recrystallized twice from CH₂Cl₂-EtOH to leave 7.5 g (55%) of colorless crystals with mp 245-247 °C dec: UV λ max 228 nm (ϵ 27 700), 258 (16 300), 309 (11 450). Anal. (C₁₈H₁₅ClN₄O₃) C, H. N.

8-Chloro-1-(1,2-dihydroxyethyl)-6-phenyl-4*H*-s-triazolo[4,3-a][1,4]benzodiazepine (11b). A mixture of 4.5 g (0.012 mol) of 11a, 200 mL of THF, 100 mL of MeOH, and 2 teaspoonfuls of Raney nickel was shaken under hydrogen at atmospheric pressure for 2 h. The catalyst was removed by filtration, the filtrate evaporated under reduced pressure, and the residue crystallized from EtOH. Recrystallization from CH₂Cl₂-EtOH yielded 3.2 g (76%) of pure colorless product with mp 211-214 °C dec: UV λ max 222 nm (ϵ 38600), sh 245 (16600), sh 290 (2900). Anal. (C₁₈H₁₅ClN₄O₂) C, H, N.

8-Chloro-1-(1,2-dihydroxyethyl)-6-(2-fluorophenyl)-4*H*s-triazolo[4,3-*a*][1,4]benzodiazepine (11d). A mixture of 10.5 g (0.035 mol) of 1d,⁹ 3.8 g (0.042 mol) of *dl*-glyceraldehyde, and 300 mL of MeOH was boiled on a steam bath for 10 min, allowed to sit at room temperature for 16 h, and filtered. The filtrate was evaporated under reduced pressure. The residue was treated with 400 mL of 1-BuOH and 12 mL (0.073 mol) of diethyl azodicarboxylate. After refluxing for 2 h, the reaction mixture was evaporated under reduced pressure. Crystallization of the residue from CH₂Cl₂-Et₂O gave 4.5 g (35%) of product with mp 203-206 °C. Recrystallization from MeOH-Et₂O yielded colorless crystals with mp 208-210 °C: UV λ max 222 nm (ϵ 36450), infl 245 (15700), sh 280 (2800). Anal. (C₁₈H₁₄ClFN₄O₂) C, H, N.

8-Chloro-6-(2-fluorophenyl)-1-vinyl-4H-s-triazolo[4,3a][1,4]benzodiazepine (12d). A solution of 5.3 g (0.017 mol) of 1d,⁹ 100 mL of CH₂Cl₂, 35 mL of MeOH, 2.5 mL of HOAc, and 3.0 mL of acrolein was stirred at room temperature for 25 min, washed with NaHCO₃ solution, dried, and concentrated under reduced pressure. The residue was treated with 2.5 mL (0.0155 mol) of diethyl azodicarboxylate and 125 mL of DMF and refluxed for 1.5 h. The reaction mixture was evaporated under reduced pressure. The residue was chromatographed over 150 g of silica gel using 3% (v/v) MeOH in CH_2Cl_2 . Clean fractions were combined and evaporated. The product crystallized from Et₂O-petroleum ether and was recrystallized from CH₂Cl₂-petroleum ether to yield 0.5 g (8.7%) of off-white prisms with mp 176-179 °C dec: UV λ sh 211 nm (ε 45 500), sh 245 (24 000), sh 290 (2750); NMR (CDCl₃) & 4.13 (d, 1) and 5.6 (d, 1) (AB system, J = 13 Hz, C₄-H), 5.6–6.8 (m, 3, CH=CH₂), 6.8–7.9 (m, 7, aromatic H). Anal. $(C_{18}H_{12}ClFN_4)$ C, H, N.

