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Photoisomerization of Aromatic Doramectin Derivatives

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Doramectin, an avermectin derivative, is an animal health drug candidate for the prophylaxis and control of endo- and ectoparasitic infections. Conversion of avermectins to highly fluorescent aromatic derivatives is used extensively for their quantitation by HPLC with fluorescent detection. It has been found that aromatic doramectin derivatives are readily equilibrated with their 8,9-*Z* isomers under ambient laboratory lighting. This may be a source of assay error which can be easily avoided by protection from light.

Doramectin (**1a**, Figure 1) is an avermectin analog with a cyclohexyl group at position 25 which can be obtained by feeding cyclohexanecarboxylic acid to a strain of *Streptomyces avermitilis* (Dutton et al., 1991; Hafner et al., 1991). Currently, it is an animal drug candidate for the prophylaxis and control of endo- and ectoparasitic infections.

The unprecedented potency of avermectins (Burg et al., 1979; Egerton et al., 1979) has mandated the development of sensitive methods for their quantitative analysis in biological and environmental matrices. Aromatization of the C2-7 ring has been used extensively to produce highly fluorescent derivatives which allow quantitation at less than the parts per billion level by HPLC with fluorescence detection. Derivatization may be accomplished under either acylation conditions (Mrozik et al., 1982; Tolan et al., 1980; Tway et al., 1981; De Montigny et al., 1990; Prabhu et al., 1991), yielding structures **2** (Scheme 1), or MnO_2 oxidation (Chabala et al., 1981) followed by dehydration (Stong, 1987), leading to structures **5**.

Although the UV-promoted isomerization of parent avermectins has been elegantly described by Mrozik et al. (1988), the photoisomerization of the aromatized derivatives has not been reported in the literature. We present herein our work on the chemistry surrounding the aromatization of doramectin and its derivatives which impact upon assay methods development.

RESULTS AND DISCUSSION

Treatment of **1a** with excess trifluoroacetic anhydride/triethylamine in acetonitrile at room temperature resulted in rapid conversion to the 4″-*O*-trifluoroacetylated aromatic derivative **2b**. Although **2b** was stable when dry, in the presence of traces of water or methanol it was slowly detrifluoroacetylated, which rendered it a poor analyte. We therefore have converted **2b** to the more stable **2a** by brief treatment with methanol/triethylamine at room temperature. In an alternative aromatization sequence, oxidation of **1a** with activated MnO_2 furnished the 5-keto derivative **4a**, which in turn, upon treatment in the dark with ethanolic ammonium acetate at reflux, yielded phenol **5a**.

During HPLC determination of the stability of **2a** in dilute solutions kept in clear glass vials, we noticed its smooth conversion to a new material (**3a**). The reaction stopped when the **2a**:**3a** ratio was approximately 3.5:1, as monitored by detection at 245 nm. Further studies revealed that this transformation is light-promoted and does not occur in the dark. In addition, **3a** was obtained pure by preparative HPLC and shown to yield the same

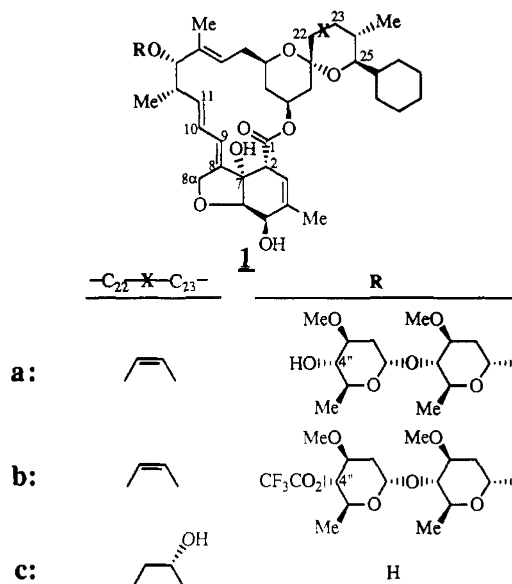

Figure 1. Structure of doramectin (**1a**) and derivatives.

3.5:1 **2a**:**3a** equilibrium mixture upon exposure to ambient laboratory lighting, within 3 h. The photoequilibration of **2a** is not readily apparent with the usual fluorometric detection. The **2a**:**3a** equilibrium ratio measured by excitation at 360 nm and emission at 470 nm was about 16:1. A similar light-promoted equilibration was observed with phenol **5a**, yielding an approximately 3:1 **5a**:**6a** mixture. In this case, both **5a** and **6a** were labile, being slowly converted to non-UV-absorbing species upon prolonged exposure to light.

