Alkylation of 2-Methyl-5-nitroimidazole. Some Potent Antiprotozoal Agents[1]

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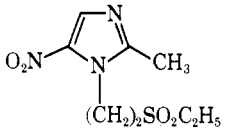
The contending forces influencing the alkylation of 2-methyl-5-nitroimidazole are discussed. A method is described which yields principally the 1-alkylated 2-methyl-5-nitroimidazole isomers preferred for their superior efficacy in the chemotherapy of certain protozoan infections. A series is reported, bearing short aliphatic side chains which incorporate electronegative groups. Several sulfones in this series are potent, well-tolerated, orally effective antiprotozoal agents. One of these agents is in clinical trial, and has been assigned the nonproprietary name, tinidazole.[2]

A real advance in the chemotherapy of protozoan infections was made about a decade ago with the introduction of metronidazole. It was the first orally effective treatment for trichomoniasis,[3,4] and, at higher dosage levels it proved to be the most efficacious and best tolerated treatment for amebiasis.[5] While well-tolerated relative to earlier drugs for the same purpose (*e.g.*, emetine for amebiasis) over the years a considerable clinical literature has accumulated on troublesome side-effects, which occur in significant percentages of patients treated.[6-8] The low toxicity of metronidazole seemed to allow margin for improvement of efficacy. An effort to do so produced ethyl [2-(2-methyl-5-nitro-1-imidazolyl)ethyl] sulfone (**1**) (tinidazole) a more potent drug than metronidazole without a demonstrable difference in toxicity.[9] It was well-tolerated in clinical trials, and it is reasonable to expect that the lower dosage regimen required will reduce the incidence of undesirable side effects. While the use of tinidazole probably will be limited to the treatment of trichomoniasis and amebiasis, some broadening of the spectrum beyond that of metronidazole was noted, perhaps due to the higher potency.



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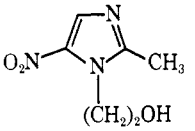

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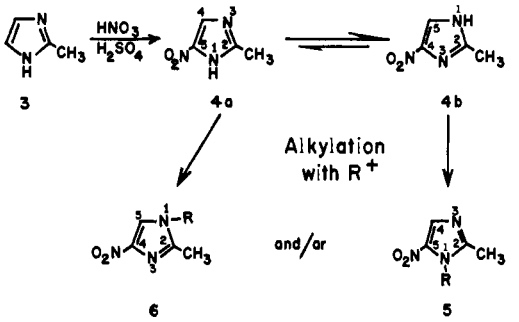
Chemistry.—Commercially available, inexpensive 2-methylimidazole **3**[10] can be nitrated conventionally,[11] and, due to its symmetry, yields a single product, 2-methyl-4(5)-nitroimidazole, which can exist in the tautomeric forms **4a** and **b**. Alkylation can then be accomplished on either the 1- or the 3-nitrogen atom to yield 1-substituted 2-methyl-5-nitroimidazoles **5** or 1-substituted 2-methyl-4-nitroimidazoles **6**, or a mixture of these isomers.



3 **4a** **4b** **6** **5**

Distinctions can be subtle, but generally the 4-nitro isomers are less basic,[12] less soluble in common organic solvents, and have uv absorption maxima which lie a few millimicrons on the blue side from those of the 5-nitro isomers **5**, although this generalization is not entirely dependable. Hydrogen atoms of the CH_2 groups attached to the 1-N atom in **5** show nmr signals measurably downfield from those of **6**. This is due to the deshielding effect of the NO_2 group attached at the C-5 of **5**. Diagnostic differences have been reported recently, too, in the chemical shifts of the NH proton in the conjugate acids of 1-substituted **5** and **6**, depending on the position of the NO_2 group.[13] Finally, at least within the range of our study, the **5** class proved to be more potent antitrichomonals than the **6**.

Alkylation of **4** by alkyl halides or sulfates under alkaline conditions yields principally **6** since in the anion (conjugate base) of **4a** the 3-N atom is the more basic and the more nucleophilic.[14] The substituent effects on the degrees of nucleophilic character of the two N atoms of the imidazole ring in **4** seem to involve induction (σ electrons) rather than conjugation (π electrons), and there is evidence that in polysubstituted imid-

(1) Some of the results of the biological evaluation of these materials were reported at the 9th Interscience Conference on Antimicrobial Agents and Chemotherapy in Washington, D. C., Oct 27–29, 1969, and will be recorded in the Proceedings of that meeting.

