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SYNTHESIS, REACTIVITY, AND BIOACTIVITY OF AVERMECTIN B_1-3,4-OXIDE

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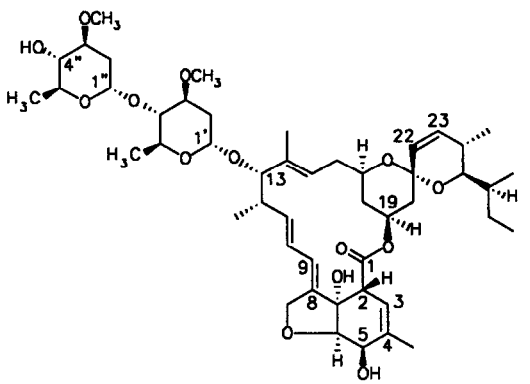
Abstract: *Avermectin B_1-3,4-oxide (2) has been synthesized from avermectin B_1 (1). While epoxide 2 is comparable to avermectin B_1 in bioactivity, opening of the epoxide leads to derivatives which are substantially less bioactive.*

The avermectins are a family of naturally occurring macrocyclic lactones with important anthelmintic and pesticidal activity.[1] The major product isolated from the fermentation of *S. Avermitilis* is avermectin B_1 (1). The 22,23-dihydro derivative of 1, known as ivermectin, is widely used as an anthelmintic agent in human and animal health. The synthesis[2] of avermectin B_1-8,9-oxide,[2,3] an analog with bioactivity[3c] comparable to that of 1 but with improved photostability[3d] (important for potential agricultural use as a pesticide), has recently been reported. The excellent bioactivity of the 8,9-oxide prompted us to examine the synthesis and reactions of other avermectin epoxides. We have recently completed and report herein the synthesis of avermectin B_1-3,4-oxide (2) and analogs derived from it. We also report herein some interesting observations regarding bioactivity of these avermectin derivatives.



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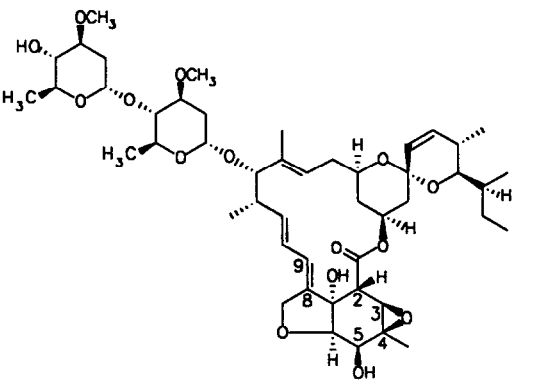
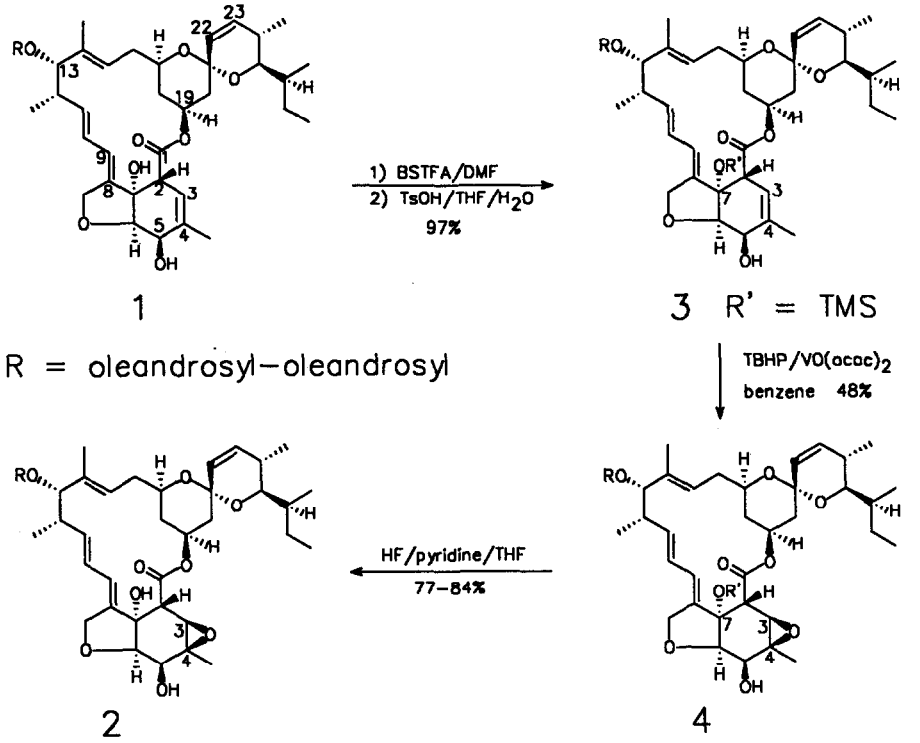


