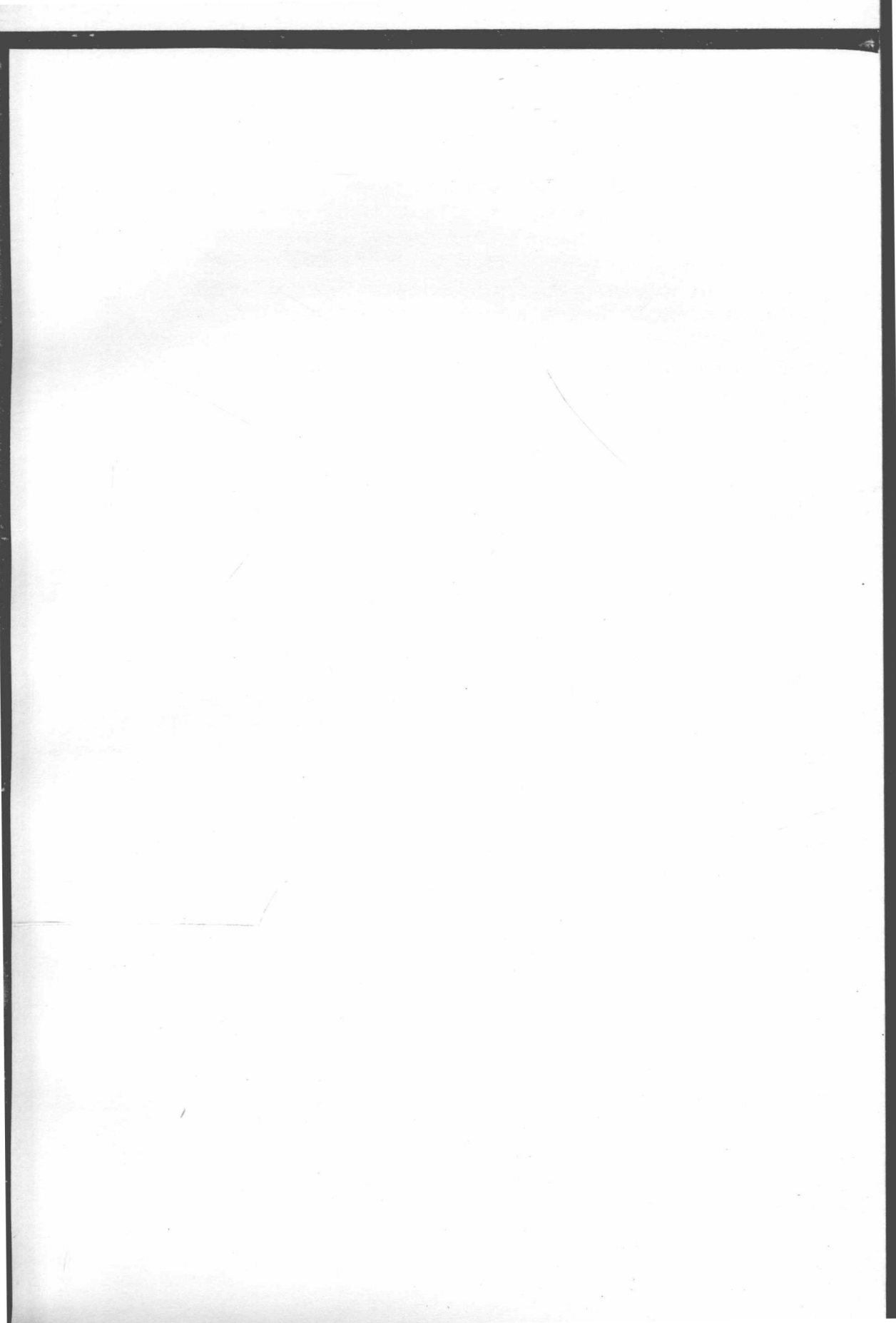
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# CONTENTS

Stereoselective Synthesis

Synthetic Routes to the Oxahydrindene Subunit of the Avermectin- Milbemycin Family of Antiparasitic Agents: A Review