8-Chloro-6-(2-chlorophenyl)-1-vinyl-4*H*-s-triazolo[4,3a][1,4]benzodiazepine (12c) and 8-Chloro-6-(2-chlorophenyl)-1-[2-[1,2-bis(ethoxycarbonyl)hydrazo]-*trans*-vinyl]-4*H*-s-triazolo[4,3-a][1,4]benzodiazepine (13c). A solution of 18.0 g (0.05 mol) of 5c, 18 mL (0.11 mol) of diethyl azodicarboxylate, and 500 mL of DMF was refluxed for 45 min and concentrated under reduced pressure. The residue was treated with Et₂O and filtered. The filtrate was evaporated, leaving a mixture according to TLC. Chromatography on 300 g of silica gel with 10% (v/v) EtOH in EtOAc did not separate the products. Two successive chromatographies over silica gel H (according to Stahl for TLC) with 5% (v/v) EtOH in CH₂Cl₂ gave 1.5 g (8%) of 12c and 1.15 g (4%) of 13c.

Recrystallization of 12c from $CH_2Cl_2-Et_2O$ yielded colorless crystals with mp 216–220 °C dec: UV λ infl 218 nm (ϵ 41 000), infl 254 (15 800), infl ~300 (1700); NMR (CDCl₃) δ 4.15 (d, 1) and 5.55 (d, 1) (AB system, J = 13 Hz, C₄-H), 5.6–6.8 (m, 3, CH=CH₂), 7–7.9 (m, 7, aromatic H). Anal. (C₁₈H₁₂Cl₂N₄) C, H, N.

Recrystallization of 13c from CH₂Cl₂-hexane yielded colorless crystals with mp 199–201 °C dec: UV λ max 217 nm (ϵ 43 600), sh 245 (22 600), max 274 (18 450); IR (KBr) 1740 cm⁻¹ (COOEt); NMR (Me₂SO) δ 1.25 (t, 6, J = 7 Hz, CH₃), 4.25 (m, 4, OCH₂), 4.25 (d, 1) and 5.25 (d, 1) (AB system, J = 13 Hz, C₄-H), 5.75 (d, 1) and 7.95 (d, 1) (AB system, J = 13.5 Hz, -CH=CH-), 7.14 (d, 1, J = 2 Hz, C₇-H), 7.3-7.9 ppm (m, 6, aromatic H). Anal. (C₂₄H₂₁Cl₂N₆O₄) C, H, N.

Acknowledgment. The authors wish to thank the following members of our Physical Chemistry Department under the direction of Dr. R. Scott: Dr. F. Scheidl for the microanalyses, Mr. S. Traiman for the IR spectra, and Dr. V. Toome for the UV spectra. We are obliged to Dr. L. O. Randall and Dr. W. Pool and their respective staffs for the pharmacological data.

References and Notes

- For paper 80, see R. Y. Ning, R. I. Fryer, and B. Sluboski, J. Org. Chem., 42, 3301 (1977).
- (2) J. B. Hester, A. D. Rudzik, and B. V. Kamdar, J. Med. Chem., 14, 1078 (1971).
- Deutsche Offenlegungsschrift 2 242 938 to The Upjohn Co. (March 15, 1973).
- (4) K. Meguro and Y. Kuwada, Tetrahedron Lett., 4039 (1970).
- (5) A. Walser and R. I. Fryer, J. Org. Chem., 40, 153 (1975).
- (6) K. Meguro, H. Tawada, H. Miyano, Y. Sato, and Y. Kuwada, Chem. Pharm. Bull., 21 (11), 2382 (1973).
- (7) S. Garattini, E. Mussini, and L. O. Randall, "The Benzodiazepines", Raven Press, New York, N.Y., 1973, p 100.
- (8) L. H. Sternbach, L. O. Randall, R. Banziger, and H. Lehr in "Drugs Affecting the Central Nervous System", Vol. 2, A. Burger, Ed., Marcel Dekker, New York, N.Y., 1968, p 239.
- (9) Mp 205-208 °C dec, prepared from the 2-(N-nitrosomethylamino) derivative as described in ref 5.

Nitroimidazoles with Antibacterial Activity against Neisseria gonorrhoeae

Robert C. Tweit,* R. D. Muir, and Sylvia Ziecina

Departments of Chemical and Biological Research, Searle Laboratories, Chicago, Illinois 60680. Received April 29, 1977

Nitroimidazoles have been prepared which show interesting activity against the bacterium, *Neisseria gonorrhoeae*, in addition to the activities usually shown by nitroimidazoles against protozoa and anaerobic bacteria. The compounds were prepared by alkylation of 1-methyl-2-mercaptoimidazole, followed by nitration. Optimum activity occurs with a 5-nitro group and a free carboxyl at the end of the group attached to the sulfur. The linkage between the sulfur atom and the carboxyl group can be alkylene or phenoxyalkylene. These compounds have only weak activity against other aerobic or facultative bacteria.

