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Research Article

Isotopic labeling of 2-desoxoparaherquamide A (PNU-141962) with deuterium

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Summary

Modifications of marcfortine A and paraherquamide A led to the discovery of 2-desoxoparaherquamide A (PNU-141962, **3**) which is as active as paraherquamide A and has an improved safety profile. In order to do preclinical studies, we wished to synthesize isotope-labeled PNU-141962. This account will describe the synthesis of [CD_3]-2-desoxoparaherquamide A (**4**). The deuterium product was prepared in anticipation of using a similar synthesis for the preparation of the corresponding ^{14}C- and ^{3}H-labeled products.

Key Words: anthelmintic; 2-desoxoparaherquamide A; deuterated 2-desoxoparaherquamide A

Introduction

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 are ideally suited for all therapeutic situations, and each class has been challenged by the

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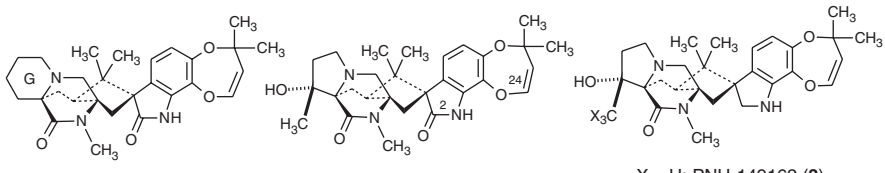
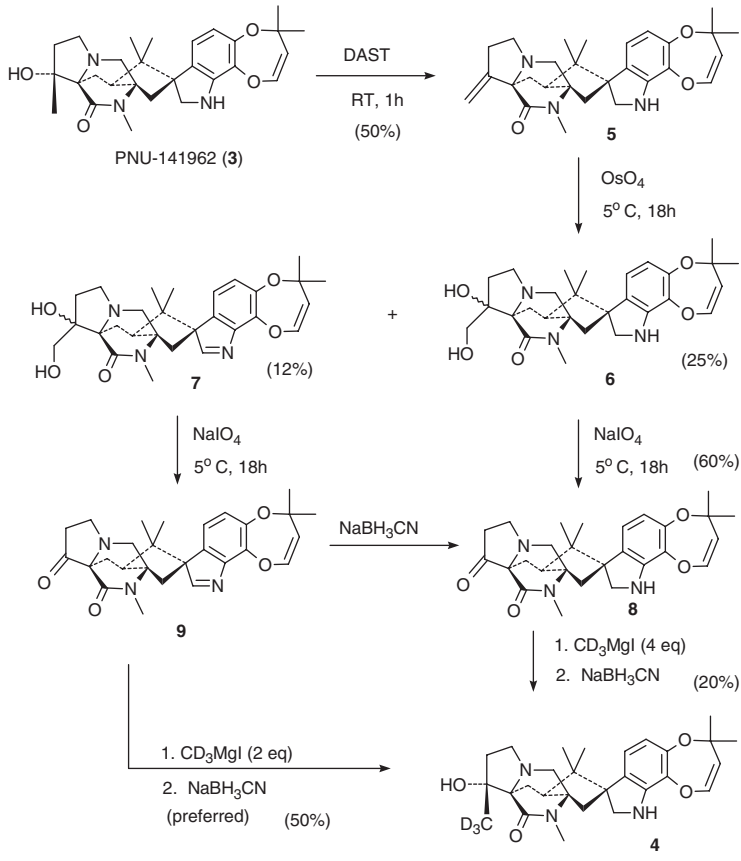


Figure 1.

development of drug-resistant nematode strains.[2] Expansion of the anthelmintic arsenal is thus an urgent goal. The discovery of a new, safe, broad-spectrum class of ruminant anthelmintic is a rare event. Despite intense efforts for >40 years, it has happened only 3 times [benzimidazoles, imidazolides and MCL (macrocyclic lactone) anthelmintics]. Pharmacia has been committed to the discovery of a new class of anthelmintics that function by a unique mode of action. We used the free-living nematode *C. elegans* in a relatively high throughput screen. In the early 1990s, we identified the natural product marcfortine A (MFA) as an active anthelmintic. The potent antiparasitic activity of marcfortine A (**1**), paraherquamide A (**2**) and their analogs has been described by scientists at Merck.[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 (**1**), a fungal metabolite of *Penicillium roqueforti* reported by Polonsky *et al.*,[4] is structurally related to paraherquamide A (**2**) which was originally isolated from *P. paraherque.*[5] Paraherquamide A (**2**) contains a five-membered G-ring possessing a hydroxyl group and a methyl group, whereas the G-ring of marcfortine A (**1**) is six membered (Figure 1).

