Synthesis and Relationship between Structure and Activity of 2-Nitroimidazole Derivatives

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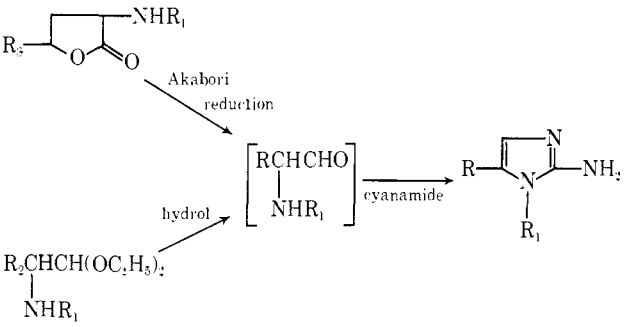
A number of 1,5-disubstituted 2-nitroimidazoles has been synthesized by diazotization and Gattermann reaction on the corresponding 2-aminoimidazoles prepared from amino acetals or amino lactones. Their antitrichomonal activity and acute oral toxicity in mice have been determined. Comparison with other series of nitroimidazoles shows that 1,5-dialkyl compounds are more active and less toxic in respect of monoalkyl or 1,4-dialkyl derivatives, whereas their activity is similar to that of 1,2-dialkyl-5-nitroimidazoles. The influence of steric effects and of partition coefficients is considered. The effect of functional substituents is also discussed.

An extensive program of synthesis of 2-nitroimidazole derivatives has been carried out in our laboratories since the observation[1] that azomycin (2-nitroimidazole) and some of its derivatives[2-4] possess interesting antiprotozoal activity. Preliminary results on the *in vivo* antitrichomonal activity of the methyl derivatives of 2-nitroimidazole (see Table III, **30**, **65**, **66**) focused our attention on the preparation of compounds disubstituted at the 1 and 5 positions of the imidazole ring. This paper is concerned with the synthesis of such compounds by diazotization and Gattermann reaction on the corresponding 2-amino derivatives, the synthesis of these latter intermediates, and a discussion of the structure–activity relationship in different nitroimidazole series.

Synthesis of 2-Aminoimidazoles.—Of the various methods available for the preparation of 2-aminoimidazoles,[5-11] two appeared suitable for the synthesis of the 1,5-disubstituted compounds: (a) the condensation of N-alkylamino acetals with cyanamide followed by cyclization of the intermediate guanidino acetal on treatment with HCl, as proposed by Lawson,[5] and (b) the condensation of N-alkylaminoaldehydes with cyanamide to obtain directly the imidazole ring.[8]

Some attempts at applying Lawson's method to the acetals of α-methylaminopropionaldehyde and butyraldehyde gave poor results mainly due to the difficulty encountered in the separation of the intermediate guanidino acetals from the dicyandiamide formed during the reaction. Better results were obtained by the second method; hence, the preparation of suitable aminoaldehydes to be condensed with cyanamide was performed essentially according to the two following routes: (a) hydrolysis of alkylamino acetals prepared by treating α-bromo acetals with monoalkylamines or α-amino acetals with bromo esters; (b) Akabori reduction of α-alkylamino lactones, prepared from the bromo lactones. In both cases the aminoaldehydes were not isolated but condensed directly in H_2O with cyanamide at pH 4.5 to give the desired 2-aminoimidazoles (Scheme I). The new amino acetals are summarized in Table I.

SCHEME I



Compounds **1–5** were obtained by treating the α-bromo acetals with alkylamine in an autoclave at 110°. The hydroxyalkylamino acetals **6–9** were prepared by refluxing the requisite α-bromo acetal with ethanolamine in cyclohexanol, and **10** and **11** by condensing the α-amino acetal with ethyl bromoacetate in PhH in the presence of NEt_3.

In Table II are reported the 2-aminoimidazoles (12–22) prepared from the corresponding acetals listed in Table I or from known N-alkylamino acetals (method D). Compound **23** and **24** were obtained by a slight modification of method D.

1-Methyl-2-amino-5-(β-hydroxyethyl)imidazole (**25**) and 1-methyl-2-amino-5-(β-hydroxypropyl)imidazole (**26**) were prepared, respectively, by reducing (Akabori reduction) α-methylamino-γ-butyrolactone and the α-methylamino-γ-valerolactone with NaHg followed by condensation of the resulting aldehydes with $NCNH_2$ (method E). Satisfactory yields were obtained in the Akabori reduction of these lactones, in contrast to the poor results observed during attempts to reduce by this method some α-alkylamino esters such as ethyl α-methylaminovalerate and ethyl α-ethylaminobutyrate. Compounds **27–29** were obtained (method F) from the hydroxy derivatives **19**, **20**, and **25** by treatment with $SOCl_2$.

All products were obtained as their hydrochlorides with the exception of **16** isolated in the form of its acetate, and **26** obtained as the picrate. Some of the products were syrups and could not be purified directly by crystallization. In these cases the best purification method involved the addition of picric acid to the syrup,

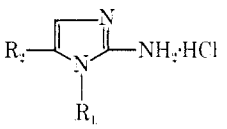
(1) H. Horie, *J. Antibiot.* (Tokyo), **A9**, 168 (1956).
(2) G. C. Lancini, E. Lazzari, and R. Pallanza, *Farmaco, Ed. Sci.*, **21**, 278 (1966).
(3) A. G. Beaman, W. Tautz, T. Gabriel, O. Keller, V. Toome, and R. Duschinsky, *Antimicrobial Agents Chemotherapy*, 469 (1965).
(4) E. Grunberg and E. Titsworth, *ibid.*, 478 (1965).
(5) A. Lawson, *J. Chem. Soc.*, 307 (1956).
(6) T. Phyl, S. Melde, and H. Beyer, *Ann.*, **663**, 108 (1963).
(7) T. Phyl, H. Lahmer, and H. Beyer, *Ber.*, **94**, 3217 (1961).
(8) G. C. Lancini and E. Lazzari, *J. Heterocyclic Chem.*, **3**, 152 (1966).
(9) B. T. Storey, W. W. Sullivan, and C. L. Moyer, *J. Org. Chem.*, **29**, 3118 (1964).
(10) A. Kreutzberger, *ibid.*, **27**, 886 (1962).
(11) R. Burtles and F. L. Pyman, *J. Chem. Soc.*, 2012 (1925).

