Biochemical Pharmacology, Vol. 36, No. 6, pp. 931–935, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

CHEMICAL AND BIOLOGICAL PROPERTIES OF ACETYL DERIVATIVES OF THE HYDROXYLAMINO REDUCTION PRODUCTS OF METRONIDAZOLE AND DIMETRIDAZOLE

WILLIAM J. EHLHARDT, BERNARD B. BEAULIEU, JR. and PETER GOLDMAN*
Department of Nutrition, Harvard School of Public Health, and the Department of Pharmacology, Harvard Medical School, Boston, MA 02115, U.S.A.

(*Received* 12 *May* 1986; *accepted* 16 *August* 1986)

Abstract—Metronidazole and related 5-nitroimidazoles undergo reduction of their nitro group apparently to produce such reactive species as 5-hydroxylaminoimidazoles. To define the role of these species we have sought ways to prepare them by the catalytic reduction of metronidazole, dimetridazole and flunidazole. Although their respective 5-hydroxylaminoimidazoles were too unstable to be isolated directly, their *O,N*-diacetyl derivatives were isolable. Of these, the diacetyl derivative of the hydroxylamine derived from dimetridazole, *O,N*-diacetyl-1,2-dimethyl-5-hydroxylaminoimidazole (DiacDMH), was used for further study. DiacDMH was converted to its monoacetyl derivative, *N*-acetyl-1,2-dimethyl-5-hydroxylaminoimidazole (AcDMH), by enzymatic deacylation. Both DiacDMH and AcDMH were examined for bactericidal activity against such strains as *Bacterioides fragilis, Clostridium perfringens*, and *Escherichi coli* strain SR58, which are known to be sensitive to dimetridazole, as well as a variety of other bacteria. No bactericidal activity was detected, even in the presence of deacetylating enzymes. As the 5-hydroxylaminoimidazole itself could not be shown to form in these bacterial incubations, it remains uncertain whether or not the hydroxylamino functionality of a 5-nitroimidazole has bactericidal activity.

Although metronidazole and related 5-nitroimidazole drugs have therapeutic value in the treatment of infections with protozoa and anaerobic bacteria [1], few details are known about how these drugs work. The drugs appear to be activated by reduction of the nitro group, suggesting that one or more compounds with a lower oxidation state, such as those exemplified in Fig. 1, are the ultimate mediators of biological activity. Of these possibly active species, V (the 6-electron reduction product) can now be excluded as a reactive form of the 5-nitroimidazole drugs. Thus, 5-amino-1-β-hydroxyethyl-2-methylimidazole (V, $R_1 = CH_2CH_2OH$, $R_2 = CH_3$), although formed during the biological reduction of metronidazole [2], is a relatively stable compound which lacks both bactericidal [2] and mutagenic [3] activities. In the search for the bactericidal form of the 5-nitroimidazole drugs, attention must therefore turn to such species as the radical anion (II), and the nitroso (III) and hydroxylamino (IV) functionalities, whose oxidation states are between those of the nitro (I) and amino functionalities (V).

The hydroxylaminoimidazole (IV) merits particular consideration by analogy to the role of phenylhydroxylamine in the mechanism of toxicity of nitrobenzene [4–6], and because of recent studies implicating this functionality in the activity of the 5-nitroimidazoles themselves [7, 8]. Unfortunately, however, the biological properties of such a postulated toxic intermediate suggest that it would be highly reactive and therefore difficult, if not impossible, to isolate. Thus, there seems to be no report in the literature of either the detection or the characterization of any 5-hydroxylaminoimidazole. On the other hand, 5-cyano-4-hydroxylamino-1-methylimidazole, prepared by reduction of the analogous 4-nitro compound, is found to be stable [9], presumably because of the electron-withdrawing cyano group. Unfortunately, the biological activity of this compound has not been reported. As a rule, however, the 4-nitroimidazoles do not have the biological characteristics that make the 5-nitroimidazoles therapeutically useful [1].