Characterization of compound **3a** proved difficult. The mass spectrum contained a peak at m/z 881 corresponding to the ammonium adduct of the structure isomeric to **2a**. Literature precedent (Mrozik et al., 1988) suggested isomerization at the C8-C11 diene with formation of either the 10,11-*Z* or 8,9-*Z* structure. The NMR spectra recorded at room temperature in $CDCl_3$ gave several very broad signals. Similar NMR peak broadening has been reported (Mrozik et al., 1988) with the 10,11-*Z* avermectin B_1 isomer but not its aglycon. To facilitate NMR interpretation, we prepared the aromatized aglycon **2c** and its photoisomer **3c**. Only when the compound was warmed to 55 °C were sharp NMR signals obtained from **3c**. The close proximity in space between H_2-8α and H-9 in the 8,9-*Z* isomer would be expected to result in a nuclear Overhauser effect (NOE) (Mrozik et al., 1988). Indeed, irradiation of the 8α-methylene of **3c** gave a 5% NOE enhancement on H-9.

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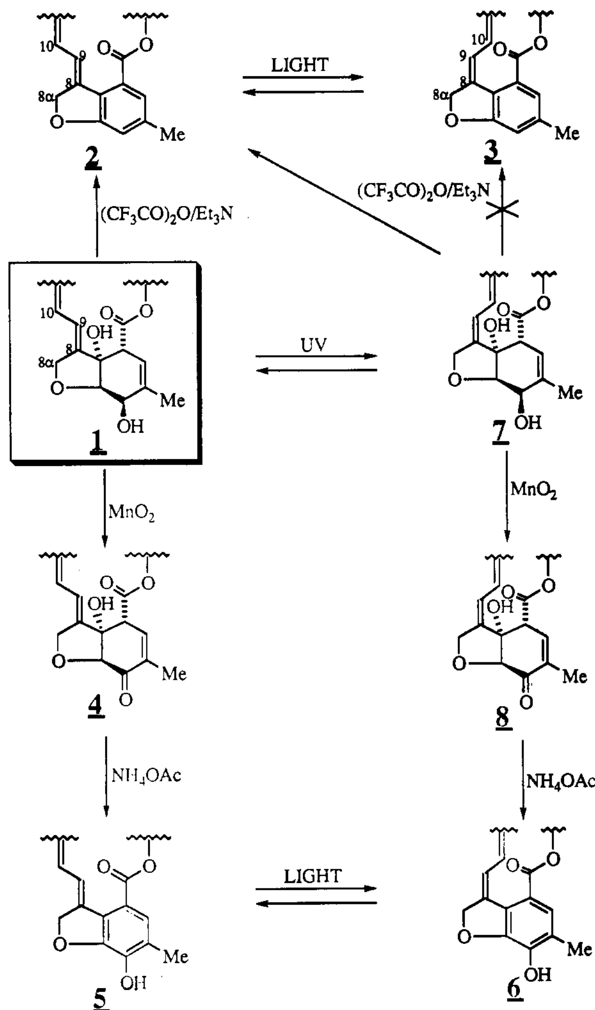
Scheme 1. Chemical Transformations



When the same NMR experiment was run on **2c**, we observed a 5% NOE on H-10, not H-9. These results are consistent with assignment of the 8,9-*Z* structure to the aromatic photoisomers **3** and **6**.

Further confirmation of this structure assignment would be obtained if doramectin (**1a**) could be converted to **6a** and/or **3a** by an alternate unambiguous route. Irradiation of **1a**, as described in the literature for avermectin B_{1a} (Mrozik et al., 1988), furnished a three-component mixture from which the 8,9-*Z* doramectin isomer **7a** was isolated by preparative chromatography. Oxidation of **7a** with MnO_2 followed by treatment with ammonium acetate in the dark furnished a product with HPLC retention time identical to that of **6a**. Furthermore, brief exposure of this product to light gave the identical **5a**:**6a** photoequilibrated mixture.

An attempt to convert **7a** to **3a** by treatment with excess trifluoroacetic anhydride/triethylamine under the usual conditions did not yield the expected product but, instead, furnished the natural **2a** derivative. A similar transformation was recently reported for the analogous 8,9-*Z* abamectin isomer (Prabhu et al., 1992). These results suggest that the aromatization in this case proceeds via a carbonium ion at C-7 which effects conversion to the more stable 8,9-*E* structure.