TABLE I
α-AMINO ACETALS
$R_2CHCH(OC_2H_5)_2$
|
NHR_1

No.	R_1	R_2	Method	Yield, %	Bp, °C (mm)	Formula[a]
1	CH_3	n-C_3H_7	A	57.6	90 (20)	$C_{10}H_{23}NO_2$
2	CH_3	i-C_3H_7	A	48.5	108 (50)	$C_{10}H_{23}NO_2$
3	CH_3	n-C_4H_9	A	73.3	122–123 (40)	$C_{11}H_{25}NO_2$
4	C_2H_5	C_2H_5	A	53.2	80–90 (15)	$C_{10}H_{23}NO_2$
5	C_2H_5	CH_3	A	66.5	74 (19)	$C_9H_{21}NO_2$
6	CH_2CH_2OH	CH_3	B	57.0	94 (1)	$C_9H_{21}NO_3$
7	CH_2CH_2OH	C_2H_5	B	45.6	99 (1.5)	$C_{10}H_{23}NO_3$
8	CH_2CH_2OH	n-C_3H_7	B	27.0	103–105 (2)	$C_{11}H_{25}NO_3$
9	CH_2CH_2OH	i-C_3H_7	B	25.0	100–102 (0.8)	$C_{11}H_{25}NO_3$
10	$CH_2COOC_2H_5$	CH_3	C	68.8	83–85 (0.5)	$C_{11}H_{23}NO_4$
11	$CH_2COOC_2H_5$	C_2H_5	C	56.0	115 (2)	$C_{12}H_{25}NO_4$

[a] All compounds were analyzed for C, H, N and the analytical values obtained were within ±0.4% of the calculated figures.

TABLE II
2-AMINOIMIDAZOLES



No.	R_1	R_2	Method	Yield, %	Mp, °C	Formula[a]
12	CH_3	CH_3	D	56	255–257[b]	$C_5H_{10}ClN_3$
13	CH_3	C_2H_5	D	65	201–203[c]	$C_6H_{12}ClN_3$
14	CH_3	n-C_3H_7	D	45	156–157[c]	$C_7H_{14}ClN_3$
15	CH_3	i-C_3H_7	D	71	170–172[c]	$C_7H_{14}ClN_3$
16	CH_3	n-C_4H_9	D	25	134–135[d] (as acetate)	$C_{10}H_{18}N_3O_2$
17	C_2H_5	C_2H_5	D	59	131–132[c]	$C_7H_{14}ClN_3$
18	C_2H_5	CH_3	D	59	192–193[c]	$C_6H_{12}ClN_3$
19	CH_2CH_2OH	CH_3	D	52	149–150[c]	$C_6H_{12}ClN_3O$
20	CH_2CH_2OH	C_2H_5	D	57.4	134–135[e]	$C_7H_{14}ClN_3O$
21	CH_2CH_2OH	n-C_3H_7	D	43	124–125[c]	$C_8H_{16}ClN_3O$
22	CH_2CH_2OH	i-C_3H_7	D	20	138–139[c]	$C_8H_{16}ClN_3O$
23	$CH_2COOC_2H_5$	CH_3	D[f]	58	215–217[c]	$C_8H_{14}ClN_3O_2$
24	$CH_2COOC_2H_5$	C_2H_5	D[f]	42	192–193[g]	$C_9H_{16}ClN_3O_2$
25	CH_3	$HOCH_2CH_2$	E	62.5	170–171[c]	$C_6H_{12}ClN_3O$
26	CH_3	CH_3CHHCH_2	E	56	195–196[a] (as picrate)	$C_{12}H_{16}N_6O_7$
27	CH_2CH_2Cl	CH_3	F	68	152–153[c]	$C_6H_{11}Cl_2N_3$
28	CH_2CH_2Cl	C_2H_5	F	52.7	136–137[c]	$C_7H_{13}Cl_2N_3$
29	CH_3	$ClCH_2CH_2$	F	65	174–175[c]	$C_6H_{11}Cl_2N_3$

[a] See footnote *a*, Table I. [b] Crystallized from EtOH–Et_2O. [c] Crystallized from MeOH–EtCOMe. [d] Crystallized as acetate from EtOAc. [e] Crystallized from *i*-PrOH. [f] Crystallized from *i*-PrOH–*i*-Pr_2O. [g] Crystallized as picrate from EtOH.

followed by recrystallization of the salt thus obtained. The latter was then reconverted to the hydrochloride by the standard procedure.[12]

Synthesis of 2-Nitroimidazoles.—Table III reports the data for the new compounds. The 1,5-dialkyl-2-nitroimidazoles (**30–36**) have been prepared essentially according to the method previously described[2] by diazotizing the aminoimidazoles in HBF_6 and treating the resulting solution of the diazo compound with an excess of $NaNO_2$ and Cu (method G). The same procedure was used to prepare the hydroxyalkyl compounds **37–40**, **47**, **48**, the chloroalkyl compounds **41**, **42**, **49**, and the imidazolyl acetic esters **52** and **53**. Compounds **43–46** were prepared by esterification of the hydroxyalkyl-2-nitroimidazoles.