S. Andrew Peak and Amos B. Smith, III

1. Introduction: Isolation, Structure Elucidation, Biosynthesis, and Biological Activity of the Avermectins

As the result of a broad, routine screening program searching for anthelmintic agents from microbial sources, researchers at Merck & Co. discovered the avermectin family of sixteen-membered-ring macrolide glycosides (Figure 1) in 1976.[1] The microorganism producing the avermectins was isolated from a soil sample obtained from Kawana, Ito City,

Figure 1. Avermectins A_{1a}-B_{2b}

1
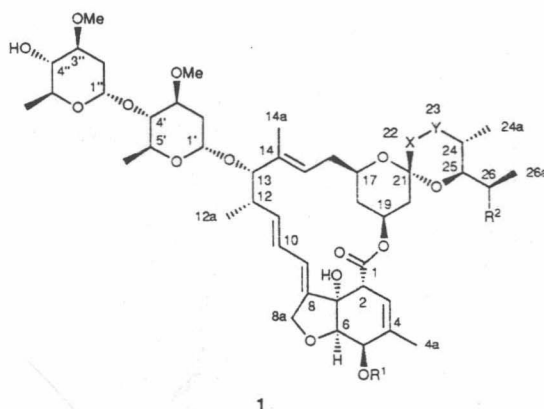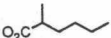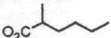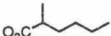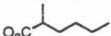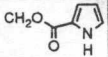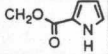
Avermectin A series: R^1 = Me	Avermectin 2 series: XY = -$CH_2CH(\alpha\text{-}OH)$-
Avermectin B series: R^1 = H	Avermectin a series: R^2 = Et
Avermectin 1 series: XY = -CH=CH-	Avermectin b series: R^2 = Me

Shizuoka Prefecture, Japan by Ōmura and co-workers at the Kitasato Institute of Tokyo; this microbe was found to be a structurally novel species of actinomycete and subsequently named *Streptomyces avermitilis*.[2] The specific bioassay that permitted detection of the avermectins and their producing organism involved the survival in mice of the gastrointestinal nematode (roundworm) *Nematospiroides dubius*, a parasite which is difficult to eradicate with standard anthelmintic agents (e.g., benzimidazoles).[3] Solvent extraction of the mycelia taken from the fermentation broth of a *Streptomyces avermitilis* culture gave a complex of eight closely related compounds. These were later termed avermectins A_{1a} through B_{2b} and found to consist of four major and four minor homologous components which could only be separated by reverse-phase high-performance liquid chromatography. The avermectin complex as a whole was active against

N. dubius in mice over a large dosage range, with the individual compounds differing in their respective anthelmintic activities. In addition, the initial testing of avermectin B_{1a} against a variety of other nematodes in experimentally infected domestic animals (i.e., sheep, cattle, dogs, and chickens) was highly successful and, importantly, produced "no grossly observable toxic reactions"[4] in the host organisms.

Determination of the structures of the avermectins[5] was facilitated by the previously published structure elucidation of the milbemycins (Figure 2),[6] a closely related series of pesticidal compounds which had been isolated a few years earlier from the fermentation broth of a different *Streptomyces* strain. Primarily by means of mass spectrometry and ^{13}C NMR

Figure 2. The α and β Milbemycins

2

Milbemycin	R^1	R^2	R^3	R^4	R^5
α_1	H	Me	H	H	Me
α_2	Me	Me	H	H	Me
α_3	H	Me	H	H	Et
α_4	Me	Me	H	H	Et
α_5	H	Me	OH	O_2C	Me
α_6	Me	Me	OH	O_2C	Me
α_7	H	Me	OH	O_2C	Et
α_8	Me	Me	OH	O_2C	Et
α_9	H	CH_2O	H	H	Me
α_{10}	H	CH_2O	H	H	Et

