OMe·HCl, 5680-80-8; H-DL-Ser-OMe·HCl, 5619-04-5; Cl_3CCH_2OCO-DL-Orn-OMe·HCl, 102921-63-1; MeOCO-L-Orn-OCH_2CCl_3·HCl, 102921-64-2; H-DL-Orn(Cbz)-OMe·HCl, 97371-32-9; H-L-Orn(Cbz)-OMe·HCl, 5874-75-9; H-L-Glu(OMe)-OMe·HCl, 23150-65-4; H-DL-Glu(OMe)-OMe·HCl, 13515-99-6; H-Gly-NH-D-CH(COOMe)CH(OH)COOMe·HCl, 103001-62-3; H-Gly-NH-L-CH(COOMe)CH(OH)COOMe·HCl, 103001-63-4; H-Gly-NH-DL-CH(COOMe)CH(OH)COOMe·HCl, 103001-64-5; H-D-Phe-NHCH(COOMe)CH(OH)COOMe·HCl, 102921-65-3; H-L-Phe-NHCH(COOMe)CH(OH)COOMe·HCl, 103001-65-6; (±)-$NH_2OCH(Me)Ph$, 102921-66-4; (±)-$NH_2OCH(Me)COOMe$, 102921-67-5.

Mercuric Acetate Oxidation of Avermectin A_{2a} as a Route to the Selective Cleavage of the Allylic C-5-Methoxy Group

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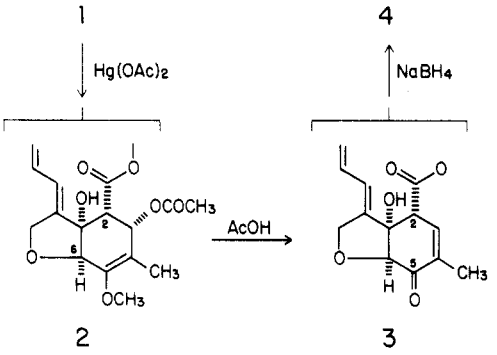
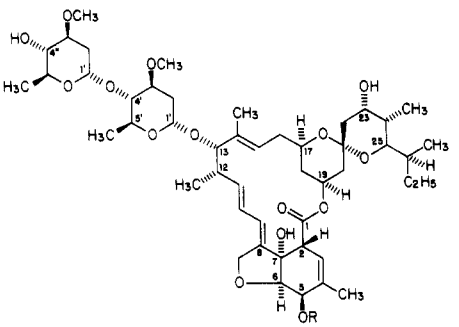
The avermectins[1] and the milbemycins[2] are two groups of closely related 16-membered macrocyclic lactones. Avermectins containing an unsubstituted allylic hydroxy group at their 5-position are of particular interest because of their potent antiparasitic[3] and insecticidal[4] effects. The fermentation of the actinomycete *Streptomyces avermitilis*, however, produces avermectins in mixtures containing both 5-hydroxy and the less potent 5-methoxy derivatives,[5] and it is therefore desirable to cleave selectively the 5-methyl ether bond to obtain the corresponding alcohols. A 5-methoxy group can also serve as a convenient protecting group in the total syntheses of avermectins and milbemycins containing the sensitive oxahydrindene part structure,[6] provided a suitable deprotection method exists. The avermectins have additional methoxy groups at the 3′- and 3″-positions and also contain glycoside bonds of 2-deoxy sugars which are highly susceptible to acid hydrolysis, so that conventional acidic ether cleavage reactions do not appear promising.[7] They are unstable under certain basic conditions due to epimerization at C-2 and double bond migration from the 3,4- to the 2,3-position into conjugation with the lactone carbonyl.[8] Therefore basic nucleophiles must be avoided. During related oxidation experiments we found that mercuric acetate reacts selectively with the 3,4-double bond resulting in substitution at C-3 and shift of the double bond into the 4,5-position, thus transforming an allylic methoxy group into a hydrolytically labile vinyl ether. Accordingly, avermectin A_{2a} (1) gives upon heating with $Hg(OAc)_2$ in toluene at 100 °C for 30–60 min the 3α-acetoxy 4,5-enol methyl ether **2** as the major product in good yield (Scheme I). The structure determination of **2** is based on the comparison of the proton NMR spectra of **1**[1a] and **2**. Compound **2** shows a new methyl singlet at δ 2.15 for the acetyl group, two sharp doublets at δ 5.73 and 2.92 with a coupling constant of 4.3 Hz for the C-3 and the C-2 protons, a singlet at δ 4.22 for C-6-H, and minor shifts of three singlets at δ 4.68, 3.77, and 1.73 for C-7-OH, C-5-OCH_3, and C-4-CH_3 groups. ^{13}C NMR and mass spectra are in agreement with the proposed structure. Epimerization of the C-2 proton during this reaction is not likely since subsequent reaction products (see below) contain the natural 2β-H configuration. The acetoxy enol ether **2** is not fully stable under the reaction conditions and is slowly transformed into a new compound which was identified as the known avermectin B_{2a} 5-ketone **3**, previously obtained from avermectin B_{2a} by MnO_2 oxidation.[9] Proton NMR, mass, and UV spectra as well as HPLC and TLC of the avermectin A_{2a} derived reaction product **3** and of authentic **3**[9] are identical in all respects. The ketone **3** can be obtained readily from acetoxy enol ether **2** or its crude reaction mixture by hydrolysis of the enol ether in glacial acetic acid at room temperature, which occurs with simultaneous elimination of the 3α-acetoxy group. The stereospecific reduction of ketone **3** with $NaBH_4$ to the naturally occurring avermectin B_{2a} (**4**) is described,[9] and when carried out with the avermectin A_{2a} derived ketone **3** afforded a product indistinguishable from natural avermectin B_{2a} by TLC, HPLC, and 400-MHz ^1H and ^{13}C NMR. Since the