More closely related biologically to the 5-nitroimidazoles are the 2-nitroimidazoles. A few 2-hydroxylaminoimidazoles, including one formed by reduction of misonidazole, have been reported and found to be unstable in aqueous solution, particularly at neutral or basic pH [10, 11]. Such instability suggests that a hydroxylamine formed from a 5-nitroimidazole is also likely to be too unstable to isolate. Indeed we have not been able to isolate such a compound using a number of methods. We have therefore prepared the *O,N*-diacetyl derivatives (VI) of the 5-hydroxylaminoimidazoles (IV) by reducing metronidazole, dimetridazole and flunidazole in the presence of acetic anhydride. In this paper we describe this synthesis, as well as our attempts to remove the acetyl group(s) in order to convert these relatively stable derivatives to their possibly active free hydroxylamines.

If the hydroxylamine (IV) is the active form of the 5-nitroimidazoles, then 5-nitroimidazoles might be considered pro-drugs which are activated by nitroreductase in the target organism. In this context, the acetyl derivatives mentioned above might also be considered pro-drugs which merely reach the active form of a 5-nitroimidazole by another pathway. Finding another pathway to the active form may prove to be a means to complement the range of antimicrobial

* Address correspondence to: Dr. Peter Goldman, Harvard University, 665 Huntington Ave., Boston, MA 02115.

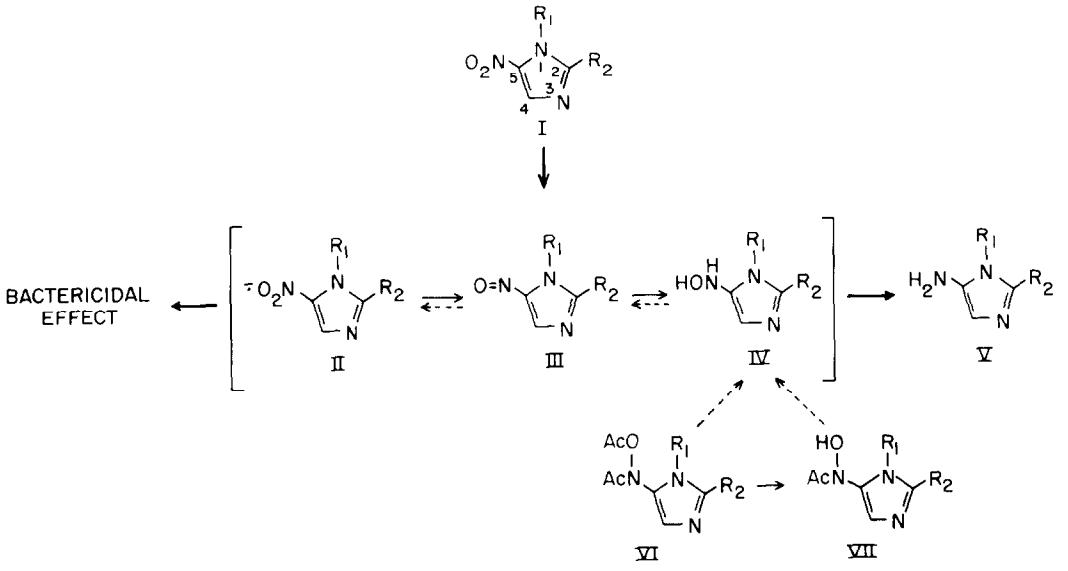

Fig. 1. Reduction of the nitro group in 5-nitroimidazoles (I) to the radical anion (II), and the nitroso (III) and hydroxylamino (IV) functionalities, which may be responsible for biological activity. The amine (V) can now be excluded as the bactericidal form of the 5-nitroimidazole drugs [2]. Also shown are the diacetyl (VI) and monoacetyl (VII) derivatives of the hydroxylamino functionality.

activity of the 5-nitroimidazoles. Thus, for example, organisms that lack nitroreductase activity, and consequently are resistant to the 5-nitroimidazoles *per se*, may nevertheless be susceptible to pro-drugs that are converted to the activated form by another pathway.

