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A Facile Synthesis of the Major Urinary Metabolite of Metronidazole

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A FACILE SYNTHESIS OF THE MAJOR URINARY METABOLITE OF METRONIDAZOLE

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Abstract: An efficient synthesis of diol **9**, a urinary metabolite of Metronidazole **5**, is described.

Metronidazole **5** is a drug used therapeutically as an antiprotazoal agent, an antibiotic against anaerobic infection[1,2] and as a radiosensitizer in cancer therapy[3-10]. During the course of a pharmacological evaluation of metronidazole, it became necessary to obtain significant quantities of its major urinary metabolites **3** and **9**[11,12]. Both of these metabolites also exhibit biological activity[13-20].

The conversion of commercially available 2-methyl-5-nitroimidazole **1** to ester **2** has been described to proceed in 12% yield[21]. Improvement of the purification procedure allowed us to obtain ester **2** from **1** in 32% yield (unoptimized), and hence carboxylic acid **3**. Although diol **9** has been synthesized previously[22-25], the precursors used were not readily available, and the procedures given were neither very efficient nor well described. We felt that ester **2** would be a reasonable precursor for diol **9**. Therefore, the next step was to introduce the hydroxyl function on the methyl group of the 2-position. We felt the best way to carry this out was to first brominate the methyl group and then displace it with a hydroxyl group. Thus, ester **2** was brominated (1 equiv. NBS, CH_2Cl_2, 275W sun-lamp, reflux, 3 days) in 72% yield. Displacement of the bromide by refluxing with potassium acetate in acetic acid for 3 days afforded the carboxylic acid **6**, which was esterified by Chan's method[26] to give ester **7** in 90% yield from bromide **4**.

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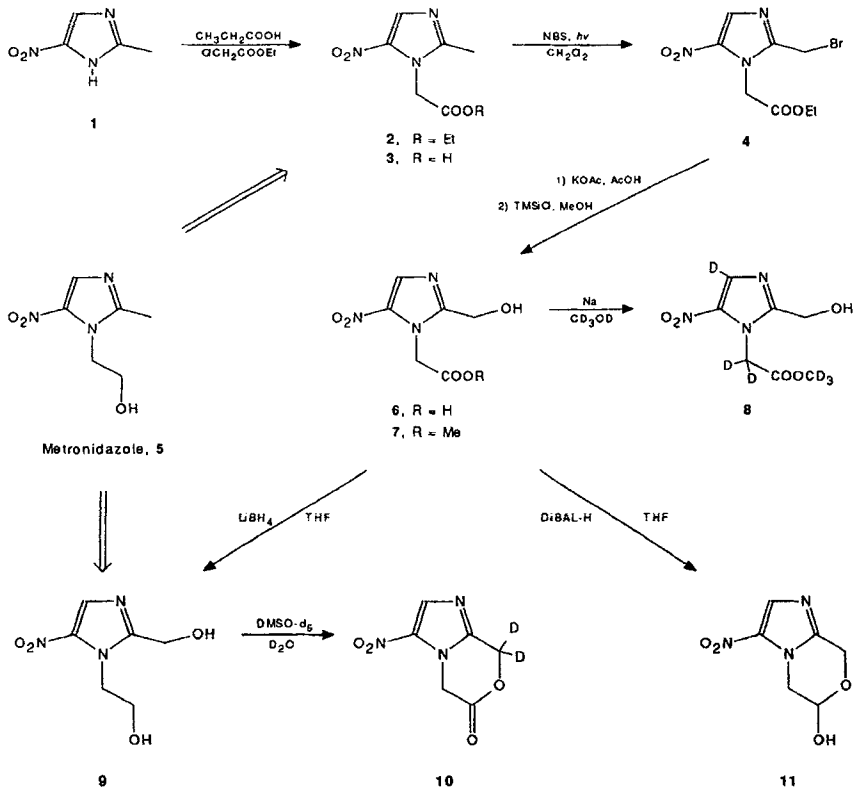

With ester **7** in hand, the only manipulation remaining was to reduce the ester functionality to the alcohol. We originally carried out the reduction using lithium aluminum hydride ($LiAlH_4$, THF, 1 h, RT) and found that the desired diol **9** could be obtained in only 40% yield after tedious flash chromatography. The low yield was due to decomposition and over-reduction. Due to the over-reduction problems encountered with lithium aluminum hydride, we next attempted to reduce ester **7** with DIBAL-H, hoping to obtain an aldehyde which then could be reduced to the diol **9** with mild reducing agents. Unfortunately, when ester **7** was subjected to di-*iso*-butylaluminum hydride (DIBAL-H, THF, 2 h, RT), only lactol **11** was isolated in 40% yield. However, reduction of ester **7** with lithium borohydride ($LiBH_4$, THF, 15 min, RT) afforded pure diol **9** in 80% yield. The structure and purity of diol **9** was supported by ^{1}H-NMR, ^{13}C-NMR, mass spectrometry and by HPLC comparison with an authentic sample.