(2) Fasigyn®

(3) C. Cosar, T. Julou, and M. Bonazet, *Ann. Inst. Pasteur*, **96**, 238 (1959).

(4) H. Beckman, *Year B. Drug Ther.*, **1963–1964**, 383 (1965).

(5) S. J. Powell, J. MacLeod, A. J. Wilmot, and R. Elsdon-Dew, *Lancet*, 1329 (1966).

(6) R. McG. Harden, C. J. S. Chisholm, and J. S. Cant, *Metab. Clin. Exp.*, **16**, 890 (1967).

(7) S. J. Powell, *Amer. J. Trop. Med. Hyg.*, **16**, 447 (1967).

(8) J. M. C. Holden and A. Keskiner, *J. Clin. Pharmacol.*, **8**, 333 (1968).

(9) H. Howes, J. Lynch, and J. Kivlin, *Antimicrob. Ag. Chemother.* 1969 (in press).

(10) Houdry Process Chemical Co., Philadelphia, Pa.; BASF AG, West Germany; and Toyo Rayon Co., Ltd., Tokushima, Japan are producers of this chemical.

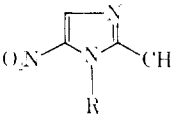
(11) Mp 248° has been reported for this substance: R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, **115**, 217 (1919). As previously described this nitration takes a violent course. Maintenance of a high temperature from the outset and gradual addition of the nitric acid permits nonhazardous operation on a large scale.

(12) The higher basicity of the 5-NO_2 isomers permits their selective removal from mixtures by salt formation as described later.

(13) J. S. G. Cox, C. Fitzmaurice, A. R. Katritzky, and G. J. T. Tiddy, *J. Chem. Soc.*, 1251 (1967).

(14) A good analysis of the kinetic and mechanistic factors controlling the alkylation of imidazole, 4 (5)-nitroimidazole and related systems over a pH range is incorporated in the following series of publications: (a) A. Grimison and J. H. Ridd, *Chem. Ind.*, 983 (1956); (b) A. Grimison, J. H. Ridd and B. V. Smith, *J. Chem. Soc.*, 1352 (**1960**); (c) J. H. Ridd and B. V. Smith, *ibid.*, 1363.

TABLE I
1-SUBSTITUTED 2-METHYL-5-NITROIMIDAZOLES



No.	R	Molecular formula[a]	Synthetic mode	Yield, %	Mp, °C	Recrystallization solvent	Uv absorption[b] λ Acid	Base	Neutral	ε
1	$(CH_2)_2SO_2CH_2CH_3$	$C_8H_{13}N_3O_4S$	A	48	127–128	PhH	302	316	311	8915
9	$(CH_2)_2SO_2CH(CH_3)_2$	$C_9H_{15}N_3O_4S$	B	8	132–133	PhH	275	315	310	8580
10	$(CH_2)_2SO_2(CH_2)_2CH_3$	$C_9H_{15}N_3O_4S$	B	8	94–95	PhMe	300	310	310	8800
11	$(CH_2)_2SO_2CH_3$	$C_7H_{11}N_3O_4S$	B	8	150–151	95% EtOH	300	309	310	8820
12	$(CH_2)_2SO_2(CH_2)_3CH_3$	$C_{10}H_{17}N_3O_4S$	B	5	89–90.5	PhH				
13	$(CH_2)_3SO_2CH_3$	$C_8H_{13}N_3O_4S$	B	3	175–177	MeOH–H_2O	309	310	309	9260
14	$(CH_2)_2SO_2C_6H_5$	$C_{12}H_{13}N_3O_4S$	B	6	119–120	MeOH–H_2O	306	306	306	7375
15	$CH_2CH_2C{\equiv}CH$	$C_7H_7N_3O_2$	B	5	78–78.5	H_2O				
16	$CH_2C{\equiv}CH$	$C_7H_7N_3O_2$	B	27	Gum[c]		300	304	303	7560
17	$CH_2C{\equiv}CCH_2OH$	$C_8H_9N_3O_3$	B	2	175–183	MeOH–H_2O	304	306	306	8690
18	$CH_2CH_2CH{=}CH_2$	$C_8H_{11}N_3O_2$	B	8	40–44	MeOH–H_2O	278	300	310	8295
19	$CH_2CH_2C_6H_5$	$C_{12}H_{13}N_3O_2$	B	2	135–137	MeOH	310	310	310	8095
20	$(CH_2)_3SO_3H$	$C_7H_{11}N_3O_4S$	Special	48	148–151	EtOH	277	310	277	4203
21	CH_2CHCl_2	$C_6H_7Cl_2N_3O_2$	B	3	147–148	95% EtOH	307	309	307	8550
22	CH_2CF_3	$C_6H_6F_3N_3O_2$	B	2	116–118	PhH	300	300	300	7860
23	$(CH_2)_2SC_2H_5$	$C_8H_{13}N_3O_2S$	Special	33	Oily[c]		280	310	310	7680