The amides of the nitroimidazolylacetic acids as well as the free acids themselves were obtained from the esters **52** and **53** by the usual methods.

Compounds **60** and **61**, 1-methyl-2-nitro-4-ethyl- and 1-(2-hydroxyethyl)-2-nitro-4-methylimidazole, were made alkylating the corresponding 4(5)-alkyl-2-nitroimidazoles.

In Vivo **Biological Activity.**—For the determination of the *in vivo* activity against *Trichomonas vaginalis*, the mouse subcutaneous infection was adopted. Evaluation was based on the presence or absence of lesions and parasites after oral treatment (see details in the Experimental Section). The data reported in Table III represent the ratio between the ED_{50} value of the compounds and that of metronidazole, in the same experiment. Actually the ED_{50} of metronidazole in our experimental conditions can vary from 8 to 17 mg/kg. The values of the oral acute toxicity reported in Table III have been determined in mice; the LD_{50} was determined by the method of Litchfield and Wilcoxon.[13]

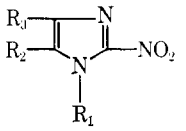
In addition to the activity of the compounds described in this paper we report the biological data of

(12) J. R. Totter and W. J. Darby in "Organic Syntheses," Coll. Vol. III, E. C. Horning, Ed., John Wiley and Sons, Inc., New York, N. Y., 1955, p 460.

(13) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).

TABLE III

2-NITROIMIDAZOLES



No.	R_1	R_2	R_3	Method	Yield, %	Mp, °C	Formula[a]	Rel ED_{50}[p] (metronidazole = 1)	LD_{50}, mg/kg
30	CH_3	CH_3	H	G	32	107–108[c]	$C_5H_7N_3O_2$	0.81	246
31	CH_3	C_2H_5	H	G	30.5	84–85[c]	$C_6H_9N_3O_2$	0.75	372
32	CH_3	n-C_3H_7	H	G	27.2	56–57[d]	$C_7H_{11}N_3O_2$	2	
33	CH_3	i-C_3H_7	H	G	36.5	83–84[e]	$C_7H_{11}N_3O_2$	0.20	265
34	CH_3	n-C_4H_9	H	G	28.7	17–18[f]	$C_8H_{13}N_3O_2$	2.5	162
35	C_2H_5	CH_3	H	G	21	65–66[e]	$C_6H_9N_3O_2$	2.6	234
36	C_2H_5	C_2H_5	H	G	27.5	61–62[h]	$C_7H_{11}N_3O_2$	2.4	
37	CH_2CH_2OH	CH_3	H	G	24.5	144–145[i]	$C_6H_9N_3O_3$	1	705
38	CH_2CH_2OH	C_2H_5	H	G	31.6	138–139[i]	$C_7H_{11}N_3O_3$	1.6	608
39	CH_2CH_2OH	n-C_3H_7	H	G	37.5	121–122[g]	$C_8H_{13}N_3O_3$	1.2	700
40	CH_2CH_2OH	i-C_3H_7	H	G	41.5	155–157[i]	$C_8H_{13}N_3O_3$	1.1	>500
41	CH_2CH_2Cl	CH_3	H	G	25.3	78[i]	$C_6H_8ClN_3O_2$	1.4	*Ca.* 400
42	CH_2CH_2Cl	C_2H_5	H	G	24.5	bp 155–160 (0.8)	$C_7H_{10}ClN_3O_2$	1	325
43	$CH_2CH_2OCOCH_3$	CH_3	H	H	84.3	100[i]	$C_8H_{11}N_3O_4$	1.5	700
44	$CH_2CH_2OCOCH_3$	C_2H_5	H	H	92.2	67–68[i]	$C_9H_{13}N_3O_4$	1.9	850
45	$(CH_2)_2OCO(CH_2)_2COOH$	CH_3	H	H	84.2	115–116[i]	$C_{10}H_{13}N_3O_8$	2.1	>1500
46	$(CH_2)_2OCO(CH_2)_2COOH$	C_2H_5	H	H	93	99–100[i]	$C_{11}H_{15}N_3O_6$	2.3	*Ca.* 1400
47	CH_3	CH_2CH_2OH	H	G	34.4	104–105[i]	$C_6H_9N_3O_3$	>3	*Ca.* 1500
48	CH_3	$CH_2CHOHCH_3$	H	G	30	123–124[i]	$C_7H_{11}N_3O_3$	1.7	670
49	CH_3	CH_2CH_2Cl	H	G	28.5	44–45[j]	$C_6H_8ClN_3O_2$	4	235
50	CH_2COOH	CH_3	H	I	99	250[k]	$C_6H_7N_3O_4$	>3	>1000
51	CH_2COOH	C_2H_5	H	I	90	142–144[k]	$C_7H_9N_3O_4$	>3	>2000
52	$CH_2COOC_2H_5$	CH_3	H	G	36.1	100[i]	$C_8H_{11}N_3O_4$	>3	>2000
53	$CH_2COOC_2H_5$	C_2H_5	H	G	36.5	85–86[i]	$C_9H_{13}N_3O_4$	>3	>2000
54	CH_2CONH_2	CH_3	H	I	95.5	199–200[i]	$C_6H_8N_4O_3$	>3	>2000
55	CH_2CONH_2	C_2H_5	H	I	89	171–172[i]	$C_7H_{10}N_4O_3$	>3	>2000
56	$CH_2CONHCH_3$	CH_3	H	L	57.5	166–168[i]	$C_7H_{10}N_4O_3$	*Ca.* 4.5	>2000
57	$CH_2CONHCH_3$	C_2H_5	H	L	78.2	171–172[l]	$C_8H_{12}N_4O_3$	2.1	*Ca.* 1500
58	$CH_2CON(CH_3)_2$	CH_3	H	I	50	156–158[i]	$C_8H_{12}N_4O_3$	3.2	*Ca.* 1500
59	$CH_2CON(CH_3)_2$	C_2H_5	H	I	70.4	94–95[i]	$C_9H_{14}N_4O_3$	2.3	>1000
60	CH_3	H	C_2H_5	M	71	49–50[e]	$C_6H_9N_3O_2$	>2[b]	33
61	CH_2CH_2OH	H	CH_3	M	57	124–125[l]	$C_6H_9N_3O_3$	1.2	172
62	H	CH_3 (or H)	H (or CH_3)	*m*				1.1	152
63	H	C_2H_5 (or H)	H (or C_2H_5)	*m*				1	212
64	H	n-C_3H_7 (or H)	H (or n-C_3H_7)	*m*				2.1	149
65	CH_3	H	H	*n*				1.1	126
66	CH_3	H	CH_3	*o*				>2[b]	39
67	CH_2CH_2OH	H	H	*n*				1	378