In conclusion, aromatic doramectin derivatives were found to be extremely light sensitive, in contrast to nonaromatic avermectins which require intense UV irradiation for isomerization (Mrozik et al., 1988). Since the aromatization of avermectins is used routinely for their quantitative determination, the observed facile photoequilibration of the aromatized derivatives under ambient laboratory lighting may be a source of significant assay error. This may be easily avoided by handling the analytical solutions in amber glass. The conversion of **7a** to **2a** (not **3a**) under the usual derivatization conditions may also result in error when avermectins are assayed in the presence of their 8,9-*Z* isomer.

EXPERIMENTAL PROCEDURES

General. High-pressure liquid chromatography was performed on a modular system consisting of an LDC Constametric III pump, a Valco injection valve equipped with a 20-μL loop, an LDC spectromonitor D variable-wavelength detector, and a Shimadzu C-R3A integrator. For preparative work, a 200-μL injection loop was used filled partially with a syringe. Column chromatographic purifications were performed with EM Science Silica Gel 60, particle size 0.063–0.2 mm. ^1H and ^{13}C NMR spectra were obtained on a Bruker AM-500 spectrometer operating at 500 and 125.76 MHz, respectively. Spectra were recorded in $CDCl_3$ with $CHCl_3$ (7.26 ppm for ^1H) or $CDCl_3$ (77.0 ppm for ^{13}C) as an internal standard. ^1H–^1H homonuclear shift correlation (COSY), ^{13}C distortionless enhancement by polarization transfer (DEPT), and ^{13}C–^1H heteronuclear shift correlation experiments were run to aid peak assignments.

Aromatic 4″-(Trifluoroacetyl)doramectin (2b). To a solution of 2 g of doramectin (**1a**) in 40 mL of MeCN and 6.5 mL of triethylamine at 0 °C was added 6.3 mL of trifluoroacetic anhydride over 30 min. The resultant mixture, protected from light, was allowed to warm to room temperature over 2 h and evaporated to dryness *in vacuo*. The residue was purified by silica gel chromatography eluting with 85:15 hexanes/ethyl acetate to give 1.65 g of **2b** (77%): HPLC (Beckman Ultrasphere ODS 5 μm, 4.6 mm × 25 cm, MeOH, 2 mL/min, 360 nm) RT = 5.5 min; ^1H NMR ($CDCl_3$) δ 6.88 (H-3), 6.74 (H-5), 6.17 (H-9), 5.73 (H-11), 5.18 (H_2-8α), 4.75 (H-4″); ^{13}C NMR ($CDCl_3$) δ 168.1 (C-1), 163.9 (C-6), 156.9 ($COCF_3$), 73.9 (C-8α).

Aromatic Doramectin (2a). To a solution of 200 mg of **2b** in 15 mL of MeOH in the dark was added 40 mg of triethylamine; the mixture was kept at room temperature for 20 min and then evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel eluting with 8:2 hexanes/ethyl acetate to give 96 mg of **2a** (53.4%): HPLC (as above) RT = 3.5 min; ^1H NMR ($CDCl_3$) δ 6.89 (H-3), 6.75 (H-5), 6.18 (H-9), 5.88 (H-10), 5.2 (H-8α), 3.25 (H-4′), 3.18 (H-4″); ^{13}C NMR ($CDCl_3$) δ 168.0 (C-1), 164.0 (C-6), 73.9 (C-8α).

5-Ketodoramectin (4a). To a solution of 24.5 g of **1a** in 900 mL of ether was added 36.7 g of activated MnO_2, and the mixture was stirred at room temperature for 18 h. The solids were filtered off, and the resultant solution was evaporated to dryness *in vacuo*. The crude oil was purified by silica gel chromatography eluting with 6:4 hexanes/ethyl acetate to give 17.7 g of **4a** (72.5%) as an off-white solid: HPLC (Beckman Ultrasphere ODS 5 μm, 4.6 mm × 25 cm, MeOH/H_2O/MeCN 86:9:5, 1.5 mL/min, UV 245 nm) RT = 7.8 min; HPLC (Waters μPorasil 3.9 × 300 mm, 6:4 EtOAc/hexanes, 1.0 mL/min, UV 254 nm), RT = 5.4 min; ^1H NMR ($CDCl_3$) δ 6.59 (H-3), 5.97 (H-9), 5.84 (H-11), 4.74 (H_2-8α), 3.6 (H-2); ^{13}C NMR ($CDCl_3$) δ 192.1 (C-5), 172.2 (C-1), 69.8 (C-8α).