Since polydeuterated metabolites are frequently used as internal standards when biological samples are analyzed by G.C.-M.S., using the specific ion monitoring technique, we

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next investigated deuterium incorporation into metabolites or metabolite precursors of metronidazole. When ester **7** was dissolved in methanol-d_4, we found that the methoxy group and the methylene group adjacent to the ester functionality underwent a partial deuterium exchange as indicated by ^{1}H and ^{13}C-NMR. This exchange proceeded to completion when ester **7** was treated with sodium in methanol-d_4, and the hexadeutero ester **8** was obtained in 75% yield. The incorporation of deuterium into ester **7** was confirmed by ^{13}C-NMR which showed carbon-deuterium couplings that were consistent with the structure. Its ^{1}H-NMR and mass spectrum also indicated deuterium incorporation. This reaction allows, in principle, for the formation of trideutero-**3** and trideutero-**9** where the deuterons are not easily exchanged. It was also observed that when diol **9** was dissolved in a mixture of dimethyl sulfoxide-d_6 and deuterium oxide, lactone **10** was formed in virtually quantitative yield after standing at room temperature for one week, permitting in principle, incorporation of a further set of deuterons into metabolite **9**.

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EXPERIMENTAL

General Methods.

Melting points (m.p.) were determined on a Gallenkamp block and are uncorrected. Low-resolution chemical ionization mass spectra were obtained on an HP-5980A quadrapole mass spectrometer in the direct-inlet mode. High-resolution chemical ionization mass spectra were obtained on a VG ZAB-2F-HS sector mass spectrometer in the direct-inlet mode. The measurements were carried out at a resolving power (res) of 10000, unless otherwise indicated. All compounds were shown to be homogeneous by tlc and to have a purity of >95% by high-field NMR and specific ion monitoring.

^{1}H-NMR spectra were obtained on either a Varian XL-200 or Varian XL-300 spectrometer at 200 MHz and 300 MHz respectively and the peak assignments were made, in some cases, with the aid of homonuclear decoupling and/or COSY experiments. Chemical shifts are given in the scale of parts per million (ppm). The residual proton signals of chloroform, methanol and methylene chloride (assigned values of δ 7.24, 3.30 and 5.32 ppm, respectively) were used as reference in these solvents. The multiplicities are recorded using the following abbreviations: **s**, singlet; **d**, doublet; **t**, triplet; **q**, quartet; **q^5**, quintet; **h^7**, heptet; **m**, multiplet; **ex**, exchangeable. ^{13}C-NMR spectra were obtained on a Varian XL-300 spectrometer at 75.4 MHz and the peak assignments were made, in some cases, with the aid of APT, COLOC and/or HETCOR experiments. The ^{13}C signals of DMSO-d_6, CD_3OD and $CDCl_3$ (assigned values of δ 39.50, 49.00 and 77.00 ppm, respectively) were used as reference in these solvents.

Methanol was distilled from magnesium. Methylene chloride was distilled from P_2O_5. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Thin-layer chromatography (tlc) was performed on silica gel (Kieselgel 60 F_{254}) aluminum-backed plates (0.2 mm thickness) and visualized by UV and/or dipping into a solution of 2.5 g ammonium molybdate and 1 g ceric sulfate in 10 mL sulphuric acid / 90 mL water, followed by heating. Kieselgel 60 (Merck 230-400 mesh) silica gel was used for column chromatography.

Ethyl-(2-methyl-5-nitro)imidazolylacetate (2).

Commercially available 2-methyl-5-nitroimidazole (25.42 g, 200.0 mmol), ethyl chloroacetate (170 mL, 1.65 mol) and propionic acid (80 mL, 1.07 mol) were refluxed for 16 h under an atmosphere of nitrogen. Excess reagent was evaporated *in vacuo* and the black residue was dissolved in methylene chloride (2 L), washed with saturated aqueous sodium bicarbonate (2 x 1 L), dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to yield a black syrup which was chromatographed over silica gel (hexanes / ethyl acetate, 1:1 v/v) to afford **2** as a yellow solid. Crystallization from ethyl acetate / hexanes (1:1, v/v) gave pure **2** (13.60 g, 32% yield, m.p. 71-73°C) as white crystals. {^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.30 (t, 3H, $CH_2\mathbf{CH_3}$), 2.39 (s, 3H, CH_3), 4.27 (q, 2H, $\mathbf{CH_2}CH_3$), 4.65 (s, 2H, CH_2COO), 7.70 (s, 1H, H4), $J_{CH_2\text{-}CH_3}$ = 7.1 Hz; ^{13}C-NMR (75.4 MHz, $CDCl_3$): δ 12.88 [$\mathbf{CH_3}$], 14.04 [$CH_2\mathbf{CH_3}$], 48.04 [$N\mathbf{CH_2}COOEt$], 62.81 [$\mathbf{CH_2}CH_3$], 120.52 [C4], 145.41 [C2], 146.27 [C5], 165.86 [**CO**]; LRMS (CI-NH_3): m/e 214 ([MH$^+$], 100%)}.