[a] See footnote *a* Table I. [b] Not effective at subtoxic doses. [c] Crystallized from Et_2O. [d] Crystallized from Et_2O–petroleum ether (bp 30–50°). [e] Crystallized from i-Pr_2O. [f] Crystallized from i-Pr_2O–hexane. [g] Crystallized from EtOAc–Et_2O. [h] Crystallized from petroleum ether. [i] Crystallized from MeOH–i-Pr_2O. [j] Crystallized from i-Pr_2O–Me_2CO. [k] Crystallized from H_2O. [l] Crystallized from EtOAc. [m] See ref 14. [n] See ref 15. [o] See ref 2. [p] The figures express the ratio ED_{50}(compound)/ED_{50}(metronidazole).

62–64 whose preparation by microbial oxidation of aminoimidazoles is described elsewhere[14] and of **65–67** previously synthesized.[2,15]

Structure–Activity Relationship.—A comparison of the biological properties of the methyl derivatives of azomycin shows that although 1-methyl-, 1,4-dimethyl-, and 1,5-dimethyl-2-nitroimidazole have similar *in vivo* activity,[2] they are significantly different in their *in vivo* antitrichomonal activity and toxicity.

The data reported in Table III on **65**, **66**, and **30** indicate that the 1,5-dimethyl derivative has the more favorable therapeutic index. The positive effect of alkyl substitution at position 5 is confirmed by a comparsion of the activity and toxicity data of **31** with that of **60** and **65**. Again **37** may be contrasted with **67** and **61**, where the introduction of an alkyl group at position 5 diminishes the toxicity with respect to the compound substituted only at position 1, whereas an alkyl group at position 4 increases the toxicity and lowers the activity.

Within the 1,5-dialkyl derivatives substantial differences in activity are observed. It is noteworthy that is not correlated with toxicity; for instance, **34** is the most toxic and one of the less active of this series.

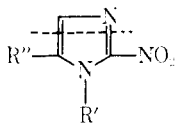
An examination of the influence of the substituent in position 5 of the N-methyl derivatives leads to the following series, in order of decreasing activity: *i*-Pr, Et, Me, *n*-Pr, and *n*-Bu. *In vivo*, exactly the same group sequence has been observed by Butler and coworkers[16] for the alkyl substituents in position 2 in the corresponding series of 1-methyl-2-alkyl-5-nitroimidazoles. Moreover data reported by the same authors show that the substitution of methyl in position 1 of 1,2-dimethyl-5-nitroimidazole with ethyl lowers the activity by a factor of 4. In the 2-nitroimidazoles a similar substitution reduces the activity of **30** and **31** to less than one-third (**35** and **36**).

(14) G. C. Lancini, E. Lazzari, and G. Sartori, *J. Antibiot.* (Tokyo), **21**, 387 (1968).

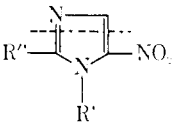
(15) G. G. Gallo, C. R. Pasqualucci, P. Radaelli, and G. C. Lancini, *J. Org. Chem.*, **29**, 862 (1964).

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

A tentative interpretation of this similarity stems from the examination of the structures shown below.



1,5-dialkyl-2-nitroimidazoles

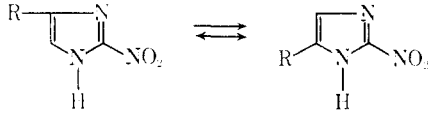


1,2-dialkyl-5-nitroimidazoles

The data discussed above and the fact that 4-nitroimidazoles are much less active than the corresponding 5-nitro isomers[17] suggest that the identical part of the molecule under the dotted lines is responsible for the activity. The latter is possibly the critical part for attachment at the microbial receptor sites. This comparison is to some extent biased by the need to compare activities determined with different techniques in different laboratories. Nevertheless this interpretation is strengthened by the finding that 1-(2-hydroxyethyl)-2-nitro-5-methylimidazole (**37**) has been found in our laboratories to have the same *in vitro* activity as 1-(2-hydroxyethyl)-5-nitro-2-methylimidazole (metronidazole).