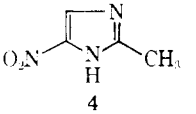
[a] All compounds were analyzed for C, H, N unless otherwise noted. Analytical results were within ±0.4% of the theoretical values. [b] The uv absorptions were determined in MeOH. The extinction coefficients were calculated from the neutral solution curves. The acidic solutions were 0.1 *N* in HCl, the alkaline solutions 0.1 *N* in NaOH. [c] Not analyzed.

azoles the electronic influences of the substituents are essentially additive.[15]

Under neutral or mildly acidic conditions a more complex kinetic mechanism is operative. Two tautomeric forms of **4** are possible, but it has been shown that in imidazoles permitting reversible 1,3-shifts of the NH proton, the tautomeric system will predominantly assume the form in which the NH bond is stronger.[15] Thus in **4** the less acidic protonated form should prevail. Contributing to formation of the desired 5-nitroimidazoles, perhaps, is the "principle of least motion."[16] Operative even in unsubstituted imidazole, the effect of this principle would be intensified by the polarizing influence of the substituents in **4**. Substitution must occur at an unprotonated nitrogen atom, so that in this instance the tautomer present in lower concentration would have the higher rate coefficient for this mechanism of substitution.

Finally, in very strong acidic solutions the conjugate acid would form at the more nucleophilic 3-N in **4**, removing it from competition for the electrophilic alkylating agent. A pH-independent factor favoring formation of the undesired 4-NO_2 isomers is the considerable steric hindrance of the 2-Me and 5-NO_2 groups on the intermediate N atom.

Fusion of **4** with alkyl tosylates often, thought not invariably, gives the desired isomer **5**. Yields tend to be low, but this method has been used to prepare many of the experimental drugs reported to date,[17–19] and indeed was used to prepare many of those reported here. A noteworthy special case of this method was the alkylation of **4** with trimethylenesultone, which gave almost exclusively 1 substitution as in **7**, while propiolactone gave principally 3 substitution as in **8**.



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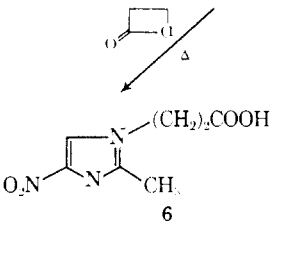

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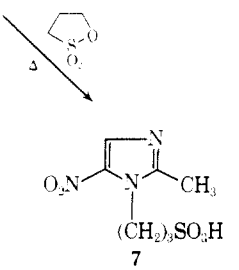

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The success of these alkylating techniques in yielding predominantly the desired isomer may be due to conjugate acid formation at the more nucleophilic N by the strong acids released during the progress of the reaction.

Consistent with the foregoing mechanistic analysis it had been reported that alkylation of 4 (5)-nitroimidazole in HCO_2H solution with Me_2SO [14a] or with ethylene oxide[20] resulted in the formation of, mainly, the 1-alkylated 5-nitro isomer. In our hands application of this method to **4** gave yields of 30–50% in contrast to yields often of 10% or less obtained by the tosylate fusion technique. In addition we found that HOAc could be used as well as HCO_2H, and that **5** could be separated from the minor quantities of **6** present by precipitation as the picrate or as the *p*-toluenesulfonate salt.

(15) G. G. Gallo, C. R. Pasqualucci, P. Radaelli, and G. C. Lancini, *J. Org. Chem.* **29**, 862 (1964).

(16) (a) J. Hine, *ibid.*, **31**, 1236 (1966); (b) F. O. Rice and E. Teller, *J. Chem. Phys.*, **6**, 489 (1938); *ibid.*, **7**, 199 (1939); (c) J.-A. Muller, *Bull. Soc. Chim. Fr.*, **45**, 438 (1886); J.-A. Muller and E. Peytral, *C. R. Acad. Sci.*, **179**, 831 (1924).

(17) C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitscheff, and R. Vanpré, *Arzneim.-Forsch.*, **16**, 23 (1966).