Ethyl-(-2-bromomethyl-5-nitro)imidazolylacetate (4).

To a stirred solution of ester **2** (10.66 g, 50.0 mmol) in dry methylene chloride (600 mL) at room temperature under an atmosphere of nitrogen was added *N*-bromosuccinimide (8.90 g, 50.0 mmol) and the solution was irradiated with a General Electric 275 W sun-lamp at reflux temperature for 3 days. Evaporation of the solvent *in vacuo* gave a yellow residue which was chromatographed over silica gel (hexanes / ethyl acetate, 2:1, v/v) affording **4** as a white solid (10.52 g, 72%). {^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.32 (t, 3H, $CH_2\mathbf{CH_3}$), 4.30 (q, 2H, $\mathbf{CH_2}CH_3$), 4.48 (s, 2H, CH_2Br), 4.85 (s, 2H, CH_2COO), 7.79 (s, 1H, H4), $J_{CH_2\text{-}CH_3}$ = 7.2 Hz; ^{13}C-NMR (75.4 MHz, $CDCl_3$): δ 13.96 [$CH_2\mathbf{CH_3}$], 20.43 [$\mathbf{CH_2}Br$], 48.34 [$N\mathbf{CH_2}COOEt$], 62.90 [$\mathbf{CH_2}CH_3$], 122.50 [C4], 142.87 [C2], 146.28 [C5], 165.51 [**CO**]; LRMS (CI-NH_3): m/e 311, 309 ([M + NH_4^+], 23.2%, 26.56%), 294, 292 ([MH$^+$], 100%, 98.2%)}.

Methyl-(-2-hydroxymethyl-5-nitro)imidazolylacetate (7).

To a stirred solution of bromide **5** (4.97 g, 17.0 mmol) in glacial acetic acid (142 mL) at room temperature under an atmosphere of nitrogen was added potassium acetate (22.79 g, 232.2

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mmol) and the reaction mixture was allowed to reflux for 3 days. Evaporation of the solvent *in vacuo* gave the crude acid **6**, which was redissolved in dry methanol (208 mL) and treated with chlorotrimethylsilane (52.0 mL, 409.7 mmol) for 42 h at room temperature under a nitrogen atmosphere. Removal of the solvent *in vacuo* gave a white residue which was chromatographed over silica gel (methylene chloride / methanol, 100:5, v/v) affording ester **6** (3.29 g, 90% yield) as a white powder. **ACID 6:** {^{1}H-NMR (200 MHz, CD_3OD): δ 4.72 (s, 2H, CH_2O), 5.21 (s, 2H, CH_2COO), 8.19 (s, 1H, H4)}. **ESTER 7:** {^{1}H-NMR (200 MHz, DMSO-d_6): δ 3.71 (s, 3H, MeO), 4.50 (d, 2H, CH_2O), 5.09 (s, 2H, CH_2COO), 5.58 (t, ex, 1H, OH), 8.32 (s, 1H, H4), $J_{CH_2\text{-}OH}$ = 5.8 Hz; ^{13}C-NMR (75.4 MHz, DMSO-d_6): δ 47.76 [NCH_2COOMe], 52.54 [**MeO**], 55.67 [CH_2OH], 123.99 [C4], 145.11 [C5], 147.62 [C2], 167.81 [**CO**]; LRMS (CI-NH_3): m/e 233 ([M + NH_4^+], 8.6%), 216 ([MH^+], 100%)}.

Perdeuteromethyl-(-2-hydroxymethyl-4-deutero-5-nitro)imidazolylacetate (8).

