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C26 DIALKYL AND SPIROALKYL ANALOGS OF MARCFORTINE A

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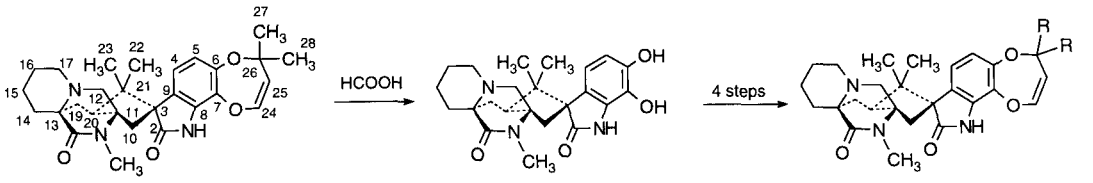
Abstract: The C26 dimethyl dioxepinoindole ring found in marcfortine A is unique among natural products. In order to ascertain the importance of the dimethyl moiety to anthelmintic activity, we prepared a variety of C26-dialkyl and spiroalkyl analogs. They include cyclobutyl, cyclohexyl, diethyl, and ethyl-methyl derivatives. This communication describes the synthesis of these compounds. © 1997 Elsevier Science Ltd.

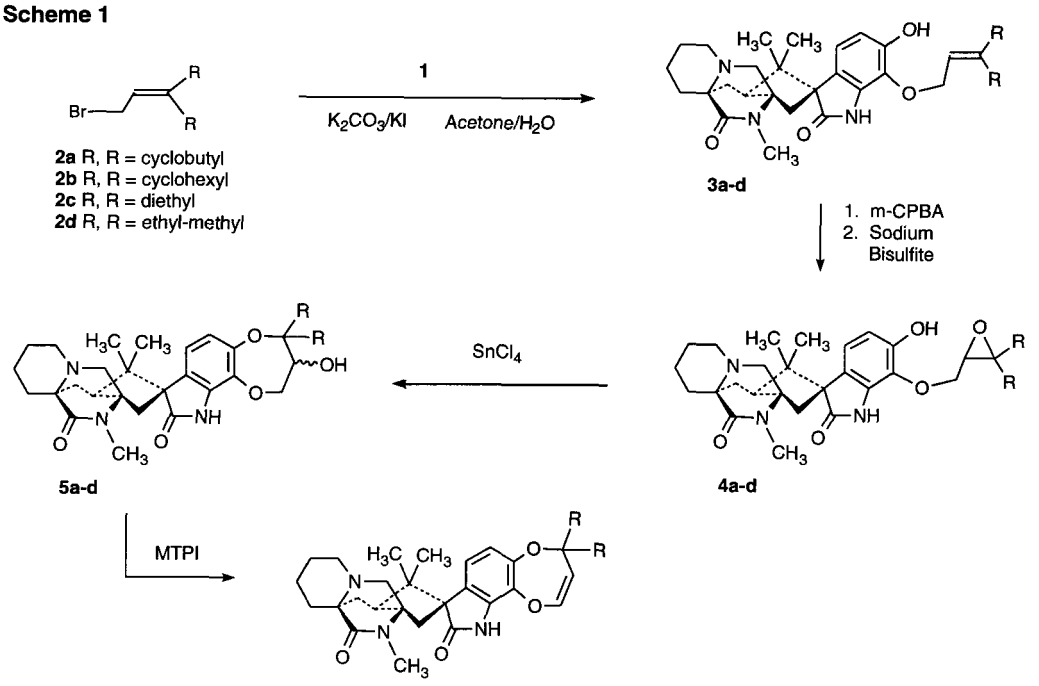
Helminths, especially parasitic nematodes, cause substantial health problems in humans and domestic animals. Currently, three distinct chemical classes are used for broad spectrum control of gastrointestinal nematodes in veterinary medicine: benzimidazoles, imidazothiazoles, and macrocyclic lactones.[1] None of these drugs is ideally suited for all therapeutic situations, and each class has been challenged by the development of drug-resistant nematode strains.[2] Expansion of the anthelmintic arsenal is thus an urgent goal. The potent antiparasitic activity of marcfortine A, paraherquamide A and their analogs was discovered by Merck scientists.[3] Because the marcfortines and paraherquamides are unique both structurally and in their mode of action, they represent a promising new class of anthelmintics. Marcfortine A (MFA), a fungal metabolite of *Penicillium roqueforti*, which was reported by Polonsky et al.,[4] is structurally related to paraherquamide A, which was originally isolated from *penicillium paraherquei.*[5] To investigate the effect of changes at the C26 position of MFA on anthelmintic activity, we synthesized several of C26-dialkyl and spiroalkyl analogs. The analogs were synthesized in four steps starting with catechol **1**, which was prepared from MFA in 80% yield by stirring in formic acid for 16 h.