For the above-mentioned series of compounds the partition coefficients (K value) have been determined in the system isooctane–aqueous buffer pH 7. A plot of the *in vitro* activity (presented as $1/ED_{50}$) against log K is shown in Figure 1. Taking into account only the compounds with an unbranched chain, a bell-shaped curve can be drawn, but the isopropyl derivative cannot be fitted into the same curve. A similar observation has been made by Butler and coworkers[16] for the 1-methyl-2-alkyl-5-nitroimidazoles; however, in this case the maximum activity was associated with a K value of about one, while an optimal value of about 0.1 is observed in our series. The striking *in vivo* activity of the branched-chain derivatives has been interpreted[16] in terms of drug metabolism by assuming a slower oxidative degradation of these products. We are inclined to attribute it to a higher *in vitro* activity as observed in our laboratory for the isopropyl derivative in comparison with the unbranched compounds.

The biological properties of the 4(5)-alkyl-2-nitroimidazoles (**62**–**64**) can be interpreted by considering that each product is, in solution, a mixture of the two tautomeric forms shown below.



Hence for these compounds biological properties intermediate between those of the compounds in which the alkyl group is fixed in position 4 and those in which it is fixed in position 5 are to be expected. In fact the activity and toxicity values of **62** are intermediate between those of **30** and **66**; this is true also for **63** with respect to **31** and **60**.

Concerning the effect of functional groups it can be seen that the introduction of OH in the β position of the alkyl chain results in a marked lowering of the toxicity (compare **35**, **37**; **36**, **38**; **31**, **47**; **32**, **48**). The activity is generally improved with the exception of **47** which is almost inactive. Acetylation of OH has no particular influence on the activity (compare **37** and **38** with **43** and **44**); the activity of the hemisuccinates (**45** and **46**) is probably due to *in vivo* hydrolysis since they were almost inactive *in vitro*.

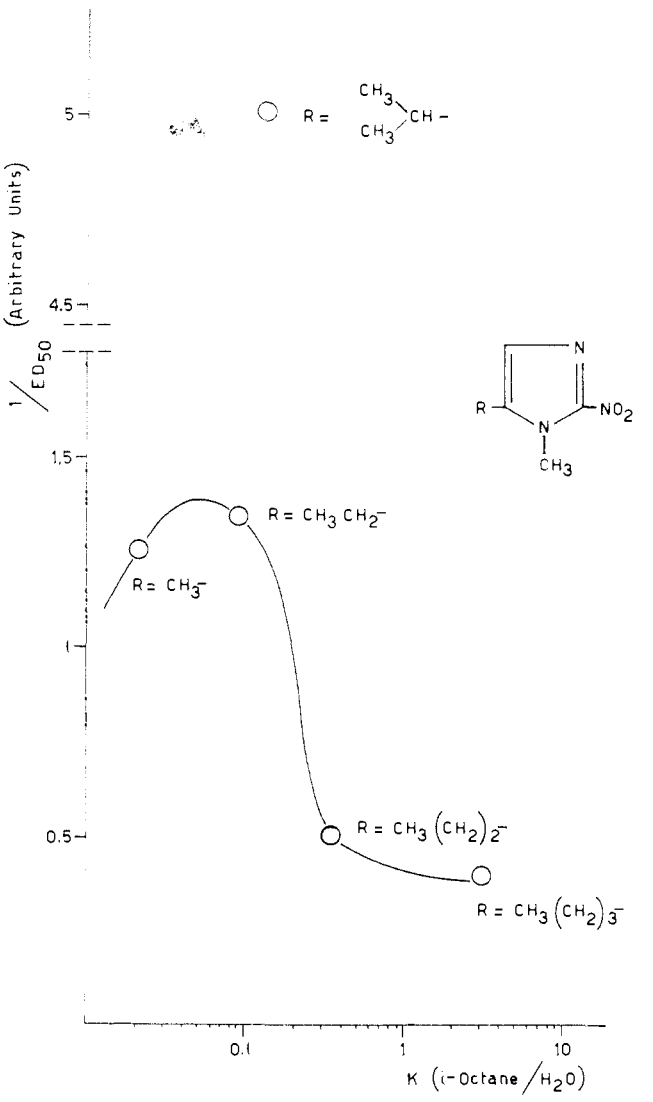


Figure 1.

The carboxylic derivatives generally show a low degree of activity. Some amides, such as the *N*-methyl- and *N*,*N*-dimethylamides of 2-nitro-5-ethyl-1-imidazoleacetic acid (**57**, **59**) appear of interest because they possess a fair degree of activity and a very low toxicity.

Experimental Section

The structures of all compounds were supported by their elemental analysis (see footnote *a* under Table I) and ir, uv, and nmr spectra. The melting points are uncorrected.

Determination of *in Vivo* Activity.—CF_1 female mice weighing 18–22 g were used. Standardized culture of *Trichomonas vaginalis* (800,000–1,000,000 protozoa/ml (1 ml) was injected subcutaneously in the dorsal area. Oral treatment was started immediately after infection and continued for 4 days (once daily). Four doses of each product were administered to groups of ten mice. Seven days after infection the animals were sacrified and examined for the presence or absence of viable parasites and then the ED_{50} was calculated according to the method of Spearman-Kärber.[18]

(17) C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitcheff, and R. Vaupré, *Arzneimittel-Forsch.*, **16**, 23 (1966).

(18) D. J. Finney, "Statistical Method in Biological Assay," C. Griffin and Co. Ltd., London, 1952, p 524.

α-Methylaminohexanal Diethyl Acetal (3). Method A.—A mixture of 107 g of α-bromohexanal diethyl acetal, 100 ml of C_6H_6, and 200 ml of dry $MeNH_2$ were heated in an autoclave for 16 hr at 110–120°. After cooling at room temperature the excess $MeNH_2$ was allowed to evaporate and the solvent was removed in Et_2O (150 ml). The suspension was mixed on cooling with 40% KOH (150 ml), the organic layer was separated, and the aqueous solution was extracted three times with Et_2O. The extracts were pooled and the solvent was evaporated. The residue was fractionated by distillation yielding 63 g of the desired product, bp 122–123° (40 mm).