5-Hydroxy Aromatic Doramectin (5a). To a solution of 107.3 mg of **4a** in 10 mL of EtOH in the dark was added 10 mL of a saturated solution of NH_4OAc in EtOH, and the mixture was refluxed for 45 min. After evaporation of the solvent *in vacuo*, the residue was dissolved in 15 mL of CH_2Cl_2, the resultant solution washed with 15 mL of H_2O and dried (MgSO4), and the solvent evaporated *in vacuo* to give 83.2 mg (79.2%) of **5a**: HPLC (Beckman Ultrasphere ODS 5 μm, 4.6 mm × 25 cm, MeOH/H_2O/MeCN 86:9:5, 1.5 mL/min, UV 360 nm) RT = 10.6 min; ^1H NMR ($CDCl_3$) δ 7.04 (H-3), 6.26 (H-9), 5.88 (H-10), 5.22 (H_2-8α), 2.15 (H_3-4α); ^{13}C NMR ($CDCl_3$) δ 167.8 (C-1), 150.8 (C-6), 74.1 (CH_2-8α).

Aromatic 23-Hydroxy-22,23-dihydrodoramectin Aglycon (2c). To a solution of 500 mg of **1c** in 20 mL of MeCN and 1.7 mL of triethylamine in the dark was added 1.5 mL of trifluoroacetic anhydride within 5 min, maintaining the temperature

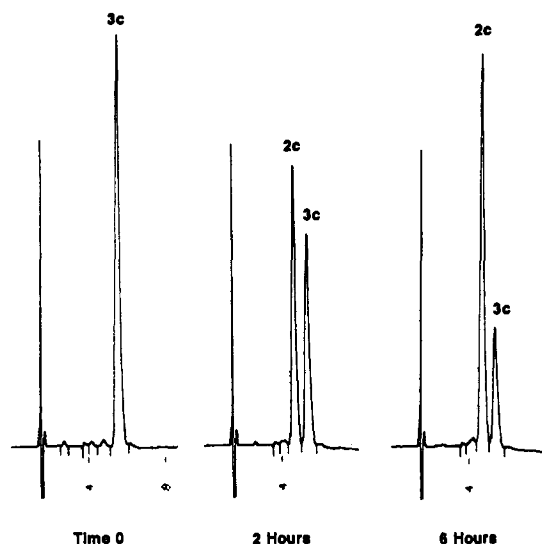


Figure 2. HPLC monitoring of photoequilibrating **3c**.

below 30 °C. The mixture was evaporated *in vacuo*, the residue taken up in 20 mL of MeOH and 0.5 mL of triethylamine, and the solution stirred at room temperature for 18 h. The solid formed was collected by filtration, washed with boiling MeOH, and dried to give 139 mg (29.5%) of **2c**: HPLC (Waters μPorasil 3.9 × 300 mm, 1:1 EtOAc/hexanes, 1.0 mL/min, UV 360 nm) RT = 4.6 min; ^{1}H NMR ($CDCl_3$) δ 6.92 (H-3), 6.72 (H-5), 6.15 (H-9), 5.87 (H-10), 5.7 (H-11), 5.17 (H_2-8α), 2.63 (H-12); ^{13}C NMR ($CDCl_3$) δ 167.5 (C-1), 164.1 (C-6), 99.8 (C-21), 74.0 (C-8α).

Aromatic (8,9-*Z*)-23-Hydroxy-22,23-dihydrodoramectin Aglycon (3c). A solution of 70 mg of **2c** in 70 mL of acetonitrile and 30 mL of methylene chloride was exposed to ambient light (near window, northern exposure) for 2 days. Monitoring by HPLC showed equilibration to a 3.2:1 **2c:3c** mixture. The solvents were evaporated *in vacuo* and the residue chromatographed in the dark on a μPorasil HPLC column eluting with 1:1 EtOAc/hexanes (1.0 mL/min, multiple injections) to obtain 12.2 mg (17.4%) of **3c**: HPLC (as for **2c**) RT = 5.3 min; ^{1}H NMR ($CDCl_3$, 55 °C) δ 6.9 (H-3), 6.74 (H-5), 6.12 (H-10), 6.06 (H-9), 5.91 (H-11), 5.03 (H_2-8α); ^{13}C NMR ($CDCl_3$, 55 °C) δ 168.2 (C-1), 164.9 (C-6), 100.1 (C-21), 78.0 (C-8α).

Photoequilibration of Aglycon 3c. A solution of 0.39 mg of **3c** in 10 mL of methanol in a clear glass vial was exposed to ambient laboratory lighting and monitored by HPLC (as described above for **2c**). After 6 h, the solution contained approximately 3:1 **2c:3c** (Figure 2). Under the same conditions, a portion of the solution kept in a brown glass vial was found to be stable.