3

Milbemycin β_1: R = Me
Milbemycin β_2: R = Et

4

Milbemycin β_3

spectroscopy (and to a lesser extent, ^1H NMR spectroscopy), the avermectins were found to have three main structural similarities with the α milbemycins: (1) a sixteen-membered-ring lactone, (2) a dioxaspiro[5.5]undecane (6,6-spiroacetal) moiety, and (3) a highly functionalized oxahydrindene subunit. These gross structural features distinguish the avermectins from previously reported anthelmintic microbial fermentation products, many of

which belong to the aminoglycoside family of antibiotics.[2] The presence of oxygenation with an accompanying disaccharide appendage at C(13) constitutes the main structural difference between the avermectins and both the α and β series of the milbemycins; the avermectin carbohydrate substituent was established as an α-L-oleandrosyl-α-L-oleandrosyl unit through a combination of spectral methods and chemical degradation. Two other important structural

Figure 3. Additional Avermectins

5

6 a: R^1 = Me; R^2 = α-L-oleandrosyl; R^3 = s-Bu
6 b: R^1 = H; R^2 = α-L-oleandrosyl; R^3 = s-Bu
6 c: R^1 = H; R^2 = α-L-oleandrosyl- α-L-
oleandrosyl; R^3 = s-Bu

7 a: X = H, OMe; R^1 = H; R^2 = H; R^3 = i-Pr
7 b: X = O; R^1 = H; R^2 = H; R^3 = s-Bu
7 c: X = O; R^1 = H; R^2 = H; R^3 = i-Pr
7 d: X = H, H; R^1 = H; R^2 = α-L-oleandrosyl-
α-L-oleandrosyl; R^3 = s-Bu
7 e: X = H, OMe; R^1 = H; R^2 = H; R^3 = s-Bu
7 f: X = H, OMe; R^1 = OH; R^2 = H; R^3 = s-Bu

8 a: X = H, OH; R = s-Bu
8 b: X = O; R = s-Bu
8 c: X = H, OH; R = i-Pr
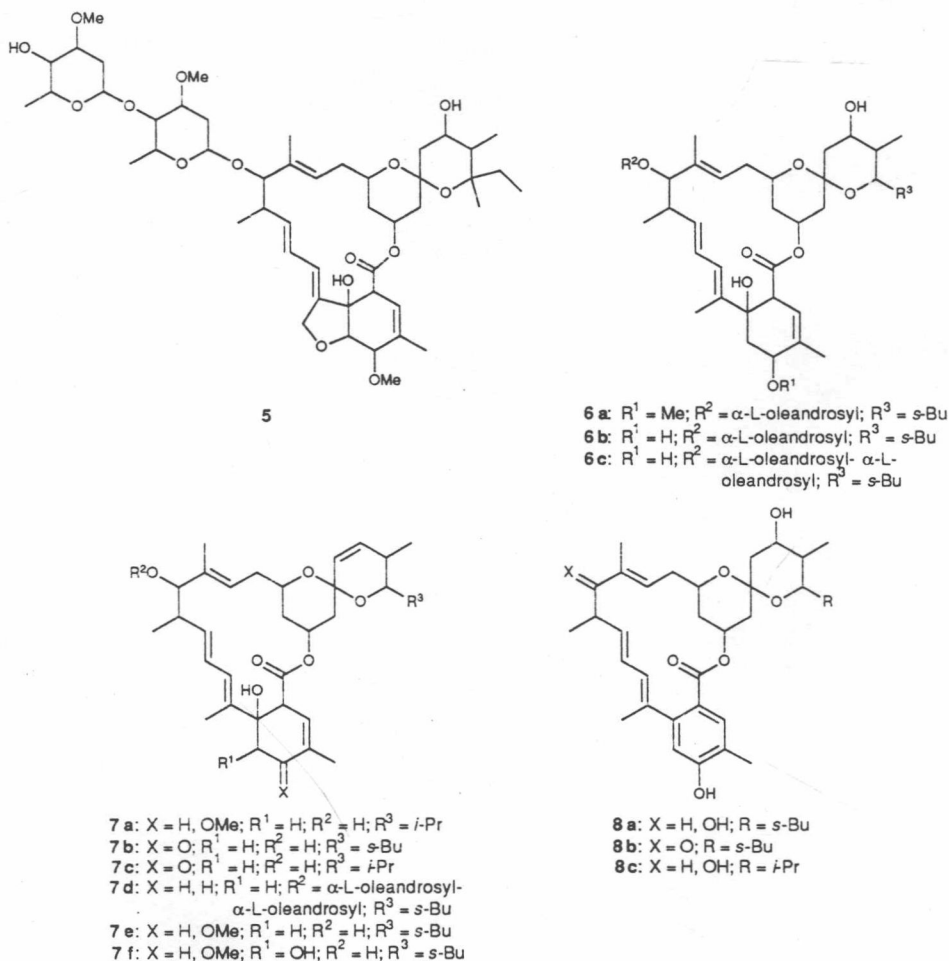
differences between the avermectins and both series of milbemycins were found in the spiroacetal moiety of these compounds: (1) C(25) of the a and b series of the avermectins is functionalized with s-butyl and isopropyl groups, respectively, whereas C(25) of the α and β milbemycins is functionalized with either an ethyl or methyl group; and (2) the 1 and 2 series of the avermectins contain a C(22)-C(23) alkene and a saturated linkage bearing a hydroxyl group at C(23), respectively, whereas the milbemycins are either oxygenated at both C(22) and C(23) or reduced at these positions. Additional avermectins have appeared in the patent literature more