Scheme I





1. R=CH_3 Avermectin A_{2a}
4. R=H Avermectin B_{2a}

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C-2 proton is obscured in the ^1H NMR spectrum of **3** but clearly visible in **4** the 2β-H configuration could be determined rigorously for **4** and thus indirectly for its precursor **3**. This three-step procedure formally accomplishes the conversion of avermectin A_{2a} into the desired avermectin B_{2a}. It should have general application for the transformation of 5-methoxy containing avermectins and milbemycins to their 5-hydroxy analogues.

Experimental Section

The natural products **1** and **4** were obtained from A. J. Kempf and Dr. K. E. Wilson, Merck Sharp and Dohme Research Laboratories, Natural Products Isolation Department. All compounds were in form of amorphous lyophilizates containing up to 6% of the 27-desmethyl lower homologues (the "b" series).[1a] Reaction products were purified by chromatography on silica gel GF Uniplates, Analtech, 0.25–1.0-mm thickness, and/or by reverse-phase high-performance liquid chromatography on a Whatman Partisil M9 10/50 ODS-3 column. Purity of products and progress of reactions were determined by analytical TLC on silica gel plates, visualized by UV fluorescence and staining with phosphomolybdic acid, and analytical HPLC on a Whatman Partisil PXS 10/25 ODS-3 column using UV absorption at 254 nm for detection. ^1H and ^{13}C NMR spectra were recorded on Varian XL-200 and XL-400 instruments in $CDCl_3$ solution with Me_4Si as internal reference. Mass spectra were obtained on an LKB Model 9000 or Varian MAT 212 mass spectrometer.