(18) K. Butler, H. L. Howes, J. E. Lynch, and D. K. Pirie, *J. Med. Chem.*, **10**, 891 (1967).

(19) F. Kajfež, V. Šunjic, H. Kolbah, T. Fajdiga, and M. Oklopčzija, *Ibid.*, **11**, 167 (1968).

(20) C. Podesva and K. Vagi, French Patent 1,379,915 (to Rhone-Poulenc) Nov 27, 1964; *Chem. Abstr.*, **62**, 9145 (1965).

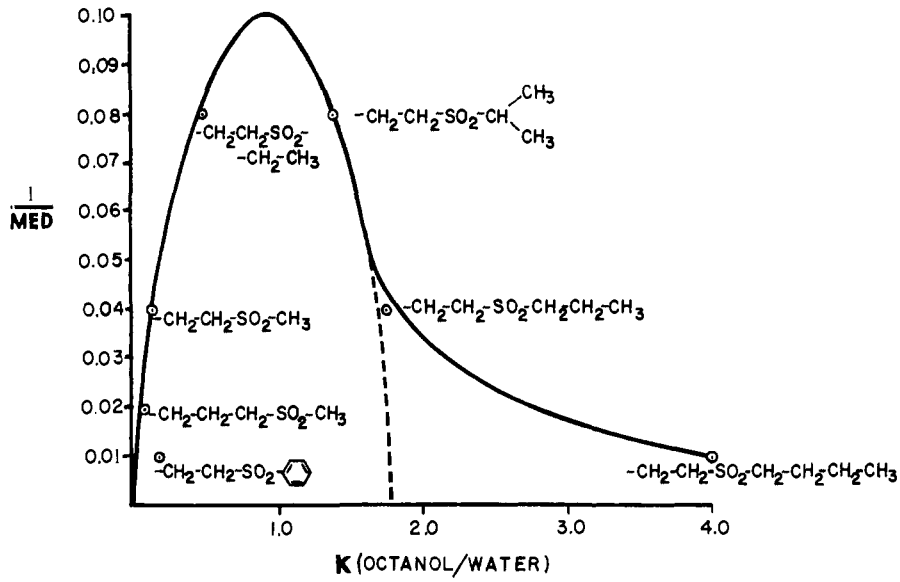

Figure 1.—Octanol-water distribution coefficients *vs.* drug efficacy.

A variety of the specific 1-alkylated 2-methyl-5-nitroimidazoles prepared are listed in Table I.

Structure–Activity Relationships.—While the details of the biological evaluation technique have been published elsewhere,[9,18] the minimum effective dose (MED) is defined as the lowest oral dose (mg/kg) of the drug which, when administered daily for 3 days, beginning 24 hr after infection, completely prevents trichomoniasis (*Trichomonas foetus*) development in 100% of the treated mice. Some of the more active 2-methyl-5-nitroimidazoles are compared in Table II with the reference drug, metronidazole (**2**) (MED 100 mg/kg).

Table II
Activity of Some 1-Substituted 2-Methyl-5-nitroimidazoles in Mice Infected with *Trichomonas foetus*

No.	MED (mg/kg)	No.	MED (mg/kg)
1	12.5	16	100
9	12.5	17	200
10	25	18	100
11	25	19	200[a]
12	100	21	50
13	50	22	200
14	100	2	100
15	25		

[a] Complete clearance at this level, but MED not established.

Electronegative groups in the 1 side chain augmented potency, especially when separated from the 1-N by 1, 2, or 3 CH_2 groups. The sulfone group was particularly effective, leading to antitrichomonal agents eightfold more potent than **2** in our mouse model infection.

Apparently both σ and π contributions of the molecular moieties were important to activity. This is evident from Figure 1 in which octanol–water distribution coefficients[21] are plotted against drug efficacy (1/-MED). The neat parabolic relationship held only over a narrow structural range. It was not possible to include the compounds bearing acetylenic side chains, for example, without destroying the relationship. By maintaining the principal σ-contributing moieties of the molecule (nitroimidazole and sulfone) constant and varying only the π contribution, that is, the R character beyond the sulfone group, it was possible to obtain the parabolic curve until the π contribution reached a certain level. This curve was useful in establishing that near-maximum potency had been obtained within the limitations of this structural series.[22]

Toxicity.[23]—The oral LD_{50} of a 10% suspension of tinidazole was >3600 mg/kg in mice.[24] Similarly the intraperitoneal LD_{50} was >2000 mg/kg in either mouse or rat. Administered orally as a 150 mg/kg dose twice daily for 30 days, tinidazole caused no changes observable clinically or at necropsy in rats or in monkeys.