Aromatic (8,9-*Z*)-Doramectin (3a). A solution of 180 mg of **2b** in 325 mL of methanol and 34.5 mg of triethylamine was exposed to ambient light for 2 days. Monitoring by HPLC showed complete detrifluoroacetylation and equilibration to a 3.5:1 **2a: 3a** mixture. The solvent was evaporated *in vacuo* and the residue chromatographed in the dark on a Beckman Ultrasphere ODS 5 μm 4.6 mm × 25 cm column eluting with 97:3 MeOH/H_2O (2 mL/min, multiple injections) to obtain 26.6 mg (14.8%) of **3a**: HPLC (as for **2b**) RT = 3.8 min; ^{1}H NMR ($CDCl_3$, 55 °C) δ 6.9 (H-3), 6.75 (H-5), 6.13 (H-10), 6.09 (H-9), 5.94 (H-11).

(8,9-*Z*)-Doramectin (7a). A solution of 70 mg of **1a** in 100 mL of cyclohexane in a quartz vessel was irradiated for 45 min with a Rayonet RPR-100 photoreactor equipped with 16 tubes emitting at 2537 Å. Monitoring by HPLC showed the presence of 50% starting material and 40% **7a**. The solvent was evaporated *in vacuo* and the residue chromatographed on a Beckman Ultrasphere ODS 5 μm 4.6 mm × 25 cm column eluting with 9:1 MeOH/H_2O (1.5 mL/min, multiple injections) to obtain 18 mg (25.7%) of **7a**: HPLC (Beckman Ultrasphere ODS 5 μm, 4.6 mm × 25 cm, MeOH/H_2O 9:1, 1.5 mL/min, 245 nm) RT = 9.9 min; ^{1}H NMR ($CDCl_3$) δ 6.44 (H-10), 5.85 (H-9), 5.76 (H-22), 5.72, (H-11), 5.57 (H-23), 4.55 (H_2-8α), 1.88 (H_3-4a); ^{13}C NMR ($CDCl_3$) δ 175.7 (C-1), 137.8 (C-11), 136.2 (C-22), 127.7 (C-23), 122.8 (C-10), 121.9 (C-15), 121.0 (C-9), 116.7 (C-3), 71.1 (C-8α).

(8,9-*Z*)-5-Ketodoramectin (8a). To a solution of 16.8 mg of **7a** in 15 mL of ether was added 84 mg of activated MnO_2 and the mixture stirred at ambient temperature for 2 h. After filtration, the solvent was evaporated *in vacuo* to obtain 14 mg (83.5%) of **8a**: HPLC (as for **4a**) RT = 5.9 min; ^{1}H NMR ($CDCl_3$) δ 6.44 (H-3 and H-10), 5.94 (H-9), 5.79 (H-11 and H-22), 4.66 (H_2-8α); ^{13}C NMR ($CDCl_3$) δ 191.8 (C-5), 174.4 (C-1), 139.4 (C-11), 137.3 (C-3), 136.3 (C-22), 127.6 (C-23), 123.1 (C-9), 122.6 (C-10), 122.1 (C-15), 73.0 (C-8α).

5-Hydroxy-Aromatic (8,9-*Z*)-Doramectin (6a). To a solution of 12 mg of **8a** in 3 mL of EtOH in the dark was added 3 mL of saturated solution of NH_4OAc in EtOH, and the mixture was refluxed for 45 min. After evaporation of the solvent *in vacuo*, the residue was partitioned between 5 mL of CH_2Cl_2 and 5 mL of H_2O, the CH_2Cl_2 layer evaporated, and the residue chromatographed on a Beckman Ultrasphere ODS 5 μm, 4.6 mm × 25 cm column eluting with MeOH/H_2O/MeCN 86:9:5 (1.5 mL/min, multiple injections) to obtain 2.6 mg (22%) of **6a**: HPLC (as for **5a**) RT = 12.2 min; ^{1}H NMR ($CDCl_3$, 55 °C) δ 7.01 (H-3), 6.08 (H-9 and H-10), 5.9 (H-11), 5.74 (H-22), 5.6 (H-23), 5.1 (H_2-8α), 2.17 (H_3-4α), 1.58 (H_3-14α), 1.15 (H_3-12α), 0.96 (H_3-24α).

NOMENCLATURE

Doramectin is a generic name for 25-cyclohexyl-5-*O*-demethyl-25-de(1-methylpropyl)avermectin A_{1a} (CAS Registry No. 117704-25-3). The term "aromatic" as used in this text denotes aromatization of the C2-5 ring of avermectins by dehydrations involving the hydroxyls at positions 5 and 7.

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra (19 pages). Ordering information is given on any current masthead page.

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