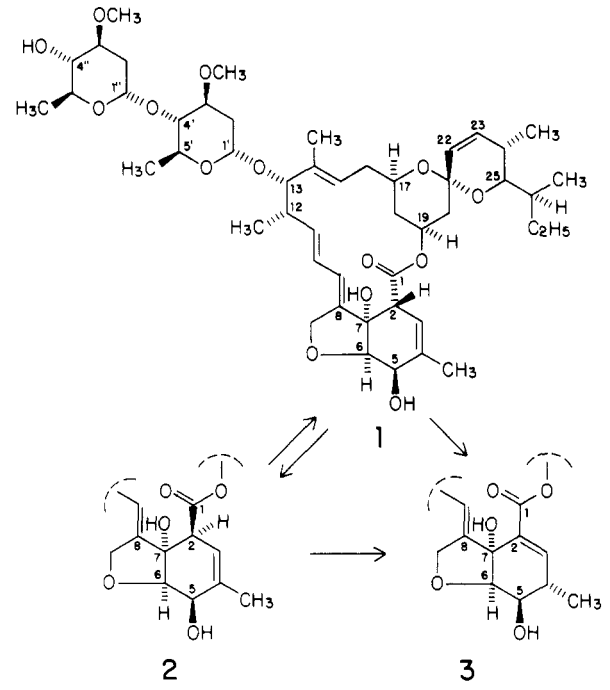
Base-Catalyzed Isomerization of Avermectins

James V. Pivnichny,* Byron H. Arison, Franz A. Preiser, Jung-Sook K. Shim, and Helmut Mrozik

Avermectin B_{1a} (1) reacts with sodium hydroxide in aqueous methanol (0.05 M) at C-2, initially tending to an equilibrium of epimers, both of which are ultimately converted to the conjugatively stabilized Δ^2 isomer **3**. The 2-epimer **2** reacts analogously to form 1 and **3**, and forward and reverse rates of epimerization are essentially equal. The Δ^2 isomer is not converted to either of the epimeric nonconjugated compounds (1 or **2**) by dilute hydroxide in aqueous methanol. Both **2** and **3** have substantially reduced biological activity relative to the parent compound.

Because of the well-documented importance of the avermectins (Putter et al., 1981) and their semisynthetic analogue ivermectin (Chabala et al., 1980) as broad-spectrum antiparasitic agents, the chemistry of this class of compounds has been of considerable interest (Fisher and Mrozik, 1984). A recent review (Strong and Brown, 1987) has cited "no fewer than four hundred publications" dealing with their insecticidal properties. The biological activity of these compounds is quite sensitive to minor molecular modifications, and in particular, structural changes at C-2 lead to substantial decreases in activity as shown below. A cursory examination of isomerization induced at this position by hydroxide ion in aqueous methanol was previously reported for ivermectin (Pivnichny et al., 1983) in connection with the preparation of the Δ^2 isomer as an internal standard for HPLC analysis. At that time kinetic evidence suggested that the conversion to the conjugated isomer proceeded through the 2-epimer as an intermediate, although the possibility of parallel conversion of both epimeric forms to the conjugated isomer, as shown in Scheme I for avermectin B_{1a} (1), was also considered. Recently interest in the total synthesis of 1 has revolved around the issue of deconjugating a Δ^2 precursor with simultaneous establishment of the proper stereochemistry at the 2-position (Fraser-Reid et al., 1987; Hanessian et al., 1986). For these reasons, we were prompted to examine more thoroughly the isomerization of avermectin B_{1a} induced at the C-2 position under the mild condition of dilute hydroxide. This report establishes the equilibrium nature of the epimerization as well as the total absence of deconjugation under equilibrium conditions and presents structural proof for both isomers **2** and **3**.