EXPERIMENTAL PROCEDURE

Materials

To prepare *O,N*-diacetyl-1,2-dimethyl-5-hydroxylaminoimidazole (DiacDMH*, VI, $R_1 = R_2 = CH_3$), a suspension of 1.0 g (7 mmoles) of dimetridazole (I, $R_1 = R_2 = CH_3$, a gift from May & Baker, Dagenham, England) and 0.60 g of 5% Pt/$CaCO_3$ (Aldrich Chemical Co., fresh bottle) in 50 ml acetic anhydride was hydrogenated (10 psi, 20°) in a Parr shaker for 1 hr. Approximately 0.45 l of H_2 (STP, 20 mmoles) was consumed. Filtration of the resulting mixture, followed by evaporation of the solvent, afforded a golden green–brown residue. This material was dissolved in 20 ml of hot benzene, filtered while hot, concentrated to 10 ml, and then allowed to cool slowly to room temperature. The solid was isolated by filtration and washed with cold benzene and ether, yielding 0.48 g (2.2 mmoles, 32%) of waxy, colorless microscopic needles with melting point 134–134.5° (sublimation at T > 120°).

^{1}H NMR (80 MHz, $CDCl_3$): δ 2.14, 2.26, 2.30 (3s, 9H, Acetyl and Me), 3.36 (s, 3H, N-Me), 7.25 (br.s, 1H, ring H). Upon addition of D_2O, the signal at δ 7.25 disappeared after 1 hr and was replaced by a DOH peak at δ 1.60. MS (FAB (+)ion): *m/e* 212 (MH^+, I = 100), 170 (MH^+-ketene, I = 45). UV (H_2O): λ_{max} 214 nm. IR (neat): 1780 (strong, ester C=O stretch), 1690 (strong, amide C=O stretch), 3250, 3190, 2990, 2970, 1640, 1370, 1330, 1280, 1200 cm^{-1}.

To further characterize the above product, 2 mg (~10 μmoles) was dissolved in 200 μl absolute ethanol in a small test tube, to which 2 mg 10% Pd-C (Aldrich Chemical Co., fresh bottle) was added. The test tube was placed in a Parr shaker bottle and treated with hydrogen at 50 psi for 1 hr. Under these conditions there was ~20% conversion to only one new product, as judged by reverse-phase HPLC under the standard conditions (retention time, 10.0 min; UV λ_{max}, 215 nm). Mass spectral analysis (FAB (+)ion) of this product showed a molecular weight of 153. The ultraviolet absorbance and molecular weight of this product were identical to those of authentic *N*-acetyl-5-amino-1,2-dimethylimidazole, which had been prepared by treating the known hydrochloride salt of 5-amino-1,2-dimethylimidazole with acetic anhydride [2]. The new product and the authentic sample also had identical retention times by reverse-phase HPLC when injected either separately or together.

A similar procedure was used to prepare the acetyl derivatives of the hydroxylamines formed by reduction of metronidazole (I, $R_1 = CH_2CH_2OH$, $R_2 = CH_3$, a gift from Searle Laboratories, Chicago, IL) and flunidazole (I, $R_1 = CH_2CH_2OH$, $R_2 = p$-F-C_6H_4, a gift from Merck Sharpe & Dohme Research Laboratories, Rahway, NJ). With these compounds, however, the triacetyl derivatives, *O,O',N*-triacetyl-1-β-hydroxyethyl-2-methyl-5-hydroxylaminoimidazole (TriacMNH) and *O,O',N*-triacetyl-1-β-

* Abbreviations: ADM, 1,2-dimethyl-5-aminoimidazole; AcDMH, *N*-acetyl-1,2-dimethyl-5-hydroxylaminoimidazole; DiacDMH, *O,N*-diacetyl-1,2-dimethyl-5-hydroxylaminoimidazole; TriacFLH, *O,O',N*-triacetyl-1-β-hydroxyethyl-2-*p*-fluorophenyl-5-hydroxylaminoimidazole; and TriacMNH, *O,O',N*-triacetyl-1-β-hydroxy-ethyl-2-methyl-5-hydroxylaminoimidazole.