Spectrum.—The efficacy of tinidazole in other protozoan infections will be reported in detail elsewhere.[9] *In vivo* and *in vitro* evaluation has shown that, beyond the high degree of efficacy against *T. foetus* and *T. vaginalis*, there is a considerable activity also toward *Eimeria tenella* and *Histomonas meleagridis*. It is as effective as metronidazole against *Entamoeba histolytica*.

As an antibacterial tinidazole was selective. The systemic model infection in mice was produced by the intraperitoneal inoculation of test organisms. Against the experimental *Streptococcus pyogenes* C203 infection the PD_{50} values were 25 and 33 mg/kg, respectively, for the oral and subcutaneous routes. There was no protection at dosages up to 100 mg/kg against infections produced by *Pasteurella multocida* or by *Staphylococcus aureus* (either antibiotic susceptible or multiple-resistant to penicillin, streptomycin, and tetracyclines)

(21) The extinction coefficients of the uv absorptions were used to determine the amount of compound in each layer. The following are references to the methodology for determination and application of distribution coefficients in structure–activity analysis: (a) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 6175 (1964); (b) J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965); (c) C. Hansch and S. M. Anderson, *J. Org. Chem.*, **32**, 2583 (1967). See also ref 18.

(22) To have doubled the potency of tinidazole would have required a 1/MED of 0.16, obviously far beyond the apparent maximum of the curve.

(23) This information was contributed by Dr. E. Gralla and his toxicology section at these laboratories.

(24) An acute oral mouse toxicity of >2000 mg/kg has been reported for metronidazole, and as a control in our test it was indistinguishable from tinidazole.

Experimental Section

General.—Melting points were taken on a calibrated Kofler hot-stage microscope. Uv absorption spectra were measured on a Cary Model 13 recording spectrophotometer. Solvents used were analytical reagent grade, and in many cases were stored over molecular sieve desiccants.

2-Methyl-5-nitroimidazole (4).—To 300 ml of 98% H_2SO_4 was added 136 g (1.66 moles) of 2-methylimidazole, allowing the temp to rise to 132°. To this hot solution was added a total of 240 ml of 70% HNO_3 at such a rate as to maintain a reaction temp of 150–170°. After the addition of HNO_3 was completed the mixture was stirred for 1.5 hr at ambient temp, cooled, and poured over ice. The pH was adjusted to 6.0 and the product collected by filtration to yield 305 g (45.2% of theory), mp 250–252°.[11]

β-Bromoethyl Ethyl Sulfide.—To a solution of 400 ml of CCl_4 and 141 g (2.27 moles) of EtSH which was cooled to −16° was added 359 g (2.27 moles) of Br_2 in 360 ml of CCl_4 so that the temp of the mixture did not exceed −10°. The resulting mixture was purged for 20 hr with N_2, then cooled again to −14°. Next 81.2 g (2.9 moles) of ethylene was added, maintaining a temperature of 0° or less. After the ethylene had been added the reaction was left at ambient temperature for 20 hr, the solvent stripped, and the residue distilled to yield 239 g of β-bromoethyl ethyl sulfide (62.5% of theory), bp 68° (18 mm).[25]

Ethyl [2(2-Methyl-5-nitro-1-imidazolyl)ethyl] Sulfide Tosylate.—A solution of 178 ml of HOAc and 250 g (1.96 moles) of 2-methyl-5-nitroimidazole was heated to 90°. Then 166 g (0.98 mole) of β-bromoethyl ethyl sulfide was added and the mixture held at 90° for 1.5 hr. The AcOH was removed at reduced pressure and the residue quenched in 260 ml of H_2O. The unreacted 2-methyl-5-nitroimidazole was removed by filtration and the pH of the aq phase adjusted to 7.0. The product was extracted with $CHCl_3$ which, after drying and concentration, gave 166 g of an oil. The oil was extracted with Et_2O and the extract cooled to yield 130.7 g of product still contaminated with the 4-nitro isomer.[26] The mixture was dissolved in 120 ml of EtOAc and added to 556 g of *p*-TSOH in 200 ml of ethyl acetate. On cooling to −2°, the tosylate salt precipitated and was collected to yield 126 g (33% of theory), mp 112–114°.