Scheme I



EXPERIMENTAL SECTION

Preparation of 2-Epiavermectin B_{1a} (2) and 2-Dehydro-4-hydroavermectin B_{1a} (3). A solution of 100 mg of avermectin B_1 (commercial product, dried in high vacuo over P_2O_5 to constant weight) in 10 mL of 0.05 M KOH in 90% aqueous MeOH was kept at room temperature under N_2 for 4 h. The reaction was stopped with 0.1 mL of AcOH, the product extracted with CH_2Cl_2, and the extract washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to 90 mg of a yellow glass. Preparative layer chromatography (0.5-mm silica gel plates; 95:5 CH_2Cl_2–MeOH) gave a mixture of 1 and **3** (60 and 35%, 50 mg) and pure **2** (35 mg): HPLC (Partisil PXS 10/25 ODS-3, 48:32:20 MeCN–MeOH–H_2O, 1.5 mL/min) t_r 8.9, 11.2 min (6, 92%); UV λ_{max} (MeOH) 245 nm (ϵ 24 400); HRMS, *m/e* found 872.4920, calcd for $C_{48}H_{72}O_{14}$ 872.4922; 400-MHz ^1H NMR ($CDCl_3$) δ (*J*, hertz) 5.95 (1 H, dt, *J* = 10, 2, C_9 H), 5.78 (1 H, dd, *J* = 10, 2, C_{22} H), 5.74 (1 H, dd, *J* = 15, 10, C_{11} H), 5.71 (1 H, m, C_3H), 5.68 (1 H, dd, *J* = 15, 10, C_{10} H), 5.58 (1 H, dd, *J* = 10, 3, C_{23} H), 5.51 (1 H, tt, *J* = 11, 4, C_{19} H), 5.41 (1 H, d, *J* = 3.8, $C_{1''}$ H), 4.94 (1 H, brd, *J* = 10, C_{15} H), 4.76 (1 H, d, *J* = 3, $C_{1'}$ H), 4.61 (1 H, dd, *J* = 13, 1.5, C_{8a} H), 4.31 (1 H, d, *J* = 3, C_6 H), 4.29 (1 H, s, C_7 OH), 4.25 (1 H, brd, *J* = 10, C_5 H), 4.13 (1 H, dd, *J* = 13, 2, C_{8a} H), 3.93 (1 H, brs, C_{13} H), 3.48 and 3.43 (2 × 3 H, 2 s, $C_{3''}$ OCH_3 and $C_{3'}$ OCH_3), 3.22 (1 H, m, C_2 H), 2.41 (1 H, d, *J* = 11, C_5 OH), 1.95 (1 H, ddd, *J* = 12, 5, 2, $C_{20_{eq}}$ H), 1.88 (3 H, qn, *J* = 1.5, C_4 CH_3), 1.84 (1 H, brd, *J* = 12, $C_{18_{eq}}$ H), 1.68 (1 H, t, *J* = 12, $C_{20_{ax}}$ H), 0.80 (1 H, q, *J* = 12, $C_{18_{ax}}$ H).

The mixture of 1 and **3** (50 mg) was separated by preparative HPLC on a Whatman Partisil M20 10/50 ODS-3 reversed-phase column (90:10 MeOH–H_2O, 9.0 mL/min), giving 13 mg of pure **3**: HPLC (Partisil PXS 10/25 ODS-3, 48:32:20 MeCN–MeOH–H_2O, 1.5 mL/min) t_r 12.7 min (90%); UV λ_{max} (MeOH) 250 nm (ϵ 22 800); HRMS, *m/e* found 872.4924, calcd for $C_{48}H_{72}O_{14}$ 872.4922; 300-MHz ^1H NMR ($CDCl_3$) δ (*J*, hertz) 6.17 (2 H, m, C_3 H, C_9 H), 5.75 (3 H, m, C_{10} H, C_{11} H, C_{22} H), 5.57 (1 H, dd, *J* = 9, 2, C_{23} H), 5.42 (1 H, d, *J* = 2, $C_{1''}$ H), 5.38 (1 H, m, C_{19} H), 4.94 (1 H, brd, *J* = 9.5, C_{15} H), 4.77 (2 H, brs, $C_{1'}$ H, C_7 OH), 4.59 (1 H, d, *J* = 12, C_{8a} H), 4.50 (1 H, d, *J* = 12, C_{8a} H), 4.06 (1 H, d, *J* = 2, C_6 H), 3.60 (1 H, dt, *J* = 2, 10, C_5 H), 3.48 and 3.44 (2 × 3 H, 2 s, $C_{3''}$ OCH_3 and $C_{3'}$ OCH_3), 2.49 (2 H, m, C_4 H and C_{12} H), 1.23 (3 H, d, *J*

Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065.