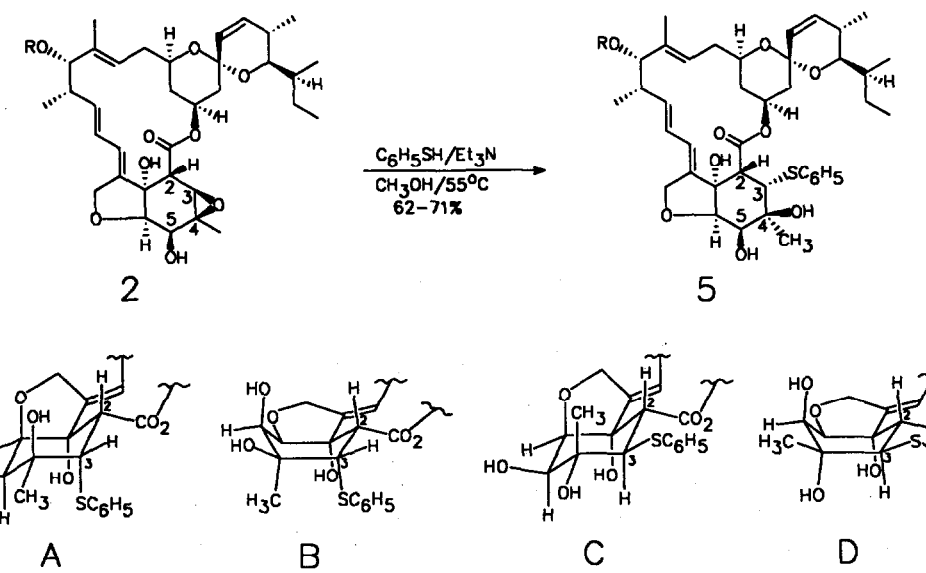
2

We began by blocking the free 7-OH of 1 as the TMS ether so that it would be unable to direct epoxidation to the 8,9-double bond. Thus, treatment of 1 with a large excess of BSTFA[4a] (15 eq BSTFA, DMF, 25°C, overnight) afforded 4",5,7-tris-O-trimethylsilyl-avermectin B_1. Brief (10 minutes at 25°C) treatment of this per-silylated material with p-toluenesulfonic acid (0.25 eq) in 9:1 THF:H_2O (to cleave the secondary TMS ethers) cleanly afforded the desired 7-O-TMS-avermectin B_1 (3) (97% yield from 1).[5a] When 3 was subjected to the vanadium catalyzed epoxidation procedure[6] (benzene, 3 eq TBHP added in portions, 0.25 eq VO(acac)$_2$, 70°C, 6 hours) previously used to prepare the 8,9-oxide[2] the expected 3,4-oxide 4 was obtained in moderate yield (ca. 48% based on unrecovered starting material; attempts to force the reaction to completion led to substantial reduction in yield).[5a] Since the epoxidation was presumably directed by the 5-OH group the stereochemistry of the epoxide was assigned as beta. Additional evidence for this assignment was later provided by NMR analysis of a derivative (*vide infra*). Removal of the TMS group with HF/pyridine/THF (25°C, overnight) then afforded the desired avermectin B_1-3,4-oxide (2) in 77-84% yield.[5a,b]