To a stirred solution of ester **7** (43 mg, 0.20 mmol) in methanol-d_4 (1.0 mL) at room temperature under an atmosphere of nitrogen was added sodium (2 mg, 0.09 mmol) and the reaction mixture was monitored by ^{1}H-NMR. After 3 h, the reaction was adjusted to pH ~ 7 with weakly acidic resin and the solvent was removed *in vacuo* to afford a solid residue. Chromatography over silica gel (methylene chloride / methanol, 100:5, v/v) gave **8** (33 mg, 75% yield) as a colourless solid. {^{1}H-NMR (200 MHz, CD_3OD): δ 5.05 (s, 2H, CH_2COO); ^{13}C-NMR (75.4 MHz, CD_3OD): δ 47.66 [q^5, NCD_2COOCD_3, $J_{C\text{-}D}$ = 21.2 Hz], 51.09 [h^7, **MeO**, $J_{C\text{-}D}$ = 20.6 Hz], 57.85 [CH_2OD], 123.79 [t, C4, $J_{C\text{-}D}$ = 34.9 Hz], 146.56 [C2], 151.83 [C5], 173.34 [**CO**]; LRMS (CI-*i*Bu): m/e 223 ([MH^+], 28.8%), 222 ([M + $2H^+$ - D^+], 97.7%), 221 ([M^+ - H^+ - D^+], 100%)}.

1-(2-Hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (9).

To a stirred solution of ester **7** (646 mg, 3.00 mmol) in dry tetrahydrofuran (60 mL) at ambient temperature under an atmosphere of nitrogen was added lithium borohydride (98 mg, 4.50 mmol). After 15 min, the reaction was quenched with methanol (10 mL) and neutralized to pH ~ 7 with weakly acidic resin. Removal of the solvent *in vacuo* gave a white solid which was chromatographed over silica gel (methylene chloride / methanol, 100:5, v/v) affording diol **9** (449 mg, 80% yield, m.p. 118-119°C) as a white powder. {^{1}H-NMR (200 MHz, DMSO-d_6): δ 3.72 (dt, 1H, CH_2CH_2OH), 4.16 (t, 2H, CH_2CH_2OH), 4.53 (d, 2H, CH_2OH), 5.01 (t, ex, 1H, CH_2CH_2OH), 5.51 (t, ex, 1H, CH_2OH), 8.27 (s, 1H, H4), $J_{CH_2\text{-}CH_2}$ = 5.4 Hz, $J_{CH_2CH_2\text{-}OH}$ = 5.3 Hz, $J_{CH_2\text{-}OH}$ = 5.9 Hz; ^{13}C-NMR (75.4 MHz, DMSO-d_6): δ 49.05 [CH_2CH_2OH], 55.37 [CH_2OH], 59.99 [CH_2CH_2OH], 123.22 [C4], 145.11 [C5], 147.56 [C2]; LRMS (CI-NH_3): m/e

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205 ([M + NH_4^+], 3.4%), 188 ([MH^+], 100%); HRMS (CI-NH_3): m/e calcd. for $C_8H_{10}N_3O_4$ [MH^+], 188.0671; found, 188.0671}.

Lactone (10).

A solution of diol **9** (19 mg, 0.10 mmol) in dimethyl-d_6-sulfoxide (1.0 mL) and deuterium oxide (100 µL) was allowed to stand for several days at ambient temperature. Removal of the solvent *in vacuo* gave the title compound in virtually quantitative yield as a white solid. {^{1}H-NMR (200 MHz, DMSO-d_6): δ 4.95 (s, 2H, NCH_2COO), 8.27 (s, 1H, H4); ^{13}C-NMR (75.4 MHz, DMSO-d_6): δ 47.92 [N**C**H_2COO], 51.26 [q^5, **C**D_2O, $J_{C\text{-}D}$ = 18.4 Hz], 123.38 [C4], 145.38 [C5], 146.26 [C2]; LRMS (CI-NH_3): m/e 203 ([M + NH_4^+], 33.6%), 186 ([MH^+], 100%); HRMS (CI-NH_3): m/e calcd. for $C_6H_4D_2N_3O_4$ [MH^+], 186.0483; found, 186.0483}.

Lactol (11).

To a stirred solution of ester **7** (11 mg, 0.05 mmol) in dry tetrahydrofuran (5 mL) at ambient temperature under an atmosphere of nitrogen was added DIBAL-H (100 µL of a 1.0 M solution in cyclohexane, 0.10 mmol). After 2 h, methanol was added until evolution of H_2 ceased. The solution was neutralized with weakly acidic resin and the solvent removed *in vacuo* to give a white residue which was chromatographed over silica gel (methylene chloride / methanol, 100:5, v/v) affording lactol **11** (4 mg, 40% yield) as a white solid. {^{1}H-NMR (200 MHz, CD_3OD): δ 3.52 (m, 1H, NCH_2), 4.46 (s, 2H, CH_2O), 5.17 (t, 1H, NCH_2**C**HOH), 7.98 (s, 1H, H4), $J_{CH\text{-}CH_2}$ = 4.9 Hz, 5.8 Hz}.

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