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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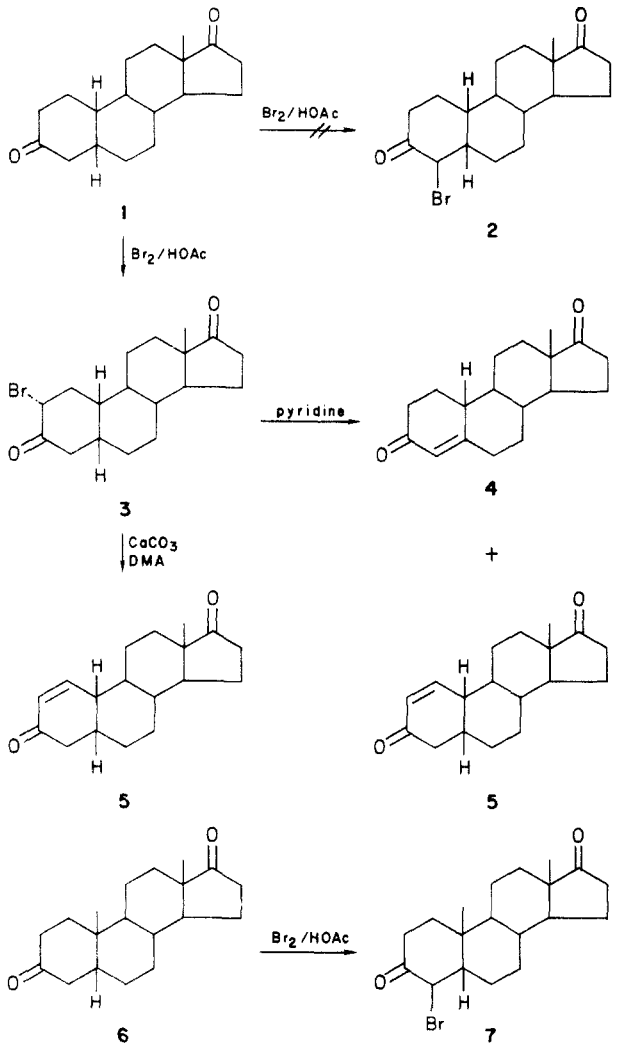
Scheme I

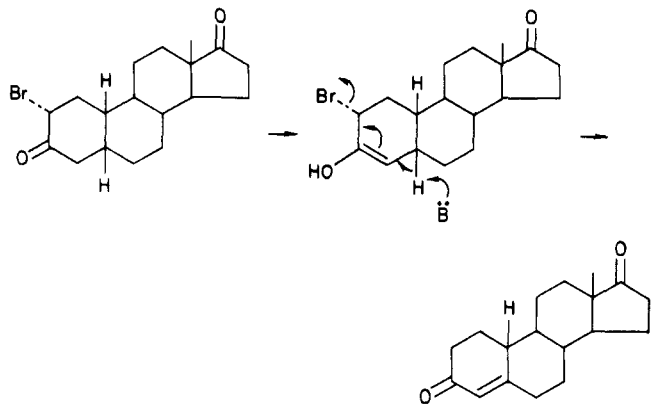


refluxing pyridine gave 4-estrene-3,17-dione (**4**).[2]

In an effort to obtain compound **2**, we repeated this work and found that the product isolated in our hands had the same melting point as that of compound **2**. However, ^{1}H NMR analysis of the isolated product showed a characteristic quartet centered at δ 4.64 (J = 4.2 Hz). This pattern is indicative of an equatorially oriented proton at C-2, which led us to the assignment of 2α-bromo-5β-estrane-3,17-dione (**3**) as the correct product obtained from bromination of dione **1** in acetic acid. Comparison of ^{1}H NMR of 4β-bromo-5β-androstane-3,17-dione (**7**) obtained from bromination of compound **6** showed a doublet at δ 4.94 (J = 11.5 Hz) for 4α-H which is completely different from that obtained for compound **3**.