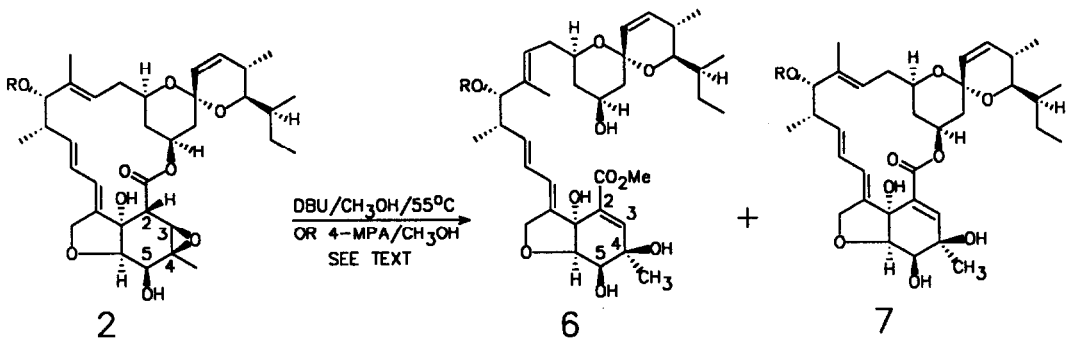
We briefly explored the reactivity of 2 towards various nucleophiles. We found that the epoxide reacted readily with thiophenol (3-4 eq) and triethylamine (3 eq) in methanol (55°C, 23 hours, 62-71%) to afford the expected epoxide opened derivative 5.[5a,c] NMR analysis of 5 provided additional support for the assignment of the epoxide stereochemistry of 2 as beta. In the 400 MHz NMR of 5 the coupling constant $J_{2,3}$ is about 4 Hz. This is consistent with partial structures A and B, which represent the chair and boat forms of the product derived from a 3,4-(β)-oxide. On the other hand, the product



derived from opening a 3,4-(α)-oxide, represented by partial structures **C** and **D**, would be expected to have a $J_{2,3}$ of about 9 Hz.[7] Thus the NMR data of **5** and the mechanism of the directed epoxidation used to prepare **2** are both consistent with the beta 3,4-epoxide stereochemistry.



In an attempt to open the epoxide with a nitrogen nucleophile 2 was treated with 4-methoxyphenethylamine (4-MPA, 5 eq.) in methanol (55°C, 41 hours). Two products were obtained, however neither was the result of epoxide opening by the amine. The major product resulted from base catalyzed beta-elimination of the epoxide oxygen to form the delta-2,3 analog 7.[5a,e] The minor product resulted from base catalyzed trans-esterification of 7 with methanol to form the corresponding methyl ester 6.[5a,d] When DBU[4b] was substituted for 4-MPA the major product was 6 (44%) with 7 isolated as the minor product (15%).



2 6 7

We found that opening of the epoxide had an interesting effect on the biological activity of the resulting derivatives (see Table I). The activity of the 3,4-oxide (2) against two-spotted spider mites[8] and brine shrimp larvae[9] is comparable to the activity of avermectin B_1 (1). However, derivatives in which the epoxide has been opened (5-7) are substantially less active. It is not clear whether this substantial loss of activity is due to a change in the conformation of the molecule or to the presence of additional substituents at C-3 and C-4. It is interesting to note, however, that analogs with a 7-OTMS group (3 and 4) are also substantially less active than 1 and 2. Further work on derivatization of the avermectins is in progress and will be reported in future publications.

TABLE I

Bioactivity of Avermectin Derivatives

Compound	*T. Urticae*[a] ED_{90}(ppm)	*A. Salina*[b] IC_{100}(ng/mL)
1	0.04	306[e]
2	0.03	430
3	>>0.25[c]	>55500[f]
4	>>0.25[c]	>55500[f]
5	>0.25[d]	>55500[f]
6	>>1.25[c]	>55500[f]
7	1.25	>55500[f]

(a) Two spotted spider mite (*T. urticae*) data obtained as described in reference 8a; (b) Brine shrimp (*A. salina*) data obtained as described in reference 9, average of 2 assays unless otherwise noted; (c) Highest level tested, <20% activity; (d) Highest level tested, <50% activity; (e) average of 103 assays; (f) Highest level tested, <100% activity.