hydroxyethyl-2-*p* - fluorophenyl - 5 - hydroxylaminoimidazole (TriacFLH), were formed; these compounds failed to crystallize, and so were purified by preparative reverse-phase liquid chromatography (retention times of 17.1 and 22.0 min respectively) using their ultraviolet absorbance for detection. The yields of these compounds were 30–40%.

For TriacFLH, ^{1}H NMR (80 MHz, $CDCl_3$): δ 1.93, 2.18, 2.30 (3s, 9H, Acetyl), 4.20 (br.s, 4H, N—CH_2CH_2OAc), 7.14 (dist'd. t, J ~ 9 Hz, 2H, phenyl H's), 7.58 (d of d, J = 5.2, 8.8 Hz, 2H, phenyl H's). IR (neat): 1750 (ester C=O stretch, sh.1780), 1700 (br., amide C=O stretch), 3260, 3200, 2980, 1640, 1540, 1370, 1230, 1200, 1150, 850 cm^{-1}. UV (EtOH): λ_{max} 256 nm. MS (NH_3 DCI): *m/e* 364 (MH^+).

For TriacMNH, ^{1}H NMR (80 MHz, $CDCl_3$): δ 2.03, 2.14, 2.27 (3s, 9H, Acetyl), 2.36 (s, 3H, Me), 4.0–4.3 (m, 4H, N—CH_2CH_2OAc). UV (EtOH): λ_{max} 214 nm. MS (FAB (+)ion): *m/e* 284 (MH^+, I = 100), 242 (MH^+-ketone, I = 70).

The ring proton was not observed in NMR spectra of either triacetyl derivative, because the small isolated samples (<1 mg) were not completely dry [2].

The monoacetyl derivative, *N*-acetyl-1,2-dimethyl-5-hydroxylaminoimidazole (AcDMH, VII, $R_1 = R_2 = CH_3$), was prepared from DiacDMH as follows: 6.0 mg (30 μmoles) of DiacDMH was treated with 2 units (approximately 0.2 mg protein) of acetylesterase (EC 3.1.1.6, Sigma Chemical Co., St. Louis, MO), in 2.0 ml of 0.1 M KPO_4 buffer, pH 7.0. During a 2-hr incubation period the absorbance maximum at 216 nm was replaced by one at 266 nm. This material was partially purified by reverse-phase liquid chromatography (ret. time, 2.5 min), but still contained some salts, presumably buffer and $(NH_4)_2SO_4$ from the enzyme preparation. ^{1}H NMR (80 MHz, D_2O): δ 2.16, 2.41 (2s, 6H, acetyl, ring Me), 3.19 (s, 3H, N-Me), 4.81 (s, 2H, DOH). Ring proton was not observed (see NMR for similar compounds). UV (H_2O): λ_{max} 266 nm, $\varepsilon \sim 1000$. IR (solid, with phosphate salts): 1680 cm^{-1} (v. broad, amide C=O stretch).

For mass spectral analysis, *N*-acetyl-*O*-propionyl-1,2-dimethyl-5-hydroxylaminoimidazole was prepared by dissolving a sample in propionic anhydride, concentrating it by rotary evaporation, and then dissolving the resulting material in approximately 1 ml of methanol. When subjected to reverse-phase preparative liquid chromatography, the mixture yielded a compound (retention time, 12.5 min) with λ_{max} = 216 nm, which by mass spectral analysis (fast atom bombardment, positive ion) showed *m/e* 226 (MH^+, I = 100), 170 (MH^+-methylketene, I = 55).