Results and discussion

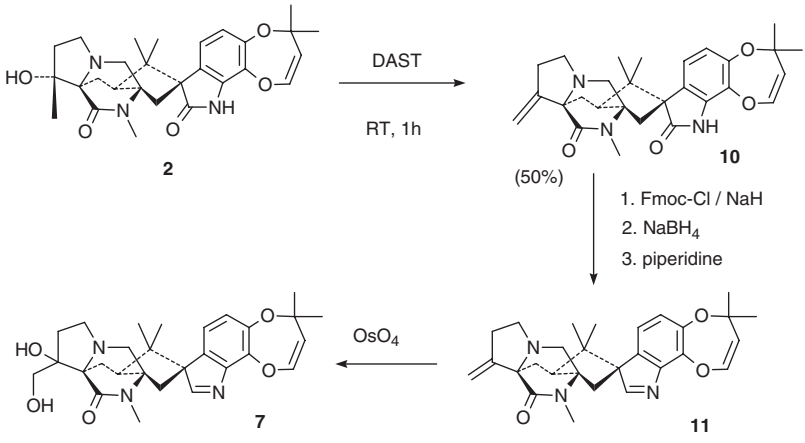
Although the synthesis of [C_{24}-^{3}H]paraherquamide has been reported,[3k] the labile nature of position-24 with respect to acid hydrolysis rendered such labeling unsuitable for our preclinical studies. Using a deuterium-labeled reagent, we have developed a synthetic strategy that is also suitable for the introduction of ^{14}C and ^{3}H into the 14-methyl group of PNU-141962 through the appropriate choice of labeling reagent (Scheme 1). The dehydration of PNU-141962 to exo-olefin 5 through the use of DAST was readily accomplished. A method has been reported for the conversion of an exo-olefin derivative of paraherquamide



Scheme 1.

to its ketone by sequential reactions involving bromination, ozonolysis and debromination with zinc.[3j] However, these procedures were long, low yielding and, worse, the benzene ring of paraherquamide was also brominated. In our hands, glycolation of the exo-olefin with osmium tetroxide followed by oxidation of the glycol to the ketone with sodium periodate proved more suitable to our purposes. Earlier, we reported a method for the stereospecific addition of MeMgI to 14-oxoparaherquamide B.[7] Using this methodology, we successfully methylated the carbonyl at position-14 of ketones 8 and 9 in a highly stereoselective manner. We also report an alternate, albeit lower yielding, synthesis using the more readily available paraherquamide A (**2**) for the preparation of labeled [CD_3]-2-desoxoparaherquamide A (Scheme 2).

The favored synthesis of [CD_3]-2-desoxoparherquamide A (Scheme 1) began with the treatment of **3** with DAST [(diethylamino)sulfur



Scheme 2.

trifluoride] in methylene chloride to provide **5** in 50% yield. Compound **5** was treated with osmium tetroxide at 5°C in the presence of NMO (4-methylmorpholine *N*-oxide) for 18 h to provide **6** and **7**, which were separated by silica-gel chromatography. Compounds **6** and **7** were treated with sodium periodate at 5°C for 18 h to provide **8** and **9**, respectively. Compounds **8** and **9** were treated with CD_3MgI followed by $NaBH_3CN$ to give **4** in 20% and 50% yields, respectively.

An alternate synthesis of **7** was important because it provided direct access to the higher yielding branch of the labeling synthesis shown in Scheme 1. Paraherquamide A (**2**) was treated with DAST[3j] to give **10** (Scheme 2) in 50% yield. Compound **10** was reduced using a published method.[6] Thus, compound **10** was reacted with 9-fluorenylmethyl chloroformate (Fmoc-Cl, 1.5 equiv) in the presence of NaH (3 equiv) at 0°C to give the intermediate, which was reduced with $NaBH_4$ in MeOH to give the amidol intermediate. This intermediate was deprotected with piperidine to give **11** in 60% yield from **10**. Compound **11** was treated with OsO_4 in the presence of NMO to give **7** in 50% yield.

Experimental

General experimental procedures

Chemical reagents and solvents were obtained from commercial sources and used directly unless otherwise stated. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX 400 NMR spectrometer at 400.13 and

100.12 MHz, respectively. FAB mass spectra were measured on a VG70-SE mass spectrometer. Thin-layer chromatography was performed on silica gel 60 F254 plates. Small-scale purifications were carried out using a Chromatotron. For larger scale work, flash chromatography over silica gel (EM Science, 230–400 mesh ASTM) was performed, and the fractions were analyzed by TLC.