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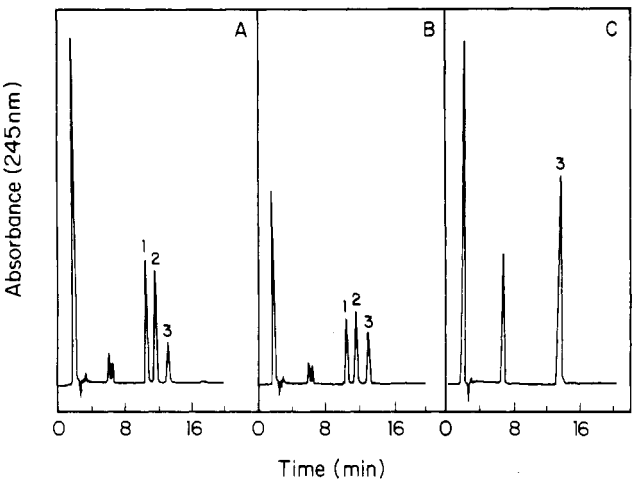

Figure 1. High-performance liquid chromatograms for the partial reaction of 1–3 (panels A–C, respectively) with 0.05 M NaOH in 50% aqueous MeOH at 25 °C. Chromatographic conditions in text.

= 7.7, C_4 CH_3), signals for C_2H and vinylic C_4CH_3 missing.

Biological Assay. Compounds 1–3 were tested against the twospotted spider mite (*Tetranychus urticae*) on 14-day-old bean plants of the variety Tendercrop by dipping the infested leaves into an aqueous solution containing the test compound, 10% acetone, and 100 ppm Triton X-155. Mortality was determined 4 days after treatment.

Kinetic Studies. Isomerization of 1–3 was carried out at 25 °C on 0.025 mg/mL solutions in 50% aqueous methanol containing 0.05 M NaOH and was followed by HPLC using MeCN–MeOH–H_2O (53:35:10) on Zorbax ODS (4.6 × 250 mm, 25 °C) with UV detection at 245 nm.

RESULTS AND DISCUSSION

The absolute stereochemistry of avermectin B_{1a} (1) has been established by X-ray crystallography (Springer et al., 1981), and the ^1H NMR spectrum of the closely related A_{2a} analogue has been thoroughly described (Albers-Schönberg et al., 1981). The identical molecular ions in the mass spectra of the three compounds described here require isomeric structures, and the close similarity of the 300- or 400-MHz ^1H NMR spectra preclude drastic structural changes. This suggested to us a C-2 epimeric and a 3,4-double-bond-shifted isomer of the natural product avermectin B_{1a}, particularly since the C-2 proton next to the lactone carbonyl group has the enhanced acidity required by basic reaction conditions. It was also shown in the experiment that compound 2 but not 3 formed the same equilibrium as 1 under the reaction conditions. The assignment of the 2-epiavermectin B_{1a} structure to 2 further rests on circumstantial NMR evidence. The largest displacements are noted for the protons attached to C-3, -6, and -8a, with lesser shifts for protons at C-2, -9, -18, -19, and -20, pointing to the lower half of the molecule for the center of change, where the C-2 proton is most easily affected by base treatment. A singlet for the vinylic C-4 methyl group combined with observation of C-5 H and OH places the double bond firmly into the 3,4-position. Observation of the C-7 hydroxyl group excludes any dehydration. Most notable is the large upfield shift for one of the C-8a protons, which is best explained by a change in conformation that brings it into close proximity to the lactone carbonyl group.