3α-Acetoxy-5-dehydro-3-hydroavermectin A_{2a} (2). A solution of avermectin A_{2a} (250 mg, 0.277 mmol) and $Hg(OAc)_2$ (250 mg, 0.784 mmol) in 4.0 mL of anhydrous toluene was stirred under N_2 in an oil bath at 100 °C for 40 min, when HPLC (8:2 MeOH–H_2O, 1.0 mL/min) indicated the completion of the reaction and a product composition of 27% of 5-ketoavermectin B_{2a} (**3**), t_r 9.5 min, less than 1% of starting material **1**, t_r 10.5 min, and 73% of product **2**, t_r 14.4 min. The reaction mixture was filtered and the solid residue washed with ca. 75 mL of EtOAc. The filtrate was washed with water, aqueous $NaHCO_3$, H_2O (2x), dried over $MgSO_4$, and concentrated in vacuo to 274 mg of yellow glass. This crude reaction mixture (30 mg) was purified on four preparative 0.25 mm thick silica gel plates, developed with a cyclohexane–acetone (7:3) mixture, giving three narrow partly overlapping bands centered at R_f 0.5. The fastest band afforded 13.5 mg of white glass, which was dissolved in benzene and freeze-dried; HPLC and TLC showed it to be a mixture consisting of 80% of **2** and 20% of 5-ketone **3**: UV λ_{max} (MeOH) 243, 236, and shoulder 251 nm (ε 31 400, 29 300, 20 600). Anal. Calcd for $C_{51}H_{78}O_{17}$ (963.180): C, 63.68; H, 8.16. Found: C, 63.73; H, 8.20. MS (field desorption), *m/e* 920 [(M + NH_4 – AcOH)$^+$], 902 [(M + NH_4 – AcOH – H_2O)$^+$], 884, 867, ~852, ~757, 740, 722, ~708, 615, 599, 597, 579, 565, 387, 323, 305, 273, 259, 257, 179, 162; 200-MHz ^1H NMR ($CDCl_3$) δ 6.00 (1 H, br d, *J* ~9 Hz, C_9H), ~5.75 (2 H, m, $C_{10}H$ + $C_{11}H$), 5.73 (1 H, d, *J* = 4.3 Hz, C_3H; s upon irradiation of δ 2.92 d), 5.45 (1 H, d, 3.3 Hz, $C_{1'}H$), 5.36 (1 H, m, $C_{19}H$), 4.96 (1 H, m, $C_{15}H$), 4.79 (1 H, d, *J* = 3.3 Hz, $C_{1'}H$), 4.68 (1 H, s, C_7OH), 4.65 (2 H, br s, $C_{8a}H_2$), 4.22 (1 H, s, C_6H), 3.98 (1 H, br s, $C_{13}H$), 3.77 (3 H, s, C_5OCH_3), 3.48 and 3.47 (2 × 3 H, 2 s, $C_{3'}$ and $C_{3''}OCH_3$), 3.28 (1 H, t, *J* = 9.0 Hz, $C_{4'}H$), 3.2 (1 H, br t, *J* = 9.0 Hz, $C_{4''}H$), 2.92 (1 H, d, *J* = 4.3 Hz, C_2H, s upon irradiation of 5.73 d), 2.15 (3 H, s, $C_{3a}OCOCH_3$), 1.73 (3 H, s, C_4CH_3), 1.53 (3 H, s, $C_{14}CH_3$).

5-Ketoavermectin B_{2a} (3). A solution of 100 mg of crude oxidation product containing 45% of **2** and 41% of **3** (HPLC, 8:2 MeOH–H_2O, 1.5 mL/min; t_r 9.5 and 6.3 min) in addition to two minor impurities (5% and 7% with t_r 5.4 and 8.1 min) in 5.0 mL of AcOH was kept at 18 °C for 5 h. The reaction mixture was diluted with 5 mL of MeOH and concentrated in vacuo. The residue was dissolved in toluene and concentrated in high vacuum to 103 mg of light glass, which was dissolved in CH_2Cl_2, and applied to a 1 mm thick silica gel plate and run in a cyclohexane–acetone (7:3) solvent system. The major band was extracted to give 68 mg crude **3** (HPLC, 85:15 MeOH–H_2O, 1.0 mL/min, t_r 8.3, 9.8, 12.8 min corresponding to 10%, 83%, 6%; 200-MHz^1 H NMR identical with that of authentic **3**[9]). Further purification was achieved by chromatography in two 28-mg batches on a Whatman M9 ODS-3 column, MeOH–H_2O (85:15), 4.0 mL/min, giving 37 mg of **3**: HPLC (8:2 MeOH–H_2O, 1.5 mL/min) t_r 11.0, 14.1 min (5%, 93%); UV λ_{max} (MeOH) 242 nm (ε 28 060); MS, *m/e* 888 (M^+), 870, 744, 726, 708, 582, 564, 547, 546, 323, 305, 259, 257, 239, 221, 145, 127, 113; 200-MHz ^1H NMR ($CDCl_3$) δ 6.58 (1 H, br dd, *J* = 1.6 and 2.6 Hz, C_3H; irradiation of C_2H at δ 3.60 gives br d, *J* = 1.6 Hz; irradiation of C_4CH_3 at δ 1.91 gives d, *J* = 2.5 Hz), 3.92 (1 H, s, C_7OH), 3.88 (1 H, s, C_6H), 3.60 (1 H, m, C_2H), 1.91 (3 H, dd, *J* = 1.6 and 2.6 Hz, C_4CH_3, irradiation of C_3H at δ 6.60 gives d, *J* = 2.6 Hz, irradiation of C_2H at δ 3.60 gives d, *J* = 1.6 Hz).