Gonorrhea is a serious public health problem in the world today.¹ Strains of the responsible organism, *Neisseria gonorrhoeae*, have arisen which are resistant to penicillin² and there is the possibility that resistance will also develop to alternative drugs. Thus, development of additional drugs with different structures is most desirable. An antigonococcal screening program in our laboratories uncovered interesting activity in some of the compounds reported in a previous paper.³ A number of additional compounds were prepared to explore this activity.

Chemistry. The compounds were made by the methods described in our previous paper.³ Yields of some of the 5-nitro compounds in Table I were low due in part to concurrent nitration of the benzene ring as seen in

Table I

	P. hominis	>100 (2)	10(6)	>100(2)	1 (4)	>100 (2)	>100 (2)	1(2)	100 (2)	>100 (2)	1 (4)	100(2)	1(2)	1 (4)	>100 (2)	100(2)	>100 (2)	1(2)	1(2)	10(2)	100 (2)	100(2)	>100 (2)	10(2)	1 (8)	
	T. foetus	>100 (2)	10(4)	>100 (2)	100(6)	>100 (2)	>100 (2)	1(2)	100(2)	>100 (2)	1 (4)	100 (2)	10(2)	10 (4)	>100 (2)	100(2)	>100 (2)	1 (2)	1(2)	100(2)	100 (2)	100(2)	>100 (2)	10(2)	1(8)	
	T. vaginalis	>100 (2)	10(6)	>100 (2)	10(6)	>100(2)	>100(2)	1(2)	10(2)	>100 (2)	1 (4)	100(2)	1(2)	1(4)	>100 (2)	100 (4)	>100 (2)	1(2)	1(2)	10(2)	100 (2)	100(2)	>100 (2)	10(2)	10 (8)	
	N. zonorrhoeae	100 (2)	>100 (4)	100(2)	>100 (4)	>100 (2)	>100 (2)	100(2)	100(2)	>100 (2)	1 (4)	100(2)	1 (8)	1 (6)	100(2)	>100 (4)	>100 (2)	100(2)	0.1(2)	>100 (2)	>100 (2)	>100 (2)	>100 (2)	>100 (2)	>100 (4)	
	C. perfringens g	>100 (2)	>100 (6)	>100(2)	100 (4)	>100 (4)	>100(2)	100(2)	>100 (2)	>100(4)	10(6)	100(2)	10(4)	10(6)	>100 (2)	>100 (2)	>100 (2)	10 (1)	10(2)	100(2)	>100 (2)	>100(2)	>100 (2)	>100(2)	10 (4)	