Methods

High pressure liquid chromatography using a C-18 reverse-phase analytical column (Supelco, Inc., Bellefonte, PA, Cat. No. 5-8294) was performed on either a Hewlett–Packard (Palo Alto, CA) 1082B liquid chromatograph equipped with a 1040A diode-array UV spectrophotometric detector, or a Waters Associates (Milford, MA) high-pressure liquid chromatograph system with UV absorbance detector (model 440). Data were recorded and areas under the curve computed by a Hewlett–Packard 52398B LC Terminal. Unless otherwise noted, compounds were eluted (1.5 ml/min) by means of a solvent program that began isocratically with 5% methanol in 0.1 M phosphate buffer at pH 7.4 for 3 min and then increased linearly to 50% methanol during 20 min. Preparative reverse-phase liquid chromatography, at a flow rate of 6.0 ml/min with the same solvent program, was carried out with a Supelco C-18 semi-preparative reverse-phase HPLC column (Supelco Cat. No. 5-8368).

Ultraviolet-visible spectra were recorded using a Hewlett–Packard model 8451A diode-array spectrophotometer. Infrared spectra were recorded on a Perkin–Elmer model 854 IR spectrophotometer, ^{1}H NMR spectra on a Varian CFT20 Fourier-transform NMR spectrometer, and mass spectra with a VG Analytical ZAB-SE mass spectrometer.

The *Escherichi coli* SR58 DNA repair mutant, $UvrB^-$ $RecA^-$, which is susceptible to metronidazole [2, 12], was propagated in brain–heart infusion supplemented broth (Scott Laboratories, Fiskville, RI). The susceptibility of this strain to DiacDMH and AcDMH under various conditions was tested in 0.1 M KPO_4 buffer at pH 7.4, a medium which enabled us to monitor the presence of the test compounds by ultraviolet absorbance. For these experiments, bacteria were harvested from their growth media by centrifugation (10,000 *g* for 20 min), washed once in 0.1 M KPO_4 buffer, and resuspended in this medium to maintain their original cell density (~10^8 cfu/ml). Bacterial viability was quantified using appropriate solid growth media.

To screen for bacteriostatic activity against potential pathogens, the minimal inhibitory concentrations (MIC) of DiacDMH were determined for the ten bacterial strains that are included in Bactrol Disk Set A (Difco Laboratories, Detroit, MI), as well as for *Bacterioides fragilis, Clostridium perfringens*, and *E. coli* strains AB1157 (wild type) and SR58 (a DNA repair mutant sensitive to metronidazole [12]). The MIC for each strain was determined in a brain–heart infusion medium (BBL, Cockeysville, MD) containing 10 μg/ml hemin and 0.5 μg/ml Vitamin K_1. After it was autoclaved, this medium was supplemented with filtered (0.45 μm Millex, Millipore Corp., Bedford, MA) solutions of cysteine · HCl and dithiothreitol to make final concentrations of 0.5 and 0.3 mg/ml respectively. DiacDMH was diluted in 2-fold increments in multiwell plates (Falcon No. 3047), which were also inoculated with approximately 10^6 cfu/ml. Duplicate determinations were made for each bacterial strain; incubations at 37° were conducted both anaerobically (BBL Gaspak jar and BBL generator envelopes, BBL Microbiology Systems, Cockeysville, MD) and aerobically.

RESULTS

Properties of DiacDMH

The 5-hydroxylaminoimidazole functionality is one possible mediator of activity of the 5-nitroimidazole drugs. Not surprisingly, such a species has not been isolated, although 5-nitroimidazoles have been reduced using a variety of methods. We found,

however, that reducing dimetridazole, metronidazole, or flunidazole with either zinc or hydrogen using a $Pt/CaCO_3$ catalyst, in the presence of acetic anhydride, enabled the acetylated form of the hydroxylamine to be isolated. These fully acetylated hydroxylamine derivatives were quite stable when stored in a dry atmosphere. In neutral or slightly acidic aqueous solutions, however, they hydrolyzed slowly to yield, within 2–3 days, compounds which have not yet been completely characterized. The mass spectroscopic analysis of these breakdown products, however, was consistent with the loss of acetic acid and the addition of two molecules of H_2O. In slightly basic solution, on the other hand, the acetyl derivatives decomposed within a few minutes, yielding products whose NMR spectra indicate hydrolysis of the imidazole ring.