The major criteria used by Rapala and Farkas for assignment of the bromo group to the 4β-position was the fact that refluxing with pyridine results in formation of 4-estrene-3,17-dione (**4**) (Scheme I). Indeed, when compound **3** was refluxed with pyridine, the major product obtained is compound **4** (66% yield). In addition, 5% yield of 5β-estr-1-ene-3,17-dione (**5**) was also isolated. While the mechanism for dehydrobromination of compound **3** by pyridine leading to the formation of compound **4** is not firmly established, the pathway shown in Scheme II is being proposed at this time. Earlier studies by Warnhoff[4] showed that dehydrobromination of 2α-bromo-5α-cholestenone with pyridine and substituted pyridines leads to a mixture of the Δ^1- and Δ^4-cholestenones. Further support for the assignment of the bromo group to the 2-position was obtained from dehydrobromination of compound **3** by refluxing with $CaCO_3$ in dimethylacetamide as described by Green and Long.[5] This method is known to be more specific than pyridine and leads to the elimination of a proton α to the bromo group. Only compound **5** was obtained from dehydrobromination of **3** with $CaCO_3$/dimethylacetamide.

Scheme II



The results obtained from this study indicate that enolization of 19-nor 3-keto 5β-steroids is directed toward C-2 as opposed to C-4 which seems to be the case with C-19 methyl 3-keto steroids.[3] These studies support our earlier findings on the direction of enolization during the phenylselenenylation of 19-nor 3-keto 5α- and 5β-steroids.[1]

In summary, the earlier report on the structural assignment of the product obtained from bromination of 5β-estrane-3,17-dione (**1**) is incorrect and that the correct structure should be 2α-bromo-5β-estrane-3,17-dione (**3**).

Experimental Section

Melting points (uncorrected) were obtained on a Fisher-Johns apparatus. NMR spectra were obtained with a JEOL-90Q spectrometer. High-resolution mass spectra were taken on LKB-9000.

General Bromination Procedure. To a solution of 3-keto steroid (350 mg, 13 mmol) in glacial acetic acid (6 mL) was added a solution (4 mL, 1 M) of bromine in glacial acetic acid, dropwise with stirring, at room temperature. The reaction mixture was kept at room temperature for an additional 10 min, after which it was poured over water and extracted with $CHCl_3$. The chloroform fraction was dried (Na_2SO_4), filtered, and evaporated to give a residue from which pure α-bromo-3-keto steroids were obtained through open-column chromatography.

2α-Bromo-5β-estrane-3,17-dione (3). 5β-Estrane-3,17-dione (**1**) gave **3**: 86% yield; mp 186–188 °C; NMR ($CDCl_3$) δ 0.90 (s, 3 H, C-18 Me), 4.64 (q, 1 H, J = 4.2 Hz, C-2 H); mass spectrum, m/z 353 (M^+).

4β-Bromo-5β-androstane-3,17-dione (7). 5β-Androstane-3,17-dione (**6**) gave **7**: 79% yield; mp 192–194 °C; NMR ($CDCl_3$) δ 0.90 (s, 3 H, C-18 Me), 1.10 (s, 3 H, C-19 Me), 4.94 (d, 1 H, J = 11.5 Hz, C-4 H).

Dehydrobromination of Compound 3 with Pyridine. 2α-Bromo-5β-estrane-3,17-dione (**3**) was dehydrobrominated by refluxing in pyridine for 12 h. The reaction mixture was poured into water and extracted with methylene chloride. The extract was washed with 1 N HCl solution and water, dried (Na_2SO_4), and evaporated to dryness. The residue was streaked over preparative silica gel (UV) plates and developed in benzene–ethyl acetate (3:1). The areas corresponding to the UV absorbing bands were separated, eluted, and characterized. The spectral data for the major product (66% yield) was found to be identical in all

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respects with an authentic sample of 4-estrene-3,17-dione (4). The minor product was isolated in 5% yield and showed the following physical characteristics: mp 185–187 °C; ^{1}H NMR δ 0.93 (s, 3 H, C-18 Me), 5.99 (d, 1 H, J = 10 Hz, C-2 H), 7.08 (dd, 1 H, J = 10 Hz, J = 2 Hz, C-1 H). These data are essentially similar to those reported earlier[1] for 5β-estr-1-ene-3,17-dione (**5**).

Dehydrobromination of Compound 3 with $CaCO_3$/Dimethylacetamide. Compound **3** (50 mg) dissolved in dimethylacetamide (0.8 ml) was added portionwise to calcium carbonate (80 mg) in boiling dimethylacetamide (3 mL) during 3 min, and refluxing was continued for 15 min. Some of the solvent was distilled under vacuum; the residue was extracted with ether and washed with HCl and water. The ether was dried and evaporated and the residue streaked over silica gel plate as described above. Only one UV absorbing band was observed, which following elution gave 42 mg (84%) of pure product, which was found to be identical in all respects with 5β-estr-1-ene-3,17-dione (**5**).