×	B. fragilis	$100(2)^{a}$	100(4)	>100(2)	100(6)	>100(2)	>100(2)		100(2)	>100(2)	10(2)	100(2)	1(2)	1 (6)	>100(2)	>100 (4)	>100(2)	10(1)	1 (2)	10 (2)	>100 (2)	100(2)	>100(2)	10(2)	1 (4)	
сн ₂) _m 0-О	Mp, °C	132-134	104-105	163 - 166	139.5-140.5	179.5 - 182	85-85.5	142.5-145	159.5 - 161	187.5-189	209-213	205.5 - 206		191 - 193	114 - 115	134 - 135	36 - 37.5	182 - 187.5	170-171	115.5-117	66-68					
	Analyses	C, H	C, H	Br, N	C, H, N	С, Н	С, Н	C, H	C, H	C, H	C, H	C, H, N	C, H, Na	C, H, N	C, H	C, H, N	C, H, N	C, H, N	C, H, N	C, H, N	С, Н, S	C, H	C, H	C, H, N		
	Formula	C ₁₂ H ₁₃ BrN ₂ OS·HBr	C ₁₂ H ₁₂ BrN ₃ O ₃ S	C ₁₂ H ₁₃ N ₃ O ₃ S HBr	C ₁₂ H ₁₂ N ₄ O ₅ S	C ₁₃ H ₁₃ N ₃ OS·HBr	C ₁₃ H ₁₃ N ₃ OS	C ₁₃ H ₁₂ N ₄ O ₃ S	C ₁₃ H ₁₂ N ₄ O ₄ S	C,H,N,O,S	C ₁₃ H ₁₃ N ₃ O ₅ S	C ₁₃ H ₁₃ N ₃ O ₆ S	C ₁₃ H ₁₂ N ₃ NaO ₅ S	C ₁₄ H ₁₅ N ₃ O ₅ S	C ₁₄ H ₁₈ N ₂ OS-HBr	C ₁₇ H ₂₂ N ₂ O ₃ S·HBr	$C_{17}H_{22}N_2O_3S$	C ₁₅ H ₁₇ N ₃ O ₅ S	$C_{17}H_{21}N_3O_5S$	C ₁₉ H ₂₄ N ₄ O ₅ S	$C_{17}H_{22}N_4O_5S$	C ₁₇ H ₂₂ N ₄ O ₆ S	$C_{17}H_{22}N_4O_7S^{-1}/C_6H_6$	C.,H.,N,O,S		
	X	4-Br-HBr	4-Br	4-NO ₂ ·HBr	$4-NO_2$	4-CN-HBr	4-CN	4-CN	4-CN	4-CO,H	4-C0 ₂ H	$4-CO_{1}H$	4-CO ₂ Na	$4-CO_2H$	H-HBr	4-C0 ₂ C ₂ H ₅ ·HBr	$4-CO_2C_2H_5$	$4-CO_2H$	$4-CO_2H$	$4-CO_2C_2H_5,2-NO_2$	$2-NO_2$	2-NO ₂	$2-NO_3$	2-NO,,4-CO,C,H,	, ,	'n.
	ш	2	2	7	8	2	2	7	2	0	0	0	2	က	4	4	4	4	9	9	7	7	2	10		Sectio
	u	0	0	0	0	0	0	0		0	0	, -	0	0	0	0	0	0	0	0	0	,1	2	0	<i></i>	ental
	R	Н	NO2	Η	NO ²	Н	Η	NO2	NO	H	ŇŐ	NO	NO2	NO2	Η	Η	H	NO ²	NO2	NO	NO	NO2	NO	Ň	nidazole	xperim
	Compd	1	8	e	4	ഹ	9	7	×	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Metror	a See E