recently,[7] including compounds with a monocyclic benzene or cyclohexene ring in place of the oxahydrindene subunit and lacking one or both of the α-L-oleandrosyl moieties (Figure 3); additional milbemycins have also been described (Figure 4).[8]

Figure 4. Additional Milbemycins

9

10

Milbemycin E: X = α-H, β-OMe; R[1] = CH$_2$OH
Milbemycin H: X = O; R[1] = Me
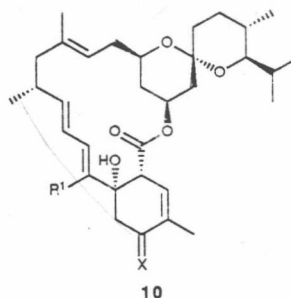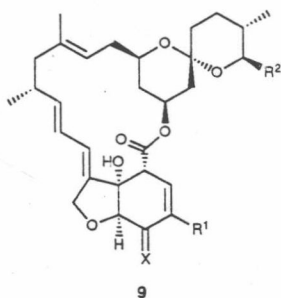
Milbemycin	X	R[1]	R[2]
D	α-H, β-OH	Me	i-Pr
F	α-H, β-OH	CH$_2$O–⟨pyrrole⟩	i-Pr
G	α-H, β-OMe	Me	i-Pr
J	O	Me	Me
K	O	Me	Et

Single-crystal X-ray analyses of avermectin B$_{1a}$ and the aglycon of avermectin B$_{2a}$ confirmed the structures and established the relative stereochemistry of the avermectins;[9] the previous structural identification of the disaccharide unit then allowed the absolute stereochemistry of these compounds to be assigned. Correlation of ^{1}H NMR data with the X-ray results indicated that the solution and solid-state conformations of the basic avermectin skeleton were virtually identical. Moreover, the absolute configurations of the avermectins and the milbemycins were shown to be identical, both by circular dichroism studies[10] and by chemical correlation of the 22,23-dihydroavermectin B$_{1b}$ aglycon with milbemycin D[11] and of the 22,23-dihydroavermectin B$_{1a}$ aglycon with its corresponding 13-deoxy derivative.[11] In the latter case, the optical rotation of the homologous milbemycin product was in agreement with the rotation of naturally occurring milbemycin α$_3$.