Marcfortine A **1**

The general route outlined below, follows the modified method of Williams[6] used in his preparation of the *gem*-dimethyl dioxepin ring of the enantiomer of paraherquamide B. By this method we were able to prepare the four dioxepin-ring analogs in which the geminal methyl groups at C26 of MFA were replaced by a cyclobutyl **6a**, cyclohexyl **6b**, diethyl **6c**, and ethyl-methyl **6d** groups (Scheme 1).

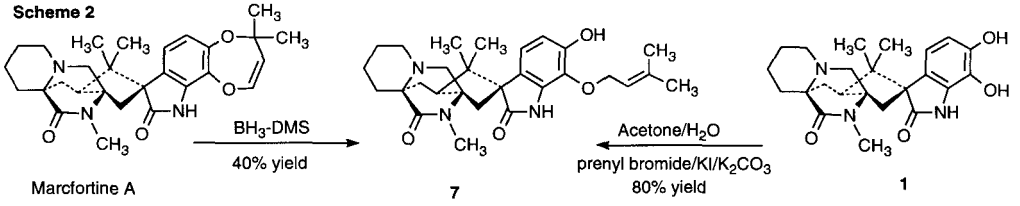
Scheme 1



The catechol **1** was coupled with the appropriate bromo reagent **2a–d** in the presence of K_2CO_3 and KI in acetone/water to give mono-alkylated products **3a–d**[7] in 29–82% yield. This was an improvement over the initial procedure[6] which employed DMF as solvent and gave low yields (5–10%). Epoxidation with m-CPBA in CH_2Cl_2 followed by workup with sodium bisulfite[8] (to remove the N-oxide) gave epoxides **4a–d** in 40–100% yield. Ring closure using $SnCl_4$ in THF provided alcohols **5a–d** (50–85%) which were dehydrated with methyltriphenoxyphosphonium iodide (MTPI) in THF/DMF to provide the final products **6a–d,** in 20–30% yield. Details of the synthesis of the cyclobutyl analog **6a** and the ethyl-methyl analog **6d** are given in the experimental section of this report.

To verify the regiochemistry of the alkylation described above we reduced marcfortine A with borane-methyl sulfide complex (see experimental) to provide in 40% yield (based on starting material) ring-opened material **7** possessing an O-prenyl group (Scheme 2). This compound was identical to the one prepared from the

Scheme 2



catechol **1** and 4-bromo-2-methyl-2-butene using the chemistry reported in step 1 of Scheme 1, thereby confirming the assigned regiochemistry of compounds **3a–d**.

The biological activity of compounds **3a–d** was evaluated in our standard anthelmintic assay which uses immunosuppressed Mongolian gerbils inoculated with Haemonchus contortus and Trichostrongylus colubriformis.[9] The compounds were administered orally at a dosage rate of 0.33 mg/gerbil. Unlike MFA, none of these compounds gave the 95% clearance of helminths we use as a criterion for determining activity, and were deemed inactive.