α-(2-Hydroxyethylamino)propionaldehyde Diethyl Acetal (6). Method B.—A mixture of 32 g of α-bromopropionaldehyde diethyl acetal, 128 ml of ethanolamine, and 400 ml of cyclohexanol was refluxed for 5 hr. The cyclohexanol and the excess ethanolamine were evaporated under reduced pressure, and the residue was dissolved in 450 ml of $CHCl_3$ and washed once with 50 ml of H_2O. The solvent, dried over $CaCl_2$, was evaporated and the residue was fractionated by distillation. The acetal was collected at 94° (1 mm), yield 16.5 g.

Ethyl N-[1-(Diethoxymethyl)propyl]glycinate (11). Method C.—To a solution of 28.8 g of diethyl α-amino acetal and 25.07 ml of Et_3N in 180 ml of anhydrous C_6H_6, 20.03 ml of ethyl bromoacetate was added dropwise and the mixture was refluxed for 8 hr under stirring. The $Et_3N \cdot HBr$ was collected and the solution was evaporated under reduced pressure. The residue was fractionated by distillation yielding 24.7 g of 11, bp 115° (2 mm).

1-Methyl-2-amino-5-*n*-propylimidazole (14). Method D.—A solution of 18.8 g of α-methylaminovaleraldehyde diethyl acetal in 300 ml of 15% HCl was stirred 3 hr at 55–60°. The cooled solution was brought to pH 4.6 by cautiously adding 50% NaOH and then 12.6 g of cyanamide was added. The mixture was warmed 2 hr at 55–60° maintaining the pH at 4.6 by addition of 10% HCl and then extracted with $CHCl_3$ in order to remove the by-products. The solution was evaporated to dryness, and the residue was washed three times with Et_2O and extracted several times with EtOH–HCl which was then evaporated. The oily residue, containing **14**·HCl, was dissolved in 100 ml of H_2O and poured into a boiling solution of 25 g of picric acid in 600 ml of H_2O. The yellow-brown picrate obtained, recrystallized from EtOH, weighed 16.9 g, mp 182–183 . It was converted to the corresponding hydrochloride by dissolving it in a boiling mixture of 250 ml of 12% HCl acid and 500 ml of C_6H_6. The organic phase was separated and the water solution, after several extractions with C_6H_6, was evaporated to dryness under reduced pressure. The residue was suspended in MeCOEt and dissolved by addition of a few milliliters of MeOH. The solution upon concentration gave 7.7 g of crystals, mp 156–157°.

Ethyl 2-Amino-5-methyl-1-imidazoleacetate (23). Method D.—A solution of 20 g of ethyl N-[1-(diethoxymethyl)ethyl]-glycinate in 300 ml of 15% HCl was stirred 4 hr at 55–60°, then decolorized and brought to pH 4.5 by cautiously adding 50% NaOH at 0 to −5°. Cyanamide (10.8 g) was added and the solution was stirred 2 hr at 50° maintaining the pH at 4.5 by addition of 10% HCl. The resulting dark solution was evaporated to dryness and the residue was triturated with Et_2O to remove excess H_2NCN. The crystalline mass was extracted several times with 200-ml portions of EtOH containing 5% HCl and the EtOH solution was concentrated to about 100 ml under reduced pressure. After addition of 100 ml of EtOH saturated with HCl the solution was refluxed 1 hr, then evaporated several times with further additions of EtOH to remove the excess HCl. The residue was taken up with 50 ml of *i*-PrOH and allowed to crystallize overnight in a refrigerator. White crystals (8.9 g) were collected. A second crop weighed 2 g. A sample recrystallized from *i*-PrOH melted at 215–217°.

1-Methyl-2-amino-5-(2-hydroxyethyl)imidazole Hydrochloride (25). Method E.—To a solution of 50 g of α-methylaminobutyrolactone hydrochloride (obtained in 85% yield by refluxing for 1 hr a mixture of α-methylamino-γ-hydroxybutyric acid in EtOH saturated with HCl) in 450 ml of H_2O and 200 ml of EtOH, 3160 g of 2.5% NaHg was added in about 2 hr keeping the temperature at from −3 to +3° and the pH at 2.5–3.5 by adding dropwise 15% HCl. The mixture was stirred 2 hr more to complete the reaction, the Hg was separated and then the pH was brought to 4.6 g by cautious addition of 20% NaOH at 0–5°. H_2NCN (42 g) was added and the resulting solution was heated for 2 hr at 55–60° maintaining the pH at about 4.5 by addition of HCl. The solution was evaporated to dryness, and the residue was washed with Et_2O and then extracted with EtOH made acidic with HCl. The EtOH solution was evaporated to dryness and the residue, treated with 50 ml of cold *i*-PrOH, gave 36.5 g of white crystals, mp 170–171° (after recrystallization from *i*-PrOH).

α-Methylamino-γ-hydroxyvaleric acid was prepared according to the procedure described by Laliberté[19] for the synthesis of the methylaminohydroxybutyric acid. Starting from 30 g of α-bromo-γ-valerolactone, 20.55 g of a white solid was obtained, mp 211° dec (from Me_2CO–H_2O). *Anal.* $C_6H_{11}NO_3$) C, H, N.