The biosynthesis of the avermectins[12] appears to involve assembly of acetate and propionate precursors via a series of transformations analogous to those occurring in the biosynthesis of saturated fatty acids. Four fermentation experiments were carried out in order to determine the biosynthetic origin of the carbon skeleton and oxygen atoms of the avermectins. First, [1-^{13}C] acetate and [1-^{13}C] propionate were fed separately to an S. avermitilis culture; avermectins A$_{1a}$, A$_{2a}$, B$_{1a}$, and B$_{2a}$ were isolated and purified. Analysis of these metabolites by ^{13}C NMR showed the avermectins to be derived from seven acetates and five propionates (Figure

5): not unexpectedly, the carbon skeletons [excluding C(25)] of milbemycins α_2, α_4, and D were independently determined to derive from an identical group of subunits by Mishima and co-workers.[13] It had already been established that L-isoleucine was the precursor of C(25) and its s-butyl substituent,[14] and these atoms were accordingly found unlabeled in both experiments; C(25) and its isopropyl substituent in the b series of the avermectins had also been shown earlier to originate from L-valine.[14] The above experiments were then repeated with [1-$^{18}O_2$, 1-^{13}C] acetate and [1-$^{18}O_2$, 1-^{13}C] propionate, and the resulting avermectin metabolites were again analyzed by ^{13}C NMR; examination of the ^{18}O isotopically shifted ^{13}C NMR resonances allowed assignment of acetate- and propionate-derived oxygens within the avermectin structure. Significantly, the presence of a propionate-derived oxygen at C(13) provided solid evidence that the avermectins are not derived from the milbemycins by a late-stage oxidation at C(13) and subsequent glycosylation; instead, this result suggested that the biosyntheses of the avermectins and the milbemycins diverge at this point during construction of the carbon chain, with additional dehydration and reduction steps required for milbemycin.

**Figure 5. Biosynthetic Origin of the Carbon Skeleton and Oxygen Atoms of the Avermectins**

11

acetate unit   propionate unit $O^* = O^{18}$
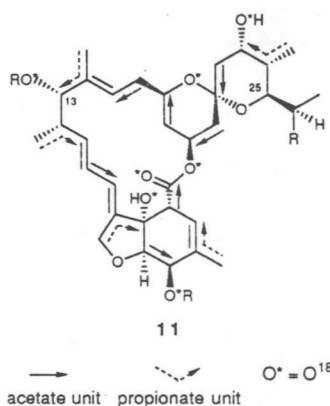
With respect to biological activity, the avermectins are effective against many species of arthropod ectoparasites (e.g., flies, ticks, and mites)[15] in addition to nematode endoparasites; the cestode (tapeworm) and trematode (fluke) classes of parasites, however, are unresponsive to treatment with these compounds. The avermectins also lack the significant antibacterial or antifungal properties commonly associated with macrolide and polyene antibiotics. They do not inhibit protein synthesis or act as ionophores;[16] alternatively, it has been proposed that the mechanism of action of the avermectins involves interference with GABA (γ-aminobutyric acid)-mediated muscular regulation in the peripheral nervous system of nematodes and arthropods. Consequently, cestodes and trematodes are insensitive to the avermectins because they do not use GABA to regulate muscular activity,[17] and it follows that the low mammalian toxicity of the avermectins is due to the inability of these compounds to penetrate the mammalian central nervous system (i.e., to cross the "blood-brain barrier"), where all of the GABA-regulated nerve

cells of mammals are located.[15]

Structure-activity relationship studies of the avermectins[1,18,19] have provided a considerable body of information as to which avermectin functionalities are important for the biological activity. It was initially observed that the avermectin B series, containing a free C(5)-OH group, was generally more active than the C(5)-OMe-substituted A series; furthermore, derivatives of avermectin B_{1a} with a t-butyldimethylsilyl group on the C(5)-OH showed a total loss of activity. The difference in potency between the avermectin 1 series, with a C(22)-C(23) alkene, and the hydrated avermectin 2 series was found to be less predictable and primarily dependent on the parasite of interest. The nature of the C(25) substituent (s-butyl or isopropyl) was seen to have little or no effect on antiparasitic activity, and mixtures of a and b avermectin components were subsequently used in biological tests without prior separation. A series of acylation experiments demonstrated the importance of a free C(23)-OH group for activity in the avermectin 2 series, whereas the C(4")-OH could be derivatized in avermectins A_{2a}, B_{1a}, and B_{2a} with essentially no loss in potency. An increasing loss of activity was observed with sequential removal of the L-oleandrosyl units; the avermectin aglycons were dramatically less active than the parent disaccharides. Hydrogenation[20] or cyclopropanation of the C(3)-C(4) alkene and aromatization of the cyclohexene moiety under forcing acetylation conditions were also accompanied by a considerable loss of activity. However, epoxidation of the C(8)-C(9) alkene or reduction of the C(22)-C(23) alkene in the avermectin 1 series gave derivatives nearly as potent as the parent olefins; in particular, 22,23-dihydroavermectin B_1, obtained by the selective hydrogenation of avermectin B_1 with Wilkinson's catalyst, was found to have a better safety profile than the natural product and, unlike avermectin B_1, proved to be comparably effective in animal tests by both oral and injected routes. 22,23-Dihydroavermectin B_1 was eventually chosen for commercial development and given the generic name ivermectin (Figure 6).[18]