Table II

Notes

10 (2)
10 (2)

P. hominis

 $T. foetus \\ 10 (2)$

 $\begin{array}{c} 10 \ (2) \\ 10 \ (2) \end{array}$

 ${10\ (2)\ >100\ (2)}$

 $10(2) \\ 100(2)$

 $\begin{array}{c}B\\fragilis\\10\ (2)\\10\ (2)\end{array}$

 $M_{p, }^{\circ}C$

Analyses

Formula

×

2 ²

2 0 7

Compd

 228^{a} 229^{a}

НО

R 5-NO₂ 5-NO₂

> 24 25

CH₃

T. vaginalis

C. N. perfringens gonorrhoeae

26	5-NO ₂	3	2	НО	230^{a}			>100 (2)	>100(2)	100 (2)	100(2)	100(2)	100 (2)
27	Н	0	4	HO	231ª			>100 (2)	>100(2)	>100 (2)	>100(2)	>100(2)	>100 (2)
28	5-NO3	0	4	НО	233^{a}			1(2)		1(2)	1(2)	1(2)	1(2)
29	5-NO2		4	НО	235^a			100(2)	>100 (2)	100(2)	100(2)	>100 (2)	100 (2)
30	$5-NO_2$	2	4	НО	237^{a}			100(2)	>100(2)	100(2)	100(2)	>100(2)	100(2)
31	5-NO ₂	0	5 2	НО	C ₁₀ H ₁₅ N ₃ O ₄ S	C, H, N	125.5 - 126.5	100(8)	10(8)	0.1(6)	1 (4)	1 (4)	1(4)
32	5-NO ₂	0	9	НО	C,H,NO,S	C, H, N	115 - 117.5	1(6)	1(6)	0.1(6)	1(6)	10(4)	1(4)
33	4-NO ₂	0	9	НО	C,H,NO,S	C, H, N	125 - 126.5	>100 (6)	>100(2)	>100(4)	100 (2)	100(2)	100(2)
34	$5-NO_2$	0	7	НО	C ₁₂ H ₁₀ N ₃ O ₄ S	C, H, N	118-119	10 (8)	0.1(6)	0.1(6)	1(4)	1(4)	1(4)
35	5-NO ₂	0	8	НО	C ₁₃ H ₂₁ N ₃ O ₄ S	C, H, N	110 - 110.5	10 (8)	10(12)	0.1(6)	10(6)	10 (4)	10(6)
36	$5-NO_2$	0	თ	НО	$C_{14}H_{23}N_3O_4S$	C, H, N	114-116	10 (10)	10(10)	0.1(6)	10 (4)	1(4)	1(4)
37	Н	0	10	OH·HBr	2384			>100(2)	>100(2)	>100 (2)	>100(2)	>100(2)	>100(2)
38	5-NO ₂	0	10	НО	239^{a}			10(2)	10(6)	0.1(2)	10(2)	10(2)	10(2)
39	5-NO ²	-	10	НО	C ₁₅ H ₂₆ N ₃ O ₅ S	C, H	119 - 122	>100(4)	>100 (4)	100(8)	10(4)	100(2)	100(2)
40	5-NO ₂	0	10	OCH ₃	240^{a}			>100(2)	>100 (2)	>100(2)	>100(2)	>100(2)	>100 (2)
41	5-NO ₂		10	OCH ₃	241^{a}			>100(2)	>100(2)	100(2)	100(2)	100(2)	100(2)
42	5-NO ₂	63	10	OCH ₃	242^{a}			>100(2)	>100(2)	>100(2)	>100 (2)	>100 (2)	>100(2)
43	5-NO ₂	0	11	ЮН	C ₁₆ H ₂₇ N ₃ O ₄ S	C, H, N	119.5 - 120.5	100(6)	> 100 (8)	>100 (8)	10(2)	10(2)	10(2)
44	5-NO ₁	•	15	НО	C ₂₀ H ₃₅ N ₃ O ₄ S	C, H, N	123 - 123.5	>100 (6)	>100 (2)	>100 (2)	100(2)	100 (2)	100 (2)
^a Comp	ound num	ber in	ref 3.	^b See Exper	imental Section.								

compounds 19 and 23. Some of the nitrations to obtain compounds such as 17 and 18 were run in CF_3COOH , followed by chromatography and ester hydrolysis in H_2SO_4 . The sodium salt 12 was obtained by dissolving the acid 10 in an equivalent amount of NaOH and concentrating the solution to dryness. The NMR spectra of the compounds were consistent with the structures assigned.

Biological Results. The assays of primary interest were the in vitro tests against N. gonorrhoeae, the protozoa Tritrichomonas foetus, Trichomonas vaginalis, and Pentatrichomonas hominis, and the anaerobic bacteria, Bacteroides fragilis and Clostridium perfringens. 1-Hydroxyethyl-2-methyl-5-nitroimidazole (metronidazole, Flagyl), the agent of choice for the T. vaginalis infections, is highly active against these two anaerobes but not against N. gonorrhoeae.

The activities against the protozoa species are essentially parallel. The prime structural requirement is a 5-nitro group on the imidazole ring. Activity is usually reduced by oxidation of the sulfide to a sulfoxide and reduced further on oxidation to the sulfone. Substituents on the phenoxy ring of the compounds in Table I can be varied somewhat, with 4-cyano and carboxyl giving activity equivalent to metronidazole and 4-bromo and nitro also showing good activity. An ortho substituent seems to reduce activity.

Among the compounds of Table II lacking the aromatic ring, the 5- NO_2 group and a free carboxyl group are necessary for activity against protozoa. Peak activity is seen with chain lengths of four to seven methylenes. Conversion of the carboxyl to an ester (40) or oxidation of the sulfur (29 and 30) reduces or eliminates activity.