In summary, we have prepared four C-26 dialkyl and spiroalkyl MFA analogs in order to assess the importance of the C-26 dimethyl group on anthelmintic activity. Unlike MFA itself, none of these analogs were active. Thus, we conclude that the C-26 dimethyl group is essential for anthelmintic activity.

Experimental Section

Compound **1**: MFA (100 mg, 0.21 mmol) was dissolved in formic acid (95% pure from Aldrich, 5 mL). The mixture was stirred at room temperature for 16 h. The volatile components were removed and the residue was redissolved in methanol (5 mL). The solution was concentrated and the residue purified by chromatography on silica gel (10% methanol in methylene chloride) to give **1** as a solid (70 mg, 80%). Selected ^{1}H NMR (300 MHz, $CDCl_3$+ CD_3OD) δ 0.80 (s, 3H), 1.09 (s, 3H), 1.4–2.0 (m, 8H), 2.2–2.8 (m, 4H), 3.10 (s, 3H, N-Me), 3.87 (d, J = 11.9 Hz, 1H, C_{12}-H), 6.52 & 6.61 (d, J = 8.2 Hz, 2H, C_4-H & C_5-H). MS(FAB): *m/e* 412. HRMS (FAB): *m/e* 412.2232 ($C_{23}H_{29}N_3O_4$ + H requires 412.2236).

Compound **3a**: To KI (40 mg, 0.24 mmol) and **2a** (40 mg, 0.24 mmol) in acetone (3 mL) at room temperature was added the catechol **1** (50 mg, 0.12 mmol) and K_2CO_3 (50 mg, 0.36 mmol). Water was then added until the turbid solution cleared (4 drops). The reaction mixture was stirred for 0.2 h diluted with saturated $NaHCO_3$ (25 mL) and extracted into CH_2Cl_2 (25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by preparative thin layer chromatography (TLC, 5% MeOH/CH_2Cl_2) to give **3a** (17 mg, 29%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H, NH), 6.81 & 6.59 (d, J = 8.2 Hz, 2H, C_4 & C_5-H), 6.69 (s, 1H), 5.50–5.35 (m, 1H), 4.36 (d, 2H), 3.8–3.65 (m, 1H), 3.12 (s, 3H, NMe), 3.05 (t, 1H, C_{20}-H), 2.80–2.55 (m, 5H), 2.50–2.11 (m, 3H), 2.00–1.25 (m, H), 1.12 (s, 3H), 0.83 (s, 3H). HRMS (FAB): *m/e* 492.2871 ($C_{29}H_{37}N_3O_4$ + H requires 494.2862).

Compound **4a**: To the olefin **3a** (0.1 g, 0.20 mmol) in CH_2Cl_2 (8 mL) at room temperature was added m-CPBA (60%, 160 mg, 1.6 mmol) in portions. The reaction mixture was stirred for 0.5 h, then quenched with sodium bisulfite (0.45 g in 5 mL of water) and stirred for 0.5 h. The reaction mixture was diluted with saturated $NaHCO_3$

(25 mL) and extracted into CH_2Cl_2 (25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified (TLC, 5% MeOH/CH_2Cl_2) to give epoxide **4a** (42 mg, 42%) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H, NH), 6.81 & 6.58 (d, 2H, C_4 & C_5-H), 6.60–6.56 (m, 1H), 4.55–4.35 (m, 2H), 3.92–3.82 (m, 1H), 3.68 & 2.39 (d, J = 11.5 Hz, 2H, C_{12}-H), 3.26–3.21 (m, 1H), 3.11 (s, 3H, NMe), 3.00 (t, 1H, C_{20}-H), 2.75–1.20 (m, 17H), 1.10 (s, 3H), 0.82 (s, 3H). HRMS (FAB): *m/e* 508.2812 ($C_{29}H_{37}N_3O_5$ + H requires 508.2811).