Authentic 5-ketoavermectin B_{2a} (3) was prepared from 100 mg of **4** by MnO_2 oxidation[9] and purified by preparative TLC (7:3 cyclohexane–acetone) giving 51 mg of **3**: HPLC (8:2 MeOH–H_2O, 1.5 mL/min) t_r 10.7, 14.0 min (16%, 84%); UV λ_{max} (MeOH) 242 nm (ε 27 080); MS, *m/e* 871 (M^+ – 17), 744, 726, 708, 582, 564, 547, 546, 323, 305, 259, 257, 239, 221, 145, 127, 113; 200-MHz ^1H NMR ($CDCl_3$) identical with that of avermectin A_{2a} (**1**) derived **3**.

Avermectin B_{2a} (4). Crude oxidation product (250 mg) containing 42% of **2** and 46% of **3** was dissolved in 5.0 mL of AcOH and kept at 18 °C for 3.5 h. The reaction mixture was diluted with 50 mL of toluene and concentrated at 18 °C under high vacuum. The residue was dissolved again in toluene, and concentrated to 280 mg of a yellow foam. TLC and HPLC (48:52:20 CH_3CN–MeOH–H_2O, 1.5 mL/min; t_r 6.5 min) shows one major component (80% of area) which was characterized by NMR as **3**. The crude ketone was dissolved in 6.0 mL of EtOH, cooled to −15 °C, and stirred under N_2. Then 12.5 mg of $NaBH_4$ was added in one portion. After 20 min 35 mL of 0.1 N aqueous AcOH was added, and the white precipitate was filtered and washed with water. The residue was dissolved in EtOAc and concentrated in vacuo to 242 mg white glass, HPLC (48:32:20 CH_3CN–MeOH–H_2O, 1.5 mL/min) t_r 5.2 min, 74% of area, identical with a sample of avermectin B_{2a} obtained by fermentation in HPLC, TLC, and NMR. The crude product was further purified on four 1 mm thick silica gel plates with CH_2Cl_2–MeOH (95:5), giving 138 mg white foam, which was freeze-dried from benzene. The 400-MHz ^1H NMR spectrum, HPLC (45:30:25 CH_3CN–MeOH–H_2O, 1.5 mL/min) t_r 6.1 and 7.4 min, 5 and 91% of area, avermectin B_{2b} accounting for 5%), and UV λ_{max} (MeOH) 245 nm (ε 28900) are identical with those of authentic avermectin B_{2a}.

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A Reinvestigation of the Reaction of Bromine with 5β-Estrane-3,17-dione

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Previous work[1] directed toward the preparation of various estrenes for estrogen binding studies showed that 5β-estrane-3,17-dione (**1**) reacts with phenylselenyl chloride with enolization toward C-2. The work of Rapala and Farkas[2] in 1958 attracted our attention since they described the synthesis of 4β-bromo-5β-estrane-3,17-dione (**2**) by bromination indicating enolization toward C-4. The assignment of the 4β-bromo group in compound **2** was based on two facts: (1) Other 3-keto 5β-steroids are known to produce predominantly the 4β-bromo products since enolization of 3-keto 5β-steroids is directed primarily towards C-4.[3] (2) Dehydrobromination of compound **2** with

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