In the NMR spectrum of DiacDMH (VI, $R_1 = R_2 = CH_3$), the chemical shift of the ring proton at C-4, δ 7.25, was between that of dimetridazole (δ 7.90) and that of 5-amino-1,2-dimethylimidazole (ADM, δ6.64) [2], a result consistent with a nitrogen functionality of intermediate oxidation state. As with ADM, the ring proton of DiacDMH is rapidly exchanged with D_2O.

As noted above, the N—O bond of DiacDMH seems to be very labile. It was, therefore, not surprising that only one product was formed under mild reducing conditions (see Experimental Procedure). That such a product was identical by mass spectroscopy, UV, and reverse-phase HPLC to authentic *N*-acetyl-5-amino-1,2-dimethylimidazole further supports our structural assignment.

We selected DiacDMH for most studies because it is both easier to prepare and is not susceptible to acetylation of the side chain at N-1 of the imidazole ring. Results with derivatives of dimetridazole should be similar to those with the comparable metronidazole derivatives, as the potencies of metronidazole and dimetridazole are similar generally [13] and specifically for *E. coli* SR58 (see Fig. 2). DiacDMH itself had no bactericidal activity at concentrations up to 2.5 mM (420 μg/ml) under conditions where both dimetridazole and metronidazole were active (Fig. 2). We confirmed that DiacDMH remained at its initial concentration during this experiment by periodically analyzing filtered aliquots of the incubation medium by HPLC. (DiacDMH had a retention time in the reverse-phase system of 9.5 min). The MIC of DiacDMH for strains of bacteria in the Bactrol Disk Set A (*E. cloacae, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhimurium, S. marcescens, S. aureus, S. epidermidis, S. pyogenes*), *E. coli* AB1157 (wild type), *B. fragilis*, and *C. perfringens* were also found to be greater than 2.5 mM. Under similar incubation conditions, dimetridazole had an MIC for *B. fragilis, C. perfringens*, and *E. coli* SR58 of approximately <0.005, 0.01, and 0.1 mM respectively. Of course, DiacDMH may fail to show bactericidal activity simply because it is unable to cross bacterial membranes. However, passage across cell membranes should not be a problem for this compound, which is less polar than the 5-nitroimidazole itself or its amino functionality, both of which pass across bacterial cell membranes [2].

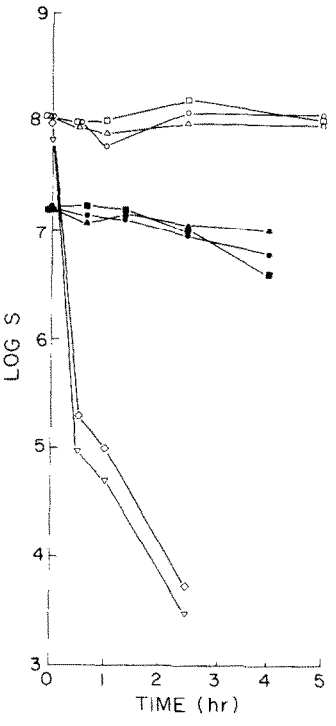

Fig. 2. Effects of DiacDMH, metronidazole and dimetridazole on the viability (S, cfu/ml) of *E. coli* strain SR58. Cultures in 0.1 M phosphate buffer (pH 7.4) were incubated at 37° with either no additions (△), 0.5 mM DiacDMH (□), 2.5 mM DiacDMH (○), 0.5 mM metronidazole (◇), or 0.5 mM dimetridazole (▽). Incubation was either aerobic (closed symbols) or anaerobic (open symbols).