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4. (a) BSTFA = Bis(trimethylsilyl)trifluoroacetamide. (b) DBU = 1,8-Diazabicylo[5.4.0]undec-7-ene

5. (a) All new compounds were characterized by ^1H NMR, MS, and ^{13}C NMR and elemental analysis where appropriate; (b) data for 2: partial ^1H NMR (300 MHz, $CDCl_3$): δ 5.65-5.8 (4H, m, H_9 & H_{10} & H_{11} & H_{22}), 5.53 (1H, dd, J = 10, 2 Hz, H_{23}), 5.35-5.50 (1H, m, H_{19}), 5.36 (1H, br d, J = 3 Hz, $H_{1''}$), 4.90-4.97 (1H, m, H_{15}), 4.73 (1H, br d, J = 3 Hz, $H_{1'}$), 4.56 (2H, br s, H_{8a}), 4.20 (1H, s, 7-OH), 4.18 (1H, dd, J = 9, 7 Hz, H_5), 3.89 (1H, br s, H_{13}), 3.87 (1H, d, J = 7 Hz, H_6), 3.66-3.90 (3H, m, $H_{5'}$ & $H_{5''}$ & H_{17}), 3.52-3.64 (1H, m, $H_{3'}$), 3.45 (1H, br d, J = 10 Hz, H_{25}), 3.40 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 3.36-3.48 (1H, m, $H_{3''}$), 3.28 (1H, d, J = 2 Hz, H_2), 3.20 (1H, t, J = 9 Hz, $H_{4'}$), 3.12 (1H, br t, J = 9 Hz, $H_{4''}$), 2.80 (1H, d, J = 2 Hz, H_3), 2.68 (1H, br s, 4"-OH), 2.48 (1H, d, J = 9 Hz, 5-OH), 2.42-2.52 (1H, m, H_{12}), 1.46 (3H, s, H_{4a}), 1.45 (3H, s, H_{14a}), 1.21 & 1.23 (2 x 3H, 2 d, J = 7 Hz, $H_{6'}$ & $H_{6''}$), 1.12 (3H, d, J = 7 Hz, H_{12a}), 0.80-0.95 (10H, m, H_{18} & H_{24a} & H_{26a} & H_{28}); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 173.2, 139.7, 138.9, 136.8, 135.7, 128.1, 125.1, 121.0, 118.7, 99.0, 96.2, 95.4, 82.3, 80.8, 80.1, 79.9, 79.8, 78.7, 76.5, 75.4, 69.3, 69.2, 68.8, 68.6, 68.1, 67.7, 60.6, 58.1, 57,0, 56., 47.8, 40.8, 40.3, 37.1, 35.6, 34.9, 34.7, 31.0, 28.0, 21.3, 20.7, 18.9, 18.2, 16.9, 15.6, 13.5, 12.5; Elem. Anal. calcd for $C_{48}H_{72}O_{15}$: C, 64.85; H, 8.15; found: C, 64.54; H, 8.44; (c) data for 5: partial ^1H NMR (400 MHz, $CDCl_3$): δ 7.32-7.36 (2H, m, Ar-H), 7.24-7.29 (2H, m, Ar-H), 7.17-7.22 (1H, m, Ar-H), 6.03 (1H, dt, J = 11, 3 Hz, H_9), 5.81 (1H, dd, J = 15, 10 Hz, H_{11}), 5.80 (1H, dd, J = 10, 2 Hz, H_{22}), 5.66 (1H, dd, J = 15, 11 Hz, H_{10}), 5.58 (1H, dd, J = 10, 3 Hz, H_{23}), 5.48 (1H, s, 4-OH), 5.41 (1H, br d, J = 3 Hz, $H_{1''}$), 5.32-5.40 (1H, m, H_{19}), 4.92-4.98 (1H, m, H_{15}), 4.76 (1H, br d, J = 3 Hz, $H_{1'}$), 4.73 & 4.57 (2 x 1H, 