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NOTE

Preparation of Paraherquamide Labeled with Deuterium or Tritium

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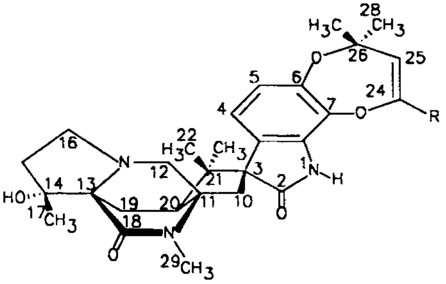
SUMMARY

Deprotonation of paraherquamide (1a) at C-24 and subsequent deuteration (or tritiation) is described. The procedure afforded 24-^{2}H-paraherquamide (1b) with 66% deuterium incorporation at C-24. Modification of the deuteration procedure to allow the introduction of tritium resulted in the preparation of 24-^{3}H-paraherquamide (1c) with specific activity 3.7 Ci/mmol.

Keywords: paraherquamide, lithiation, deuteration, tritiation.

INTRODUCTION

Paraherquamide (1a) is an unusual oxindole alkaloid first isolated by Yamazaki et al.[1] It was later discovered that 1a and several natural analogs were potent anti-parasitic agents[2] and a number of synthetic analogs were prepared.[3] In order to facilitate mode of action studies tritium-labeled paraherquamide (1c) was synthesized as described herein.



1a R = ^{1}H PARAHERQUAMIDE
1b R = ^{2}H
1c R = ^{3}H

EXPERIMENTAL

General: Deuterium Oxide (^2H_2O, minimum isotopic purity 99.8%) was obtained

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from MSD Isotopes. Tritium oxide (^3H_2O, specific activity 47.6 Ci/mmol) was obtained from New England Nuclear as a 0.0105 M solution in THF. Analytical thin layer chromatography (TLC) was performed on 2.5 x 10 cm plates coated with 0.25 mm thickness of silica gel containing PF 254 indicator (Analtech). Preparative TLC was performed on 20 x 20 cm plates coated with 0.5 or 1.0 mm of silica gel containing PF 254 indicator (Analtech). Compounds were visualized with shortwave UV light or by staining with iodine vapor. For preparative TLC compounds were eluted from the silica gel with ethyl acetate. All chromatography solvents were HPLC grade or better.

24-Deutero-paraherquamide (1b): Potassium hydride (6 drops of 20-25% oil suspension) was added to a solution of paraherquamide (20 mg, 0.0405 mmol) in 1 mL of anhydrous THF. The resulting mixture was stirred at room temperature for 2 hours then cooled to -78° C as *tert*-Butyllithium (0.100 mL of 2.0 M pentane solution) was added.[4,5] The yellow mixture was warmed to 0° C and stirred at 0° C for 45 minutes. Deuterium oxide (^2H_2O, 0.100 mL) was then added slowly (gas evolution) and the mixture stirred at 0° C for an additional 10 minutes. Water (1 mL) was then added and the mixture extracted with dichloromethane (3 x 2 mL). The combined extracts were dried ($MgSO_4$), filtered and evaporated to a light yellow oil. The crude product was purified by preparative layer chromatography on a 1.0 mm silica gel plate eluted with 7% methanol in dichloromethane to afford a colorless oil (13 mg, 65% yield, R_f 0.35) which was identified by ^1H NMR as 24-deutero-paraherquamide (**1b**). The ^1H NMR spectrum clearly showed ca. 66% deuterium incorporation at C-24 with no significant incorporation elsewhere in the molecule.

24-Tritio-paraherquamide (1c): Potassium hydride (6 drops of 20-25% oil suspension) was added to a solution of paraherquamide (20 mg, 0.0405 mmol) in 1 mL of anhydrous THF. The resulting mixture was stirred at room temperature for 2 hours then cooled to -78° C as *tert*-Butyllithium (0.100 mL of 2.0 M pentane solution) was added. The yellow mixture was warmed to 0° C and stirred at 0° C for 1 hour. An aliquot of the mixture (0.200 mL, *corresponding to ca. 4 mg of paraherquamide*) was then removed and added to a 0.0105 M solution of ^3H_2O in THF (30 mL of THF containing ca. 0.0069 g of ^3H_2O).[6] After 5 minutes the solvent was evaporated under vacuum and the residue evaporated several times from THF to remove volatile tritium. Water (2 mL) was added to the residue and the mixture was extracted with dichloromethane. The extract was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by preparative layer chromatography on two 0.5 mm silica gel preadsorbent plates eluted with 7% methanol in dichloromethane. The product band was eluted with ethyl acetate to afford 7.3 mCi of crude product. *A portion* of this crude product was purified by HPLC on a 9.4 mm x 10 cm Whatman ODS-3 column eluted with 25:75 acetonitrile:0.1% aqueous H_3PO_4 to yield 130 μCi of 24-^{3}H-paraherquamide (1c) of 92% radiochemical purity and specific activity 3.7 Ci/mmol. The chromatographic (TLC and HPLC) properties of the labeled material thus obtained were identical to those of natural (unlabeled) paraherquamide.

CONCLUSION

A convenient procedure for the preparation of paraherquamide labeled with either deuterium or tritium has been developed. The availability of tritium-labeled paraherquamide has allowed mode of action studies which will be published separately.[7]

ACKNOWLEDGMENTS

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REFERENCES

1. (a) Yamazaki M., Okuyama E., Kobayashi M., Inoue H.-Tetrahedron Lett. 22: 135 (1981); (b) Yamazaki M., Fujimoto H., Okuyama E., Ohta Y.-Maikotokishin (Tokyo) 10: 27 (1980) (Chemical Abstracts 95: 19321p (1981); (c) The Chemical Abstracts name for paraherquamide is Spiro[4H,8H-[1,4]dioxepino[2,3-g]indole-8,7'(8'H)-[5H,6H-5a,9a](iminomethano)[1H]cyclopent[f]indolizine]-9,10'(10H)-dione,2',3',8'a,9'-tetrahydro-1'hydroxy-1',4,4,8',8',11'-hexamethyl-(1'α,5'aβ,7'β,8'aβ,9'aβ)-(-). CAS Registry No. 77392-58-6). In the interest of brevity and clarity we have used trivial names based on paraherquamide rather than Chemical Abstracts names throughout this paper.

2. (a) Ondeyka J.G., Goegelman R.T., Schaeffer J.M., Kelemen L., Zitano L.-manuscript submitted to J. Antibiotics; (b) Shoop W.L, Egerton J.R., Eary C.H., Suhayda D.-manuscript submitted to Research in Veterinary Science; (c) Ostlind D.A., Mickle W.G., Ewanciew D.V., Andriuli F.J.-manuscript submitted to Research in Veterinary Science.

3. Blizzard T.A., Marino G., Mrozik H., Fisher M.H., Hoogsteen K., Springer J.P.-J. Org. Chem. 54: 2657 (1989).

4. Deprotonation of vinyl ethers is a known reaction: Baldwin J.E., Hofle G.A., Lever O.W.-J. Am. Chem. Soc. 90: 7125 (1974).

5. Potassium hydride is added to convert the OH and NH groups into soluble potassium salts: Moyer M.P., Shiurba J.F., Rapoport H.-J. Org. Chem. 51: 5106 (1986).

6. A relatively large amount of anion mixture was generated and an aliquot removed for quenching due to the difficulty of reproducibly generating the anion on extremely small scale.

7. Schaeffer J.-manuscript in preparation.