Preparation of AcDMH

Attempts were made to remove the acetyl groups from DiacDMH by both enzymic and non-enzymic methods. When treated with acetylesterase, DiacDMH yielded a product which was separated in impure form from the reaction mixture by preparative reverse-phase liquid chromatography (LC). Structural assignment of this compound indicated that the *O*-acetyl moiety had been removed, resulting in AcDMH (VII, $R_1 = R_2 = CH_3$). IR spectroscopy indicated that the sole remaining acetyl group was in an amide rather than ester linkage. The NMR spectrum was also compatible with this structure. The ring proton at C-4 was not observed, which, as noted above with respect to similar derivatives, was attributed to the rapid exchange of the proton in this position.

Confirmation of the structure of AcDMH was also aided by its conversion to *N*-acetyl-*O*-propionyl-1,2-dimethyl-5-hydroxylaminoimidazole by treatment with propionic anhydride. Mass spectral analysis of this compound (fast atom bombardment [+] ion) showed a molecular ion at *m/e* 226 (MH^+), and a peak at *m/e* 170 (I = 55) corresponding to the loss of methylketene, which is comparable to the loss of ketene that had been observed with DiacDMH, and is, therefore, consistent with a labile propionate ester linkage in one compound and a labile acetate in the other.

The shift in UV absorbance maximum from 216 to 266 nm when DiacDMH was hydrolyzed to yield AcDMH is also noteworthy, as it suggests that the imidazole ring and the hydroxylamino group were better conjugated in a derivative with one rather than two acetyl groups. In comparison, 2-hydroxylamino-1-methylimidazole shows an absorbance maximum in aqueous solution at 230–240 nm [11].

Properties of AcDMH

To maximize the possibility of detecting any bactericidal activity of AcDMH, this monoacetyl derivative was formed from DiacDMH and tested against the SR58 mutant strain of *E. coli*. For this experiment, 3.0 mg (15 μmoles) DiacDMH and one unit of acetylesterase in 1 ml of 0.1 M phosphate buffer, pH 7.0, were used as described above (see Materials), and the resulting solution, containing AcDMH, was incubated aerobically at 37° together with 10 μl of a thick suspension of *E. coli* strain SR58 (~10^8 cfu/ml final concentration) for a period of 4 hr. At intervals, aliquots of the incubation mixture were withdrawn, either to determine bacterial viability or to monitor AcDMH by ultraviolet absorbance. To obtain ultraviolet spectra the aliquot was first filtered to remove bacteria and then diluted 1:40 in water. Under these conditions, the spectra indicated that more than 80% of AcDMH remained at 4 hr, and there was no loss of bacterial viability.

Hydrolysis of DiacDMH

Aryl acyl amidase from *Fusarium* species (EC 3.5.1.13, Sigma Chemical Co.) proved capable of hydrolyzing DiacDMH. Thus, in a 1.0-ml mixture that contained 0.1 M KPO_4 buffer at pH 7.0 and one unit of aryl acyl amidase, incubation at 37° resulted in the loss of more than 90% of 2.0 mM DiacDMH in 1 hr (as monitored by reverse-phase LC). Of the products that formed, one appeared to be AcDMH (in a yield of <10%).

To determine whether the hydrolysis of DiacDMH by this enzyme yielded any products that were bactericidal, DiacDMH (4.0 mM) was incubated aerobically at 37° with *E. coli* SR58 (~10^8 cfu/ml) and 1 unit of aryl acyl amidase in 1.0 ml of KPO_4 buffer, pH 7.4; no bactericidal activity was observed.