The presence of a 5-nitro and a carboxyl does not ensure activity since Landmark and Müller⁴ report that 1-carboxymethyl-2-methyl-5-nitroimidazole is inactive vs. T. foetus and T. vaginalis.

The structural requirements for antianaerobe activity against *B. fragilis* and *C. perfringens* are narrower, with 4-carboxyl being the only acceptable substituent and with chain length restricted to m = 2-6 in the compounds of Table I. In Table II a free carboxyl is required with chain lengths of 2-10 acceptable and peak activity at m = 4-6for *B. fragilis* and m = 7 for *C. perfringens*.

Activity against N. gonorrhoeae also has specific structural requirements. Besides the 5-NO₂ group on the imidazole ring, a carboxyl group on the end of the side chain is necessary to obtain activity at 10 μ g/mL or less. The carboxyl cannot be esterfied and oxidation of the sulfur is deleterious.

The number of carbon atoms in the chain is important, being limited to 2–10 in Table II for good activity. Among the compounds in Table II, activity is lost when the chain length reaches 15 methylenes. Peak activity occurs with 5-10 methylenes as shown in Table II.

Some of these compounds were also screened against the bacteria, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermidis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Streptococcus faecalis, Propionibacterium acnes, and Klebsiella pneumoniae, and the fungus, Candida albicans, by methods described before.³⁵ Activity was seen against some of the bacteria, but only at a concentration of 100 μ g/mL. No activity was observed against C. albicans.

Conclusions

Addition of a carboxyl group to the side chain attached to the 2 position of a nitroimidazole ring maintains the in vitro activity reported previously³ for similar compounds. More importantly, a new activity against N. gonorrhoeae is found in these compounds.

Experimental Section

Melting points (corrected) were taken in a Thomas-Hoover capillary apparatus. Analyses indicated by symbols of the elements represent results within $\pm 0.4\%$ of the theoretical values. Most compounds were prepared by the methods of reference 3. Sample preparations are given for the compounds prepared by different methods.

Ethyl 4-[6-(1-Methyl-5-nitro-2-imidazolylthio)hexyloxy]benzoate (45). Ethyl 4-[6-(1-methyl-2-imidazolylthio)hexyloxy]benzoate, 5.8 g, was dissolved in 15 mL of CF₃COOH with 2.5 mL of HNO₃ (concentrated). The solution was kept at room temperature for 18 h, heated to 90 °C for 0.5 h, and then poured into H₂O. The pH was adjusted to 6 with NaHCO₃ and the aqueous layer was decanted from an oil which was chromatographed on Woelm silica in a low-pressure system using 20% ethyl acetate-benzene. Fractions containing 45 (as indicated by thin-layer chromatography) were combined and evaporated to yield a solid which was triturated with ether to give 0.5 g (8%), mp 75-78 °C. Compound 19 was eluted next to yield 0.6 g (10%).

4-[6-(1-Methyl-5-nitro-2-imidazolylthio)hexyloxy]benzoic Acid (18). Compound 45, 0.5 g, was dissolved in 4 mL of H_2SO_4 (concentrated). After 15 min the solution was diluted to 40 mL with H_2O . The solid which separated was recrystallized twice from acetone to give 0.15 g (30%) of 18.

Sodium 4-[2-(1-Methyl-5-nitro-2-imidazolylthio)ethoxy]benzoate (12). Compound 10, 1.8 g, was mixed with less than 1 equiv of NaOH solution and the mixture was stirred for several hours and then filtered. The filtrate was concentrated under vacuum. The residue was 12, 0.9 g (47%).