*14,17-Anhydro-2-desoxoparaherquamide A (**5**)*

Compound **3** (0.4 g, 0.83 mmol) and DMAP (70 mg, 0.57 mmol) were dissolved in CH_2Cl_2 (20 ml) and treated with DAST (0.13 ml, 1 mmol) at room temperature (rt) for 1 h. The mixture was taken up in CH_2Cl_2 (50 ml) and washed with 5% aqueous solution (30 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (acetone/hexanes, 1/1) to give **5** as a white solid (190 mg). Physical characteristics: FABHRMS: *m/z* 462.2776 ($C_{28}H_{35}N_3O_3$ + H requires 462.2754). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (d, *J*=8.1 Hz, 1 H), 6.42 (d, *J*=8.1 Hz, 1 H), 6.30 (d, *J*=7.7 Hz, 1 H), 5.25 (brs, 1 H), 5.10 (brs, 1 H), 4.80 (d, *J*=7.7 Hz, 1 H), 3.95 (d, *J*=8.8 Hz, 1 H), 3.40 (d, *J*=9.4 Hz, 1 H), 2.96 (s, 3 H), 1.5-3.9 (m, 12 H), 1.43 (s, 6 H), 0.94 (s, 6 H).

*17-Hydroxy-2-desoxoparaherquamide A (**6**) and 17-hydroxy-2-desoxo-1,2-anhydroparaherquamide a (**7**)*

Compound **5** (1.27 g, 2.75 mmol) and NMO (2.2 g, 18.8 mmol) were dissolved in a mixture of THF/water (2:1, 100 ml). The mixture was treated with OsO_4 (10 ml, 2.5 wt% solution in *t*-BuOH) at 0°C. After 30 min, the mixture was placed in a refrigerator (5°C) for 16 h. The mixture was taken up in EtOAc (100 ml) and washed with 5% aqueous solution (50 ml) of $NaHCO_3$ containing $NaHSO_3$ (1 g). The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (3% MeOH in CH_2Cl_2) to give **6** as a white solid (300 mg) and **7** as a white solid (150 mg). Three hundred milligrams of **5** was recovered. Physical characteristics of **6**: FABHRMS: *m/z* 496.2787 ($C_{28}H_{37}N_3O_5$ + H requires 496.2811). ^{1}H NMR ($CDCl_3$) δ 6.68 (d, *J*=8.1 Hz, 1 H), 6.41 (d, *J*=8.1 Hz, 1 H), 6.30 (d, *J*=7.7 Hz, 1 H), 4.80 (d, *J*=7.7 Hz, 1 H), 4.03 & 3.85 (ABq, 2 H), 3.94 (d, *J*=8.8 Hz, 1 H), 3.40 (d, *J*=9.4 Hz, 1 H), 2.96 (s, 3 H), 2.30 & 2.22 (ABq, 2 H), 1.5–3.4 (m, 12 H), 1.43 (s, 6 H), 0.93 (s, 3 H), 0.91 (s, 3 H).

Physical characteristics of **7**: MS (ESI+) for *m/z* 494 $(M+H)^+$. Selected ^{1}H NMR ($CDCl_3$) δ 8.18 (s, 1 H, imine proton), 6.99 (s, 2 H), 6.49 (d, *J*=7.7 Hz, 1 H), 4.94 (d, *J*=7.7 Hz, 1 H), 3.07 (s, 3 H), 1.48 (s, 3 H), 1.46 (s, 3 H), 0.90 (s, 3 H), 0.74 (s, 3 H).