Compound **5a**: To the epoxide **4a** (60 mg, 0.12 mmol) in THF (5 mL) at room temperature was added $SnCl_4$ (0.06 mL). The turbid reaction mixture was stirred for 0.25 h, quenched with KF (10% aqueous, 10 mL) diluted with water (10 mL) and extracted into EtOAc (25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified (TLC, 5% MeOH/CH_2Cl_2) to give **5a** as a mixture of diastereomers (27 mg, 50%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 & 7.73 (s, 1H, NH), 6.90–6.69 (m, 2H), 4.35–3.65 (m, 4H), 3.12 (s, 3H, NMe), 3.05 (t, 1H, C_{20}-H), 2.75–1.22 (m, 20H), 1.12 (s, 3H), 0.83 & 0.80 (s, 3H). HRMS (FAB): *m/e* 508.2812 ($C_{29}H_{37}N_3O_5$ + H requires 508.2811).

Compound **6a**: To the alcohol **5a** (17 mg, 0.035 mmol) in THF (2 mL) and DMF (0.2 mL) was added methyltriphenoxyphosphonium iodide (MTPI, washed with Et_2O to remove impurities, 50 mg, 0.1 mmol) at room temperature. The reaction mixture was warmed to 60 °C in an oil bath, maintained at this temperature for 0.25 h, then cooled to room tepmerature. The reaction was quenched with MeOH (1 mL), and $Na_2S_2O_3$ (saturated aqueous solution, 10 mL) then extracted into EtOAc (20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified (TLC, 5% MeOH/CH_2Cl_2 eluted 7 times) to give **6a** (4.0 mg, 25%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H, NH), 6.82 & 6.75 (d, J = 8.2 Hz, 2H, C_4 & C_5-H), 6.37 & 5.20 (d, J = 7.7 Hz, 2H, C_{24} & C_{25}-H), 3.80–3.60 (brs, 1H), 3.12 (s, 3H, NMe), 3.03 (t, 1H, C_{12}-H), 2.75–2.55 (m, 2H), 2.45–2.25 (m, 3H), 2.30–1.20 (m, 16H), 1.13 (s, 3H), 0.85 (s, 3H). HRMS (FAB): *m/e* 490.2747 ($C_{29}H_{35}N_3O_4$ + H requires 490.2705).

Compound **3d**: To KI (0.24 g, 1.4 mmol) **2d** (0.23 g, 1.4 mmol) in acetone (5 mL) at room temperature was added K_2CO_3 (0.26 g, 1.9 mmol) and catechol **1** (0.2 g, 0.48 mmol) dropwise in acetone/water (3 mL/2 mL). More water was then added dropwise until all solids dissolved. After 0.2 h of stirring, the reaction was diluted with water (25 mL) and extracted into EtOAc (35 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to give a quantitative yield of crude product that was purified by silica gel chromatography (30% acetone/CH_2Cl_2) to give **3d** (0.19 g, 82%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H, NH), 6.81 & 6.60 (d, J = 8.2 Hz, 2H, C_4 & C_5-H), 5.51 (t, J = 7.4 Hz, 1H), 4.52 (d, J = 7.4 Hz, 2H), 3.80–3.65 (m, 1H), 3.12 (s, 3H, NMe), 3.0 (t, 1H,

C_{20}-H), 2.70–1.3 (m, 16H), 1.62 (s, 3H), 1.15 (s, 3H), 1.02 (t, 3H), 0.82 (s, 3H). HRMS (FAB): *m/e* 494.3023 ($C_{29}H_{39}N_3O_4$ + H requires 494.3018).

Compound **4d**: To **3d** (0.1 g, 0.2 mmol) in CH_2Cl_2 (12 mL) at room temperature was added m-CPBA (60%, 180 mg, 0.6 mmol) in portions. The reaction mixture was stirred for 0.5 h, then treated with sodium bisulfite (0.95 g in 10 mL of water) and stirred for 0.5 h. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL) and extracted into CH_2Cl_2 (25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo for 4 h to give a quantitative yield of the crude epoxide **4d** (100 mg) as a mixture of diastereomers. Selected ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.80 (m, 1H, C_5-H), 6.60 (d, 1H, C_4-H), 4.70–4.50 (m, 1H), 4.20–4.15 (m, 1H), 3.30–3.15 (m, 1H), 3.13 (s, 3H) 3.10–2.95 (m, 1H), 2.80–2.60 (m, 2H), 0.99 (t, 3H), 0.82 (s, 3H). MS (FAB): *m/e* 510. HRMS (FAB): *m/e* 510.2977 ($C_{29}H_{39}N_3O_5$ + H requires 510.2968).