Microbiology. Antimicrobial tests were conducted by tenfold serial dilution in suitable liquid or solid media, the highest concentration of compounds used being $100 \ \mu g/mL$. Activities against the anaerobic bacteria, *C. perfringens* ATCC 13124 and *B. fragilis* ATCC 23745, were determined in Fluid Thioglycollate

Broth (Bioquest, Cockeysville, Md.) while those against N. gonorrhoeae ATCC 19424, an aerobic bacterium, were determined on Chocolate Agar [GC Agar Base (Bioquest) supplemented with Bacto Hemoglobin and Bacto Supplement C (Difco Laboratories, Detroit, Mich.)]. Diamond medium⁶ with 5% Dubos medium serum (Difco Laboratories) was used for antiprotozoal tests with T. vaginalis ATCC 30001, T. foetus 1002-96-27, and P. hominis ATCC 30000. The C. perfringens and B. fragilis test preparations were incubated for 24 h and the slower growing N. gonorrhoeae and the trichomonads were incubated for 48 h, all at 37 °C. After incubation the bacterial preparations were observed visually for the presence of growth and the trichomonad preparations were observed microscopically for the presence of motile cells. Minimal inhibitory concentrations (MIC values) are reported as the lowest concentrations of the compounds, in $\mu g/mL$, which prevented development of visible growth or resulted in the absence of motile organisms. Replicate tests were done (two to eight replicates) when the quantities of compounds available permitted, and in cases where variations in the results occurred the highest MIC values are reported.

Acknowledgment. We wish to thank Dr. William J. Marsheck⁷ for developing the antigonococcal screen and Mr. Alan E. Moormann and Ms. Bobbie Coleman for excellent synthetic work.

References and Notes

- (1) A. H. Rudolph, J. Am. Med. Assoc., 220, 1587 (1972).
- (2) B. J. Culliton, Science, 194, 1395 (1976).
- (3) R. C. Tweit, E. M. Kreider, and R. D. Muir, J. Med. Chem., 16, 1161 (1973).
- (4) D. G. Lindmark and M. Müller, Antimicrob. Agents Chemother., 10, 476 (1976).
- (5) R. C. Tweit, R. D. Muir, and S. Mizuba, J. Med. Chem., 12, 343 (1969).
- (6) L. S. Diamond, J. Parasitol., 48, 488 (1957).
- (7) W. J. Marsheck and J. Lee, Antimicrob. Agents Chemother., submitted for publication.

Chemoimmunotherapy of Cancer. 3.¹ Analytical Measurement of Chemical Half-Lives of Monofunctional Alkylators

J. E. Wright,* M. J. Hayes, V. Subramanyam, and A. H. Soloway

Department of Medicinal Chemistry and Pharmacology, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, Massachusetts 02115. Received June 6, 1977

The objective of this study is the measurement of the rates of hydrolysis of a series of chloroethyl sulfide derivatives, under simulated physiological conditions. Interferences encountered with the conventional spectrophotometric method prompted the use of a rapid-response, chloride selective electrode. This probe was readily capable of monitoring the hydrolytic rate, which is identical with the rate of chloride ion formation. Since the desired subsecond half-lives were not achieved by any of the compounds, factors influencing the rates were investigated. The results suggest that the rate-controlling cyclization step may be inhibited, due to coordination of undissociated protonic functional groups on the aromatic portions of the structures with the lone-pair electrons on sulfur.

After three decades of clinical experience with cytotoxic alkylating agents in the treatment of cancer, myelosuppressive side effects remain a major pitfall of chemotherapy.² Approaches to this problem have included a number of techniques intended to permit the shunting of anticancer agents through the diseased region, while bypassing sensitive hemopoietic tissues.³⁻⁷ The goal of developing such methods had prompted Seligman and his associates to synthesize a number of alkylators which may be rapidly deactivated by hydrolysis, following a single passage through a tumor's capillary bed. Kinetic studies of these agents showed that the desired subsecond halflives were achieved by a number of compounds in the chloroethyl sulfide series.⁸

Our recent efforts have sought to incorporate this capability of rapid deactivation into the design of alkylators suitable for chemoimmunotherapy of cancer.¹ In this report, kinetic studies on the hydrolysis of such alkylating immunogens are presented. The structures are shown in Figure 1.

Previously, kinetic studies of alkylating agents have utilized the Epstein reagent, 4-(4'-nitrobenzyl)pyridine, to measure the unhydrolyzed mustard remaining after suitable time intervals.^{8,9} Our initial studies showed this