*14-Oxo-2-desoxo-17-norparaherquamide A (***8***)*

Compound **6** (0.26 g, 0.53 mmol) was dissolved in a mixture of THF/water (2:1, 45 ml). The mixture was treated with $NaIO_4$ (0.4 g, 1.87 mmol) at 0°C. After 30 min, the mixture was placed in a refrigerator (5°C) for 16 h. The mixture was taken up in CH_2Cl_2 (50 ml) and washed with 5% aqueous solution (30 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (3% MeOH in CH_2Cl_2) to give **8** as a white solid (140 mg, 58%). Seventy milligrams of **6** was recovered. Physical characteristics: FABHRMS: *m/z* 464.2572 ($C_{27}H_{33}N_3O_4+H$ requires 464.2549). ^{1}H NMR ($CDCl_3$) δ 6.70 (d, *J*=8.0 Hz, 1 H), 6.42 (d, *J*=8.0 Hz, 1 H), 6.31 (d, *J*=7.7 Hz, 1 H), 4.81 (d, *J*=7.7 Hz, 1 H), 3.95 (d, *J*=9.4 Hz, 1 H), 3.65 (d, *J*=11.2 Hz, 1 H), 3.40 (d, *J*=9.4 Hz, 1 H), 3.32 (t, *J*=7.1 Hz, 1 H), 2.95 (s, 3 H), 2.4–2.7 (m, 4 H), 2.2–2.3 (m, 4 H), 1.4–1.7 (m, 2 H), 1.43 (s, 6 H), 0.93 (s, 3 H), 0.90 (s, 3 H). ^{13}H NMR (100 MHz, $CDCl_3$) δ 208.4, 169.7, 146.3, 143.7, 139.7, 137.1, 126.9, 120.0, 114.9, 114.3, 79.7, 70.5, 65.5, 61.6, 60.9, 59.3, 56.0, 49.9, 45.5, 42.0, 36.9, 32.0, 30.3, 25.8, 25.1, 23.3, 19.7.

*14-Oxo-2-desoxo-1,2-anhydro-17-norparaherquamide A (***9***)*

Compound **7** (0.13 g, 0.26 mmol) was dissolved in the mixture of THF/water (2:1, 20 ml). The mixture was treated with $NaIO_4$ (0.2 g, 0.93 mmol) at 0°C. After 30 min, the mixture was placed in a refrigerator (5°C) for 16 h. The mixture was taken up in CH_2Cl_2 (30 ml) and washed with 5% aqueous solution (20 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (3% MeOH in CH_2Cl_2) to give **9** as a white solid (50 mg, 41%). Eleven milligrams of **7** was recovered. Physical characteristics: MS (ESI+) for *m/z* 462 $(M+H)^+$. ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1 H), 6.98 (d, *J*=8.1 Hz, 1 H), 6.95 (d, *J*=8.1 Hz, 1 H), 6.47 (d, *J*=7.7 Hz, 1 H), 4.91 (d, *J*=7.7 Hz, 1 H), 3.78 (d, *J*=11.2 Hz, 1 H), 3.36 (t, *J*=7.1 Hz, 1 H), 3.02 (s, 3 H), 2.4–2.8 (m, 4 H), 2.27 (dd, *J*=12.8, 10.8 Hz, 2 H), 2.04 (d,

J=15.8 Hz, 1 H), 1.6–1.8 (m, 2 H), 1.47 & 1.44 (2 s, 6 H), 1.24 (s, 3 H), 0.72 (s, 3 H).

*Conversion of 14-oxo-2-desoxo-1,2-anhydro-17-norparaherquamide A (**9**) to 14-oxo-2-desoxo-17-norparaherquamide A (**8**)*

Compound **9** (10 mg, 0.02 mmol) was dissolved in MeOH (2 ml). The mixture was treated with $NaBH_3CN$(10 mg, 0.16 mmol) at 0°C. After 30 min, the mixture was taken up in CH_2Cl_2 (10 ml) and washed with 5% aqueous solution (5 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (3% MeOH in CH_2Cl_2) to give **8** as a white solid (5 mg, 50%). ^{1}H NMR analysis of this compound was the same as that of previously prepared material.

*[CD_3]-2-Desoxoparherquamide A (**4**) from (**8**)*

Compound **8** (23 mg, 0.05 mmol) was dissolved in THF (2 ml) at −78°C. The mixture was treated with CD_3MgI (0.2 ml, 0.2 mmol, 1 M solution in Et_2O) at −78°C. After 30 min, the mixture was warmed to 0°C and stirred for an additional 30 min. The mixture was taken up in CH_2Cl_2 (10 ml) and washed with 5% aqueous solution (5 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was redissolved in MeOH (2 ml) and treated with $NaBH_3CN$ (10 mg, 0.16 mmol) at 0°C. After 30 min, the mixture was taken up in CH_2Cl_2 (10 ml) and washed with 5% aqueous solution (5 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (3% MeOH in CH_2Cl_2) to give **4** as a white solid (5 mg, 20%). FABHRMS: m/z 483.3035 ($C_{28}H_{34}D_3N_3O_4$ + H requires 483.3050). ^{1}H NMR analysis of this compound was identical to that of **3** except there is no resonance at δ 1.65.