Compound **5d**: To the crude epoxide **4d** (40 mg, 0.076 mmol) in THF (5 mL) at room temerature was added $SnCl_4$ (0.05 mL) dropwise. The turbid reaction mixture was stirred for 1 h, quenched with KF (10% aqueous, 10 mL) diluted with water (10 mL) and extracted into EtOAc (25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified (TLC, 5% MeOH/CH_2Cl_2) to give alcohol **5d** as a mixture of diastereomers (33 mg, 82%) as a white solid. Selected ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 & 8.07 (s, 1H, NH), 6.80–6.70 (m, 1H), 6.65–6.50 (m, 1H), 4.30–3.70 (m, 3H), 3.80–3.75 (m, 1H), 3.12 (s, 3H, NMe), 3.15–3.00 (m, 1H, C_{20}-H), 2.41 (d, 1H). MS (FAB): *m/e* 510.

Compound **6d**: To the alcohol **5d** (15 mg, 0.029 mmol) in THF (2 mL) and DMF (0.2 mL) was added methyltriphenoxyphosphonium iodide (MTPI, washed with Et_2O to remove impurities, 20 mg, 0.1 mmol) at rt. The reaction mixture was warmed to 60 °C in an oil bath, maintained at this temperature for 0.5 h, treated with more reagent (20 mg), heated an additional 0.5 h, then cooled to rt. The reaction was quenched with MeOH (0.25 mL), and $Na_2S_2O_3$ (saturated aqueous solution, 10 mL) then extracted into EtOAc (20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified (TLC, 5% MeOH/CH_2Cl_2) to give product **6d** as a mixture of diastereomers (3.5 mg, 25%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 & 7.62 (s, NH), 6.80 & 6.67 (d, 1H, J = 8.2 Hz, 2H, C_4 & C_5-H), 6.39 (d, 1H, C_{24} -H), 4.87–4.84 (m, 1H, C_{25}-H), 3.75–3.65 (m, 1H), 3.12 (s, 3H), 3.08–3.0 (m, 1H), 2.75–2.10 (m, 5H), 1.90–0.80 (m, 22H). MS (FAB): *m/e* 492. HRMS (FAB): *m/e* 492.2872 ($C_{29}H_{37}N_3O_4$ + H requires 492.2862).

Compound **7**: To marcfortine A (50 mg, 0.1 mmol) in THF (5 mL) at room temperature under a nitrogen atmosphere was added BH_3-DMS (10 M, 0.03 mL, 0.3 mmol) dropwise. The reaction mixture was stirred for 1 h at

rt, quenched with MeOH (1 mL) stirred for 10 min, then partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified (TLC, 5% MeOH/CH_2Cl_2 eluted 7 times) to give **6a** (4.0 mg, 25%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, NH), 6.81 & 6.59 (d, J = 8.2 Hz, 2H, C_4 & C_5-H), 5.67 (s, OH), 5.51 (t, 1H), 4.49 (d, 2H), 3.68 (d, 1H), 3.12 (s, 3H), 2.97 (t, 1H, C_{20}-H), 2.67 (d, 1H), 2.64 (d, 1H), 2.39 (d, 1H), 2.30 (dd, 1H), 2.15 (d, 1H), 1.95–1.50 (m, 8H), 1.78 & 1.63 (s, 6H), 1.10 & 0.82 (s, 6H). MS (ES+): *m/e* 480; MS (ES-): *m/e* 478.

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