DISCUSSION

Our experiments have shown that 5-nitroimidazole drugs (I, Fig. 1) can be partially reduced in the presence of acetic anhydride to yield stable compounds with a hydroxylamino functionality (VI). As these compounds are almost certainly derived directly from 5-hydroxylaminoimidazoles (IV), there must be at least transient formation of the 4-electron reduction species. It seems reasonable, therefore, that this species also forms during the biological reduction of the 5-nitroimidazoles, making it a likely candidate for the active form of these drugs. Although, under certain conditions, there occurs an average of 4-electron reduction of the nitroimidazoles [14, 15], it is not justified to conclude that the hydroxylamine is actually formed biologically. Reduction of 5-nitroimidazoles is a complex process [2, 16] in which overall stoichiometry may be misleading. The uptake of electrons helps to define the product only in cases where there is a clean conversion of one compound to another. In our synthesis of DiacDMH, for example, three equivalents of hydrogen were taken up to make a product that requires only two equivalents, but the yield of DiacDMH was only 35%.

Both the *N*-acetylhydroxylamine (AcDMH, VII) and *O,N*-diacetylhydroxylamine (DiacDMH, VI) derivatives of dimetridazole are reasonably stable under biological conditions and neither has detectable bactericidal activity. It is unclear, however, whether the hydroxylamine itself has any bactericidal activity, as we were uncertain as to whether this compound was liberated from either of the acetyl derivatives. It remains possible, therefore, that the bactericidal activity of a 5-nitroimidazole can be attributed to the hydroxylamine (IV). Indeed, we cannot exclude the possibility that compounds such as DiacDMH and AcDMH would be potent prodrugs for any pathogens with appropriate deacylases.

Acknowledgements—This work was supported by U.S. Public Health Service Grant CA34957 from the National Cancer Institute (DHHS). We would like to thank S. Santikarn of the mass spectrometry facility of the Harvard School of Public Health (Boston, MA) for mass spectral analyses and the Department of Chemistry of Harvard University (Cambridge, MA) for use of their NMR facility.

REFERENCES

1. P. Goldman, R. L. Koch, T-C. Yeung, E. J. T. Chrystal, B. B. Beaulieu, Jr., M. A. McLafferty and G. Sudlow, *Biochem. Pharmac.* **35**, 43 (1986).
2. W. J. Ehlhardt, B. B. Beaulieu, Jr. and P. Goldman, *Biochem. Pharmac.* **36**, 259 (1987).
3. C. E. Sullivan, F. P. Tally, B. R. Goldin and P. Vouros, *Biochem. Pharmac.* **31**, 2689 (1982).
4. M. Mitchard, *Xenobiotica* **1**, 469 (1971).
5. R. O. Beauchamp, Jr., R. D. Irons, D. E. Rickert, D. B. Couch and T. E. Hamm, Jr., *CRC Crit. Rev. Toxic.* **11**, 33 (1982).
6. A. R. Becker and L. A. Sternson, *Proc. natn. Acad. Sci. U.S.A.* **78**, 2003 (1981).
7. G. T. Miwa, R. Wang, R. Alvaro, J. S. Walsh and A. Y. H. Liu, *Biochem. Pharmac.* **35**, 33 (1986).
8. D. I. Edwards, *Biochem. Pharmac.* **35**, 53 (1986).
9. E. C. Taylor and P. K. Loeffler, *J. org. Chem.* **24**, 2035 (1959).
10. R. A. McClelland, J. R. Fuller, N. E. Seaman, A. M. Rauth and R. Battistella, *Biochem. Pharmac.* **33**, 303 (1984).
11. R. A. McClelland, R. Panicucci and A. M. Rauth, *J. Am. chem. Soc.* **107**, 1762 (1985).
12. T-C. Yeung, B. B. Beaulieu, Jr., M. A. McLafferty and P. Goldman, *Antimicrob. Agents Chemother.* **25**, 65 (1984).
13. C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitcheff and R. Vaupré, *Arzneimittel-Forsch.* **16**, 23 (1966).
14. S. N. J. Moreno, R. P. Mason and R. DoCampo, *J. biol. Chem.* **259**, 8252 (1984).
15. N. F. LaRusso, M. Tomasz, M. Müller and R. Lipman, *Molec. Pharmac.* **13**, 872 (1977).
16. E. J. T. Chrystal, R. L. Koch and P. Goldman, *Molec. Pharmac.* **18**, 105 (1980).