Figure 6.   Ivermectin (22,23-Dihydroavermectin B_1)

12
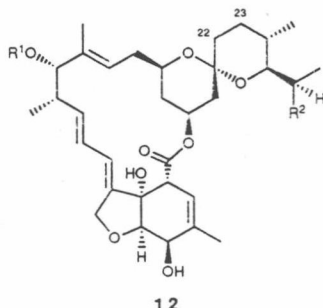
$R^1 = \alpha$-L-oleandrosyl-α-L-oleandrosyl
$R^2 \geq 80\%$ Et, $\leq 20\%$ Me

Ivermectin is currently licensed in many countries for the treatment and control of parasites in various animals; for example, the Food and Drug Administration approved its use in the United States for the prevention of heartworm in dogs in 1986.[21]   Potential human

applications of ivermectin therapy[22] have been demonstrated in the treatment and control of human onchocerciasis (a.k.a. "river blindness") and lymphatic filariasis,[23] a mosquito-transmitted parasitic disease chiefly endemic to parts of India, China, and Indonesia. Avermectin B_1, the most generally active member of the naturally occurring avermectins, has itself been commercialized under the generic name abamectin for agricultural use in controlling several species of mites and insects.[22]

2. Synthetic Routes to the Oxahydrindene Subunit of the Avermectins

The tremendous medical importance of the avermectins and milbemycins, particularly ivermectin, has engendered extensive effort directed toward the total syntheses of these molecules and their subunits. A comprehensive analysis of all of the synthetic and degradative work on the avermectins and milbemycins is beyond the scope of this review, and in illustration of the rapid pace of this field, we note that the 1988 survey of this chemistry by Crimmins[24] is already outdated. Most researchers in the synthetic community regard the construction of the highly oxygenated C(1)-C(9) subunit of the avermectins and α milbemycins (Figure 7) as the paramount challenge of avermectin-milbemycin synthesis. Consequently we have focused on the previous synthetic approaches to this subunit, of which at least sixteen have appeared in the literature, and our summary of these routes is organized alphabetically by principal investigator. We use the Merck avermectin numbering scheme (Figure 7) in the discussion that follows.

Figure 7. The Oxahydrindene Subunit

13
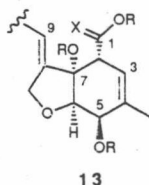
2.1 Barrett Approaches

Barrett's latest synthetic work on the oxahydrindene subunit of the avermectins (Scheme 1)[25] showcases his recently published annulation methodology for tetrahydrofuran synthesis,[26] employing a two-step condensation of the dianion derived from 4-[(*tert*-butyldiphenylsilyl)oxy]-2-(tributylstannyl)-(*E*)-2-buten-1-ol (**23**) with α,β-epoxy cyclohexanone derivative **22**. The synthesis of **22** begins with the Diels-Alder reaction of acetoxy diene **14** with acrylic acid, and the relative stereochemistry at C(2), C(5), and C(6) is set by a subsequent series of functional group transformations. Although the annulation product **25** is formed in only modest yield, the relative stereochemistry of the contiguous stereocenters at C(2), C(7), and C(6) is correctly established, presumably via stereoselective initial attack of the vinyl anion anti to the bulky orthoester substituent at C(2), followed by an intramolecular S_N2 displacement of the C(6) epoxide center by the C(8a) alkoxide. The *E* C(8)-C(9) exocyclic