Registry No. **1**, 5696-51-5; **3**, 102922-53-2; **4**, 734-32-7; **5**, 101469-27-6; **6**, 1229-12-5; **7**, 4588-83-4.

Effect of pH on the Regioselectivity of Pictet–Spengler Reactions of 3-Hydroxyphenethylamines with Formaldehyde and Acetaldehyde

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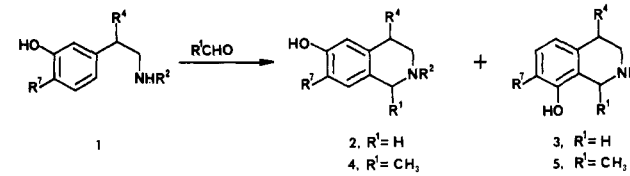
The Pictet–Spengler condensation of 3-alkoxyphenethylamines or 3-hydroxyphenethylamines **1** with aldehydes is widely utilized in the synthesis of tetrahydroisoquinolines[1] and also serves as the biosynthetic route to these alkaloids.[2] Cyclization has generally been reported to proceed para to the activating 3-alkoxy or 3-hydroxy group, thereby generating 6-alkoxy- or 6-hydroxytetrahydroisoquinolines (i.e. **2** or **4**), respectively.[1,3–5] With formaldehyde, cyclization ortho to the alkoxy or hydroxy group, forming 8-alkoxy- or 8-hydroxytetrahydroisoquinolines (i.e., **3**) has been reported to accompany para cyclization.[1,6–11] However very few instances of ortho cyclization with aldehydes other than formaldehyde have been reported.[8,9,12,13] and a systematic quantitative investigation of regioselectivity has not been performed.

We recently observed that condensation of norepinephrine (**1g**) and epinephrine (**1h**) with formaldehyde and acetaldehyde in neutral to mildly acidic aqueous solution affords 20–50% of the unexpected tetrahydro-4,7,8-isoquinolinetriols (**3g**, **3h**, **5g**, and **5h**) respectively, as well as the expected tetrahydro-4,6,7-isoquinolinetriols (**2g**, **2h**, **4g**, and **4h**).[8,9] We now report a systematic investigation of the regioselectivity of Pictet–Spengler condensations of a series of 3-hydroxyphenethylamines (**1**) with formaldehyde and acetaldehyde.

Results and Discussion

A series of 3-hydroxyphenethylamines **1** was treated with 8 equiv of aqueous formaldehyde or acetaldehyde at pH 2–8.5 at 20 °C. The reaction progress and product distribution were followed by thin-layer chromatography and liquid chromatography. In accord with Pictet–Spengler reactions of other 3-hydroxyphenethylamines,[1,5,8,9,13,14] the reaction rate was strongly influenced by pH. For example, the half-life for the reaction of **1d** with formaldehyde was 12 min at pH 2 and less than 1 min at pH 7. The half-life for the reaction of **1d** with acetaldehyde was 1.5 h at pH 2 and less than 1 min at pH 7. At pH 2, the yield of tetrahydroisoquinolines was nearly quantitative. At pH 7, the *N*-methyltetrahydroisoquinolines were obtained in high yields, while the yields of the N-unsubstituted tetrahydroisoquinolines were somewhat lower (60–80%) due to competing side reactions with excess aldehyde.[9]

The regioselectivities of the Pictet–Spengler cyclization of **1** (including those investigated previously[8,9]), determined by analytical liquid chromatography, are summarized in Table I as percentage of ortho cyclization. The regioselectivity is influenced only subtly by the aldehyde or the substituents on **1**. At pH 2, cyclization occurs exclusively or primarily para to the activating aromatic hydroxy group, affording **2** and **4**. At pH 5, significant cyclization ortho



1 2, R¹ = H; 4, R¹ = CH₃ 3, R¹ = H; 5, R¹ = CH₃

to the activating hydroxy group occurs, affording **3** and **5** as well as **2** and **4**. The amount of ortho cyclization is greatest at pH 7, and generally somewhat less at pH 8.5. Since the ratio of isomeric products does not vary significantly during the course of the reaction, selective destruction of one isomer apparently does not occur.

All of the tetrahydroisoquinoline products except **4b** and **5d** were previously known (see references in Table I) and were chromatographically and spectrally identical with

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