*[CD_3]-2-Desoxoparherquamide A (**4**) from (**9**)*

Compound **9** (20 mg, 0.043 mmol) was dissolved in THF (2 ml) at −78°C. The mixture was treated with CD_3MgI (0.12 ml, 0.12 mmol, 1 M solution in Et_2O) at −78°C. After 30 min, the mixture was warmed to 0°C and stirred for an additional 30 min. The mixture was taken up in CH_2Cl_2 (10 ml) and washed with 5% aqueous solution (5 ml) of

$NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was redissolved in MeOH (2 ml) and treated with $NaBH_3CN$ (5 mg, 0.08 mmol) at 0°C. After 30 min, the mixture was taken up in CH_2Cl_2 (10 ml) and washed with 5% aqueous solution (5 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (3% MeOH in CH_2Cl_2) to give **4** as a white solid (10 mg, 50%). ^{1}H NMR analysis of this compound was the same as that of previously prepared material.

*14,17-Anhydroparaherquamide A (**10**)*

Compound **2** (2.09 g, 4.24 mmol) and DMAP (0.7 g, 5.74 mmol) were dissolved in CH_2Cl_2 (70 ml) and treated with DAST (0.67 ml, 5.1 mmol, 1.2 equiv) at rt for 1 h. The mixture was taken up in CH_2Cl_2 (150 ml) and washed with 5% aqueous solution (100 ml) of $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (acetone/hexanes, 4/6) to give **10** as a white solid (1.14 g). Physical characteristics: MS (ESI+) for *m*/*z* 476 $(M+H)^+$. ^{1}H NMR analysis of this compound was the same as previously reported.[3j]

*14,17-Anhydro-2-desoxo-1,2-anhydroparaherquamide A (**11**)*

Compound **10** (475 mg, 1 mmol) was dissolved in THF (30 ml) and treated with NaH (60% in oil, 0.1 g, 2.5 mmol) at rt. After 30 min, the mixture was cooled to 0°C and treated with 9-fluorenylmethyl chloroformate (0.4 g, 1.5 mmol). After 10 min, the reaction was quenched with buffer solutions (pH 7, 20 ml and pH 4, 20 ml). The mixture was extracted into ethyl acetate (2×30 ml) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was dissolved in MeOH (20 ml) and treated with $NaBH_4$ (0.15 g, 4.0 mmol) at 0°C. After 15 min, the reaction was quenched with $NaHCO_3$ (sat, 50 ml) and extracted into EtOAc (2×30 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 ml) and treated with piperidine (2 ml) at rt. After 2 h, the mixture was concentrated and the residue was purified on a silica plate (3% MeOH in CH_2Cl_2) to give **4** as a white solid (273 mg, 60%). Physical characteristics: ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 1 H), 7.00 (d, J=8.1 Hz, 1 H), 6.98 (d, J=8.1 Hz, 1 H), 6.49 (d, J=7.7 Hz, 1 H), 5.32

(brs, 1 H), 5.15 (brs, 1 H), 4.93 (d, J=7.7 Hz, 1 H), 3.06 (s, 3 H), 1.5–3.9 (m, 11 H), 1.46 & 1.48 (2 s, 6 H), 0.90 (s, 3 H), 0.76 (s, 3 H); FABHRMS: m/z 460.2601 ($C_{28}H_{33}N_3O_3$ + H requires 460.2600).

*17-Hydroxy-2-desoxo-1,2-anhydroparaherquamide A (**7**) from 14, 17-anhydro-2-desoxo-1,2-anhydroparaherquamide A (**11**)*

Compound **11** (0.20 g, 0.44 mmol) and NMO (4-methylmorpholinyl *N*-oxide, 0.4 g, 3.4 mmol) were dissolved in a mixture of THF/water (2:1, 20 ml). The mixture was treated with OsO_4 (1.6 ml, 2.5 wt% solution in *t*-BuOH) at 0°C. After 30 min, the mixture was placed in a refrigerator (5°C) for 16 h. The mixture was taken up in EtOAc (20 ml) and washed with 5% aqueous solution (10 ml) of $NaHCO_3$ containing $NaHSO_3$ (200 mg). The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified on a silica plate (5% MeOH in CH_2Cl_2) to give **7** as a white solid (70 mg). Seventy milligrams of **11** was recovered. ^{1}H NMR analysis of this compound was the same as previously prepared samples.

Conclusion

Isotopic labeling of 2-desoxoparaherquamide A (PNU-141962) with deuterium was achieved from PNU-141962 in four steps. The same approach may be used for the preparation of the corresponding ^{14}C- and ^{3}H-labeled products.

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