The 2-dehydro-4-hydroavermectin B_{1a} structure was assigned to isomer 3 on the basis of following key observations: (1) major chemical shift displacements confined to the cyclohexene structure part; (2) absence of the C-4 allylic methyl signal at δ 1.83 and appearance of a new

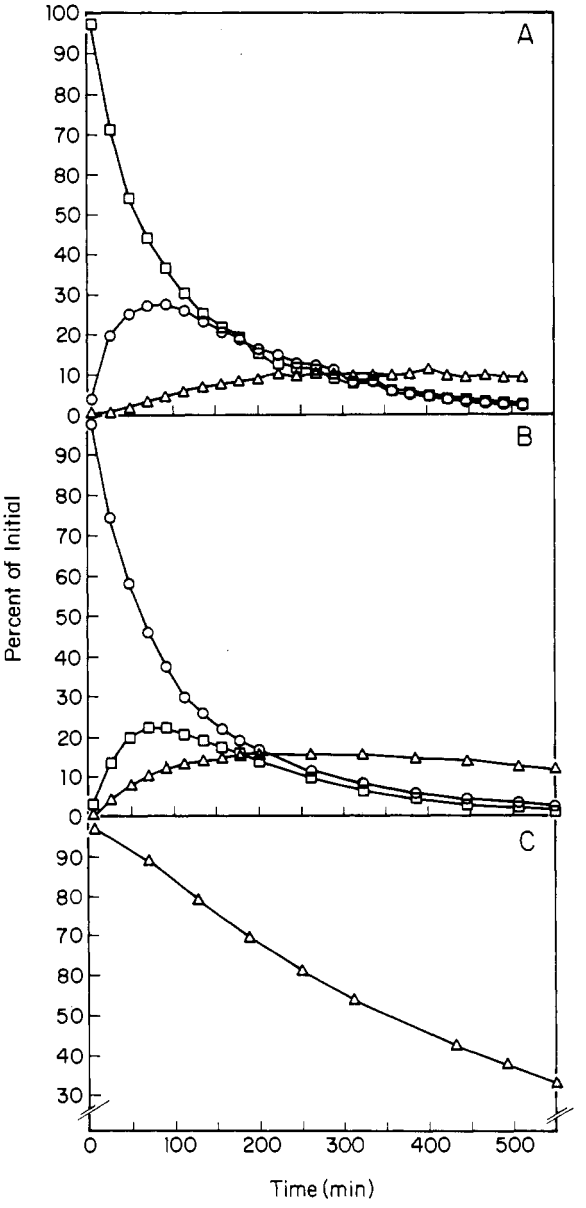

Figure 2. Kinetic profiles for the reaction of 1–3 (panels A–C, respectively) with 0.05 M NaOH in 50% aqueous MeOH at 25 °C: □ = 1; ○ = 2; △ = 3.

methyl doublet near δ 1.20; (3) no detectable signal for the C_2 H and no signals in the regions characteristic for C_3 H, C_5 H, and C_6 H, but the appearance of four new methine resonances at δ 6.17 (C_3 H), 4.06 (C_6 H), 3.60 (C_5 H), and 2.49 (C_4 H); (4) retention of the signal assigned to the C_7 hydroxyl group. Only one of the possible epimers at the new tetrahedral C-4 was obtained, the coupling constant of H-4 and H-5 of 10 Hz requiring a trans hydrogen arrangement placing the C_4 CH_3 group into the equatorial 4-α position. The absence of the 4-β form was also indicated by the lack of a second HPLC peak under conditions of high resolution (ca. 50 000 theoretical plates/m).