Ethyl [2-(2-Methyl-5-nitro-1-imidazolyl)ethyl] Sulfone (1) (Method A).—A mixture of 530 ml of H_2O, 126 g (0.326 mole) of tosylate salt from the preceding procedure, and 200 ml of $CHCl_3$ was cooled to 7°, and 136 ml of 12.5% NaOCl was added in one portion. The resulting mixture was stirred for 15 min, then extracted with $CHCl_3$. The combined extracts were washed with satd $NaHCO_3$ and the $CHCl_3$ was replaced with *i*-PrOH. The solids were collected and dried to yield 39 g (48% of theory), mp 125–126°.

Ethylsulfonylethyl *p*-Toluenesulfonate.—To 69 g (0.5 mole) of ethylsulfonylethanol in 150 ml of C_5H_5N at 0° was added 95 g (0.5 mole) of *p*-TsCl portionwise so that the temperature was held at 0–10°. After addition was complete (10 min), the reaction mixture was stirred at 0–10° for 2 hr. Then 250 ml of H_2O was added slowly and the mixture extracted with $CHCl_3$. The extract was washed with 2 *N* HCl until the aq phase remained acidic, then once with H_2O. Drying (Na_2SO_4) followed by concentration yielded an oil which crystallized to give 113.2 g (77.5%) of product, mp 57–58°.

Ethyl [2-(2-Methyl-5-nitro-1-imidazolyl)ethyl] Sulfone (1) (Method B).—A mixture of 58.4 g (0.2 mole) of the above tosylate and 12.7 g (0.1 mole) of 2-methyl-5-nitroimidazole was heated at 145–150° under N_2 with stirring for 4 hr. The reaction mixture was extracted with 500 ml of hot H_2O. The aq solution was adjusted to pH 9 with 10% Na_2CO_3, then extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4), and concd to a gummy solid. Recrystallization from C_6H_6 yielded 4.36 g (18%) of colorless crystals, mp 127–128°.

Trifluoroethyl *m*-Nitrobenzenesulfonate.—To a mixture of 110.8 g (0.5 mole) of *m*-nitrobenzenesulfonyl chloride and 50 g (0.5 mole) of 2,2,2-trifluoroethanol in 130 ml of $(ClCH_2)_2$ was added a 40% aq solution of 22 g (0.54 mole) of NaOH at a rate permitting maintenance of the reaction temp at 15–25°. After standing overnight at ambient temp, the mixture was diluted with 100 ml of H_2O and 50 ml of $(ClCH_2)_2$. The resulting emulsion was broken by centrifugation, the ethylene chloride layer separated and washed with 300 ml of 2 *N* HCl then with H_2O. Drying of the washed extract (Na_2SO_4), followed by concentration, yielded 100 g of oily tosylate which was used in the following experiment without purification.

Preparation of 1-(2,2,2-Trifluoroethyl)-2-methyl-5-nitroimidazole (22).—A mixture of 57.2 g of the crude tosylate from the preceding experiment, 12.7 g (0.1 mole) of 2-methyl-5-nitroimidazole was heated to 150° under N_2 for 6 hr. Since chromatography (tlc) indicated unchanged starting materials still present, heating was continued for 50 hr at 160°, then 10 hr at 165°. The reaction mixture was worked up as in method B above to yield 4.1 g of crystalline product (21% of theory), mp 118–119°.

Preparation of 3-(2-Methyl-5-nitro-1-imidazolyl)propanesulfonic Acid (20).—A mixture of 12.7 g (0.1 mole) of **4** and 12.2 g (0.1 mole) of propanesultone in 50 ml of DMF was heated at 140–150° for 4 hr. After cooling, dilution with H_2O caused separation of unchanged **4**, which was removed by filtration. Removal of solvents from the filtrate at reduced pressure left a dark gum. The gum was triturated with $CHCl_3$, then with 95% EtOH until it crystallized. The crude product, mp 135–139°, weighed 15.6 g. Recrystallization from EtOH–H_2O yielded colorless crystals, mp 148–151°.

Acknowledgment.—We are grateful to Mr. Robert Martingano, Mrs. Annette Silva, Mr. Herbert Slater, and Mr. John Kivlin for technical assistance. We appreciate reviews of the manuscript by Dr. John Lynch and by Dr. Richard Koch.

(25) Bp 78–81° (21 mm) has been reported for this compound: R. M. McCurdy and S. H. Prager, U. S. Patent 2,925,406 (1960); *Chem. Abstr.*, **55**, 11921 (1961).

(26) The contaminant was detected by tlc.