1-Methyl-2-amino-5-(2-hydroxypropyl)imidazole Picrate (26). Method E.—α-Methylamino-γ-valerolactone hydrochloride (45 g) (mp 171–172°) (obtained in 80% yield by refluxing for 1 hr a solution of α-methylamino-γ-hydroxyvaleric acid in EtOH saturated with HCl) was reduced and condensed with H_2NCN according to the procedure above described for the corresponding 5-β-hydroxyethyl derivative. An oily residue was obtained which was dissolved in 150 ml of H_2O and poured into a boiling solution of 62.6 g of picric acid in 1300 ml of H_2O. The crude precipitate was recrystallized from EtOH, yield 58.5 g, mp 195–196°.

1-(2-Chloroethyl)-2-amino-5-methylimidazole Hydrochloride (27). Method F.—A solution of 9.6 ml of $SOCl_2$ in 37 ml of C_6H_6 was added dropwise to a well-stirred mixture of 12 g of 1-hydroxyethyl-2-amino-5-methylimidazole hydrochloride and 150 ml of C_6H_6, maintaining the temperature from 0 to 5°. After stirring 1 hr at room temperature, the mixture was refluxed 1 hr. The dark mixture was extracted three times with H_2O, which, decolorized with Norit, was evaporated to dryness. The solid residue was crystallized from MeOH–EtCOMe yielding 9 g of a white product, mp 152–153°.

1-Methyl-2-nitro-5-ethylimidazole (31). Method G.—A solution of 7.2 g of $NaNO_2$ in 40 ml of H_2O was added dropwise at −20° in 30 min to a stirred mixture of 16.2 g of 1-methyl-2-amino-5-ethylimidazole hydrochloride, 50 ml of H_2O, and 68 ml of 50% fluoroboric acid; the stirring was continued for 30 min at −10°. The solution was poured into a well-stirred mixture of 21.2 g of Cu powder, 74 g of $NaNO_2$, and 2000 ml of H_2O. After 2 hr the Cu was filtered off and the solution, brought to pH 2, was extracted with Et_2O (four 2000-ml portions). The combined extracts were concentrated to 500 ml and washed with a 5% $NaHCO_3$ solution (two 100-ml portions) which was reextracted with Et_2O. The combined organic phases on concentration gave 3.9 g of crystals, mp 84–85°. A further crop of 0.8 g was obtained from the mother liquor by column chromatography (silica gel–Et_2O).

1-(2-Acetoxyethyl)-2-nitro-5-ethylimidazole (44). Method H.—A mixture of 1.5 g of 1-(2-hydroxyethyl)-2-nitro-5-ethylimidazole and 15 ml of Ac_2O was heated at 90–100° for 7 hr. Evaporation of the solution gave an oily residue which solidified on standing and weighed 1.7 g. A sample recrystallized from *i*-Pr_2O containing a few drops of MeOH melted at 67–68°.

1-(2-Hydroxyethyl)-2-nitro-5-ethylimidazole Monosuccinyl Ester (46). Method H.—A mixture of 1.5 g of 1-(2-hydroxyethyl)-2-nitro-5-ethylimidazole, 0.81 g of succinic anhydride, and 1.31 ml of pyridine was warmed for 1 hr on a steam bath. The mixture was then evaporated under reduced pressure and the oily residue was dissolved in a few milliliters of AcOEt, absorbed in a 2 × 40 mm column of silica gel 0.05–0.2 mm, and eluted with AcOEt. Fractions of 150 ml were collected. From the first and the second fractions, respectively, 2.07 and 0.08 g of the pure succinyl ester were obtained. A sample crystallized from *i*-PrO_2 containing a few drops of CH_3OH melted at 99–100°.

2-Nitro-5-methyl-1-imidazoleacetic Acid (50). Method I.—To 7 g of ethyl 2-nitro-5-methyl-1-imidazoleacetate suspended in 20 ml of H_2O, 1 mole of 10% NaOH was added and the mixture was gently heated to complete dissolution of the product. The cooled solution acidified with 10% HCl acid gave a precipitate which was collected and washed with 5 ml of cold H_2O, yield 6.2 g, mp 250°.

2-Nitro-5-ethylimidazoleacetamide (55). Method I.—In a solution of 1.55 g of ethyl 2-nitro-5-ethylimidazoleacetate in 40 ml of EtOH, anhydrous NH_3 was bubbled for 6 hr. After 2 days at room temperature the solution was evaporated and the residue crystallized from MeOH–*i*-Pr_2O, yield 1.20 g of light yellow crystals, mp 171–172°.

N-Methyl-2-nitro-5-ethyl-1-imidazoleacetamide (57). Method L.—To a solution of 1.5 g of 2-nitro-5-ethyl-1-imidazoleacetic acid in 16 ml of anhydrous THF 0.72 ml of ethyl chloroformate was

(19) R. Laliberté and L. Berlinguet, *Can. J. Chem.*, **40**, 1960 (1962).

added at 0 to −5°. After stirring for 30 min, $MeNH_2$ was slowly bubbled for 3 hr in the mixture maintained from 0 to 5°.

The mixed anhydride, which had separated, slowly redissolved and the clear solution was allowed to stand overnight at room temperature and then evaporated. The residue was crystallized from EtOAc and weighed 1.25 g, mp 171–172°.

N,N-Dimethyl-2-nitro-5-ethyl-1-imidazoleacetamide (59). Method I. To a solution of 5 g of ethyl 2-nitro-5-ethylimidazole-acetate in 150 ml of MeOH, 68 g of Me_2NH was added. The mixture was heated 8 hr at 40° and, after one night at room temperature, refluxed for 6 hr. Evaporation of the solvent afforded an oily residue which solidified on standing. After crystallization (MeOH–i-Pr_2O) 3.5 g of yellow crystals was obtained, mp 94–95°.