It was of great interest to investigate the effect of these minor structural changes on the biological activities of the avermectins, particularly since changes of this nature occur under relatively mild chemical reaction conditions and therefore must be considered in agricultural formulation of such highly active agents. A contact activity assay against the twospotted spider mite on bean plants was used because of the very high potency of avermectin B_{1a} (1) against mites (Table I). The LC_{90} for the natural product 1 is 0.04 ppm, for the 2-epimer 2 it is 4.0 ppm, which amounts to a reduction in potency of 100, and for the Δ^2

Table I. Efficacy of Avermectin Isomers against *T. urticae*

compd	mortality, % 6.25 ppm	1.25 ppm	0.25 ppm	0.05 ppm	0.01 ppm	LC_{90}, ppm
1				100	66	0.038
2	100	78	17	3		4.0
3	100	100	97	35		0.23

isomer 3 is 0.2 ppm, only one-fifth of the natural product.

Chromatograms corresponding to partial isomerization of the individual compounds are shown in Figure 1, and the reaction profiles are plotted in Figure 2, with peak areas normalized to that of the initial. The isomerization previously reported for ivermectin is confirmed here for avermectin B_{1a}. In addition, the behavior of the 2-epimer 2 is seen to be analogous: Initial epimerization to the natural product is followed by ultimate formation of the Δ^2 isomer 3. Both forward and reverse epimerizations appear to follow pseudo-first-order kinetics during the initial stage of the reaction with equal rate constants (~0.011 min^{-1} in 0.05 M hydroxide in 50% MeOH–H_2O), indicating that there is no significant energy difference between the two forms. This tendency toward formation of an equilibrium mixture of epimers is overridden, however, by conversion to the energetically favored conjugated isomer 3. Compound 3 is itself unstable in hydroxide, but there is no measurable conversion to either of the two epimeric Δ^3 compounds (1 or 2), a consequence of the lack of a proton sufficiently acidic for removal under these mild conditions. The kinetic profile shown in Figure 2B demonstrates that Fraser-Reid's specified reaction time of 1 h produces an optimum conversion of 20–25% for the specific conditions 0.05 M NaOH in 50% aqueous methanol at room temperature. Presumably the yield can be increased by establishing conditions where epimerization is kinetically more favored relative to the conjugation reaction and by isolating and recycling unepimerized 2 in a subsequent hydroxide-catalyzed equilibration.

Registry No. 1, 65195-55-3; 2, 106434-14-4; 3, 110415-68-4; avermectin B_1, 71751-41-2.

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[4 + 2] Cycloaddition of Conjugated Azomethines to Aryl Isothiocyanates and Fungitoxicity of the Resulting 6,7-Dihydro-1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5-thiones

Lal Dhar S. Yadav,*[1] Atma R. Misra, and Harendra Singh

[4 + 2] cycloaddition of conjugated azomethines, 5-aryl-2-[(*p*-fluorobenzylidene)amino]-1,3,4-oxadiazoles IIIa–d, to aryl isothiocyanates affords a novel class of compounds, 2,6,7-triaryl-6,7-dihydro-1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5-thiones IVa–l. Condensation of 2-amino-5-aryl-1,3,4-oxadiazoles IIa–d with *p*-fluorobenzaldehyde furnished the requisite azomethines IIIa–d. The compounds IVa–l have been compared with Dithane M-45, a commercial fungicide, for their fugitoxic action against *Aspergillus niger* and *Fusarium oxysporium*, and the results correlated with their structural features.

Encouraged by the significant fungitoxicity displayed by some 1,3,4-oxa(thia)diazolo[3,2-*a*]-*s*-triazine-7-thiones reported in our earlier communications (Bhattacharya et al., 1982; Singh et al., 1981, 1983b), we considered it of interest to synthesize more compounds of this class with certain structural modifications. Thus, the title compounds with partial saturation in the *s*-triazine nucleus and thione function at position 5 instead of at position 7 have been synthesized and evaluated for their antifungal activity. The presence of the fluoroaryl moiety in these compounds is expected to enhance their fungitoxicity (Filler and Kobayashi, 1983). The investigation appeared

Department of Chemistry, University of Gorakhpur, Gorakhpur 273009, India.

[1] Present address: Department of Chemistry, M.G. Degree College, Gorakhpur 273001, India.

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