2 dd, J = 14, 3 Hz, H_{8a}), 4.12 (1H, dd, J = 10, 4 Hz, H_5), 4.05 (1H, d, J = 4 Hz, H_6), 3.96 (1H, s, 7-OH), 3.92 (1H, br s, H_{13}), 3.62 (1H, d, J = 4 Hz, H_3), 3.48 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 3.37 (1H, d, J = 4 Hz, H_2), 3.25 (1H, t, J = 9 Hz, $H_{4'}$), 3.17 (1H, td, J = 9, 2 Hz, $H_{4''}$), 2.48 (1H, d, J = 2 Hz, 4"-OH), 2.26 (1H, d, J = 10 Hz, 5-OH), 1.47 & 1.42 (2 x 3H, 2 s, H_{4a} & H_{14a}); (d) data for 6: partial ^1H NMR (400 MHz, $CDCl_3$): δ 6.70 (1H, s, H_3), 6.29 (1H, br d, J = 11 Hz, H_9), 5.80-5.96 (2H, m, H_{10} & H_{11}), 5.70 (1H, br d, J = 10 Hz, H_{22}), 5.53 (1H, dd, J = 10, 2 Hz, H_{23}), 5.36 (1H, t, J = 7 Hz, H_{15}), 5.26 (1H, br d, J = 3 Hz, $H_{1''}$), 4.70 (1H, br d, J = 3 Hz, $H_{1'}$), 4.65 & 4.56 (2 x 1H, 2 br d, J = 14 Hz, H_{8a}), 4.58 (1H, s, 4-OH), 4.12 (1H, s, 7-OH), 4.04-4.12 (1H, m, H_{19}), 3.77 (3H, s, CO_2CH_3), 3.38 (3H, s, OCH_3), 3.34 (3H, s, OCH_3), 3.14 (2H, d, J = 9 Hz, $H_{4'}$ & $H_{4''}$), 1.51 & 1.37 (2 x 3H, 2 s, H_{4a} & H_{14a}); Elem. Anal. calcd for $C_{49}H_{76}O_{15}{\cdot}H_2O$: C, 62.67; H, 8.37; found: C, 62.65; H, 8.24; (e) data for 7: partial ^1H NMR (400 MHz, $CDCl_3$): δ 6.23 (1H, s, H_3), 6.13 (1H, br d, J = 11 Hz, H_9), 5.75-5.83 (2H, m, H_{11} & H_{22}), 5.65 (1H, dd, J = 14, 11 Hz, H_{10}), 5.57 (1H, dd, J = 10, 2 Hz, H_{23}), 5.40 (1H, br d, J = 3 Hz, $H_{1''}$), 5.34-5.42 (1H, m, H_{19}), 4.94 (1H, m, H_{15}), 4.75 (1H, br d, J = 3 Hz, $H_{1'}$), 4.58 (2H, br s, H_{8a}), 4.17 (1H, d, J = 2 Hz, H_6), 3.78 (1H, dd, J = 10, 2 Hz, H_5), 3.47 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.24 (1H, t, J = 9 Hz, $H_{4'}$), 3.16 (1H, br t, J = 9 Hz, $H_{4''}$), 2.73 (1H, d, J = 10 Hz, 5-OH), 1.46 & 1.35 (2 x 3H, 2 s, H_{4a} & H_{14a}); Elem. Anal. calcd for $C_{48}H_{72}O_{15}{\cdot}0.5\ H_2O$: C, 64.19; H, 8.19; found: C, 64.18; H, 8.16.

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7. Assuming a chair or boat conformation in which the ester carbonyl (C-1) is equatorial. In the alternative chair or boat conformations (C-1 axial) both isomers would be expected to have the same $J_{2,3}$ (about 2 Hz).

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