1-Methyl-2-nitro-4-ethylimidazole and 1-Methyl-2-nitro-5-ethylimidazole (60–31). Method M. A suspension of 1.6 g of silver 4(5)-ethyl-2-nitroimidazole and 2 ml of CH_3I in 60 ml of Me_2CO was refluxed for 4 hr. The inorganic precipitate formed during the reaction was filtered off and the solution was evaporated to dryness. The oily residue on the silica gel, Et_2O–petroleum ether (bp 30–50°) 1:1) showed one spot (R_f 0.1) corresponding to 1-methyl-5-ethyl-2-nitroimidazole and a further spot having R_f 0.2. The two products were separated through a 2 × 20 cm silica gel column eluted with petroleum ether containing increasing quantities of Et_2O. The fractions were pooled according to the analysis. Those containing the product having R_f 0.2 gave 780 mg of 1-methyl-2-nitro-4-ethylimidazole after recrystallization from i-Pr_2O, mp 49–50°. From those containing the compound having R_f 0.1 was obtained 195 mg of 1-methyl-2-nitro-5-ethylimidazole, identical with a sample prepared from 1-methyl-2-amino-5-ethylimidazole according to method G.

1-(2-Hydroxyethyl)-2-nitro-4-methylimidazole (61). Method M. To a suspension of 1.17 g of silver 4(5)-methyl-2-nitroimidazole, in 90 ml of $CH_3C_6H_5$, 9.5 ml of bromoethanol was added and the mixture was refluxed 7 hr under stirring. The inorganic salt was filtered off and the organic phase was evaporated. The residue was extracted several times with boiling H_2O and, after purification with charcoal, the solution was evaporated to an oily syrup which solidified on standing. By recrystallization from EtOAc was obtained 490 mg of yellow crystals, mp 121–125°.

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Lincomycin. VIII. 4′-Alkyl-1′-demethyl-4′-depropylclindamycins. Potent Antibacterial and Antimalarial Agents[1]

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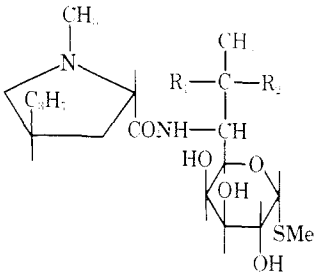
The preparation of methyl 7(*S*)-chloro-7-deoxythiolincosaminide (**4**) is described and evidence favoring the 7(*S*) configuration for the 7-chloro substituent is presented. This compound was used in the preparation of 4′-alkyl-1′-demethyl-4′-depropylclindamycins shown to be potent antibacterial and antimalarial agents.

Replacement of the 7(*R*)-hydroxyl group of lincomycin (**1**)[2] and of a number of its 4′-alkyl-4′-depropyl analogs[3] by chlorine afforded derived antibiotics which



1, R_1 = OH; R_2 = H
2, R_1 = H; R_2 = Cl

possessed enhanced antibacterial potency. As part of a program to delineate the potentiating effect of this substituent the synthesis of 1′-demethyllincomycin analogs containing a 7(*S*)-chloro-7-deoxy grouping was undertaken. The 7(*S*)-chloro substituent was introduced into lincomycin[2] and 4′-alkyl-4′-depropyllincomycin analogs by treatment of the parent antibiotic with $SOCl_2$.[3] This procedure was not applicable to the synthesis of certain types of chlorinated lincomycins, particularly those possessing reactive groups in the amino acid moiety. Maximum flexibility in analog synthesis appeared to be attainable by chlorination of methyl thiolincosaminide (MTL) (**3**), followed by condensation of the resulting chloro sugar (**4**) with an appropriately substituted proline derivative. To this end, our initial efforts were directed toward the synthesis of methyl 7(*S*)-chloro-7-deoxythiolincosaminide (**4**).

Although lincomycin can be cleaved with hydrazine hydrate to yield the sugar, methyl thiolincosaminide (**3**),[4] similar treatment of clindamycin[5] (**2**) did not afford the corresponding chloro sugar **4**. Therefore the direct introduction of Cl into methyl thiolincosaminide (**3**) was investigated. Treatment of **3** with excess triphenylphosphine dichloride in MeCN afforded a monochloro substituted product in 40% yield. Condensation of this product with *trans*-1-methyl-4-*n*-propyl-L-proline[6] yielded clindamycin (**2**) identical, both chemically and microbiologically, with that prepared from lincomycin (Chart I). This conversion indicated that the Cl in **4** was in the same position and configuration as the 7-Cl of clindamycin (**2**).

Since the configuration about the 7-carbon of clindamycin (**2**) was uncertain at the time that methyl 7-chloro-7-deoxythiolincosaminide (**4**) was first prepared, experiments were directed toward establishing the

(1) Presented in part at the 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 9–14, 1967, and at the Eleventh Medicinal Chemistry Symposium, Quebec, Canada, June 1968.

(2) (a) R. D. Birkenmeyer, Abstracts of Papers, Fifth Interscience Conference on Antimicrobial Agents and Chemotherapy and IVth International Congress of Chemotherapy, Washington, D. C., Oct 17–21, 1965, p 17; (b) R. D. Birkenmeyer and F. Kagan, to be published; (c) B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *Antibacterial Agents Chemotherapy*, 727 (1966).

(3) B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *J. Med. Chem.*, **10**, 355 (1967).

(4) W. Schroeder, B. Bannister, and H. Hoeksema, *J. Am. Chem. Soc.*, **89**, 2448 (1967).

(5) Clindamycin is the generic name for 7(*S*)-chloro-7-deoxylincomycin.

(6) B. J. Magerlein, R. D. Birkenmeyer, R. R. Herr, and F. Kagan, *J. Am. Chem. Soc.*, **89**, 2459 (1967).