spectrum, major signals at m/e 197, 182, 155, 140, 127, 125, 113, 111, 98, and 97. *Anal.* ($C_{10}H_{19}N_3O$) C, H, N.

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Deuterium Isotope Effect in Chloramphenicol Action

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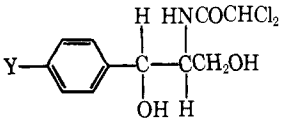
α-Deuteriochloramphenicol was synthesized and its activity against *Escherichia coli* was tested in comparison with chloramphenicol itself. α-Deuteriochloramphenicol had only approximately 80% of the chloramphenicol activity. This finding supports the hypothesis that the benzylic C–H bond is broken in the kinetically determining step of chloramphenicol action. This is consistent with the idea that the antibiotic blocks an enzyme covalently.

Several hypotheses have been developed to explain the mode of action of chloramphenicol in bacterial cells.[1,2] Evidence is increasing that chloramphenicol interferes with the synthesis of proteins essential in cell division.[3] However, the biochemical studies on the mechanism of action of chloramphenicol and the structure–activity studies in the chloramphenicol series of compounds essentially remained unrelated.[2] An interesting exception is the work of Jardetzky and Julian.[4] These authors suggest that the antibiotic acts *via* a competitive inhibition mechanism because of some structural similarities between chloramphenicol and pyrimidine nucleotides. However, Richmond recently pointed out,[5] that antibacterial substances which act solely by competitive inhibition of a single enzyme or receptor have proved to be relatively ineffective as chemotherapeutic agents. Even in cases where antibacterial compounds affect the operation of regulatory processes in the cell in a competitive manner the evolutionary flexibility of bacterial populations ensures that a process of mutation followed by selection allows the bacterial population to survive. Therefore, the most effective bacterial inhibitors are compounds that bind irreversibly to an enzyme active center or are bound covalently to an enzyme product that is used for a subsequent biosynthetic step. Azaserine, puromycin, and penicillin, for example, act in this manner. From a chemical point of view it is not difficult to detect the chemically reactive site in azaserine, puromycin, or penicillin. Chloramphenicol is a very potent antibiotic, too. However, compared to other antibiotics chloramphenicol appears to be quite unreactive (Figure 1).

Recently, Hansch, *et al.*,[6] reported a good quantitative correlation with a set of chloramphenicols of structure I obtained using regression analysis. In eq 1

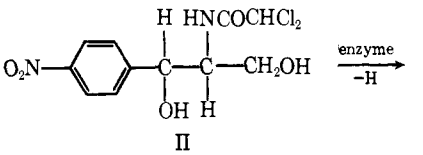


I

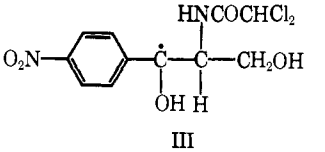
$$\log A = [3.069\ (\pm 1.2)]E_R + [0.227\ (\pm 0.16)]\pi + 0.769\ (\pm 0.25) \quad (1)$$

$$n = 8 \qquad s = 0.140 \qquad r = 0.954$$

log A represents variations in chloramphenicol activity on the growth of *Escherichia coli* due to changes of the substituent Y.[7] E_R is a radical parameter[8] and π a hydrophobic constant,[9] both dependent on the properties of Y. Although substituent effect analysis *per se* cannot elucidate a particular mechanism of action it still can provide valuable information about the nature of drug–receptor interactions. From eq 1 it was concluded that changes in activity are proportional to the ability of the substituents to stabilize a free radical provided the lipophilic character of the substituents is kept constant. This led Hansch, *et al.*, to postulate that chloramphenicol may act *via* a hydrogen radical transfer mechanism.[6] Therefore the benzylic CH bond



II



III

is proposed to be the point of chemical attack at the site (Figure 1). The enzyme involved could be in-

(1) T. D. Brock, *Exp. Chemother.*, **3**, 119 (1964).
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(4) O. Jardetzky and G. R. Julian, *ibid.*, **201**, 397 (1964).
(5) M. H. Richmond, International Symposium on the Rational Development and Application of Drugs, Nijmegen, the Netherlands, July 8–10, 1969.
(6) C. Hansch, E. Kutter, and A. Leo, *J. Med. Chem.*, **12**, 746 (1969).
(7) E. R. Garrett, O. K. Wright, G. H. Miller, and K. L. Smith, *ibid.*, **9**, 203 (1966).
(8) T. Yamamoto and T. Osu, *Chem. Ind. (London)*, 787 (1967).
(9) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

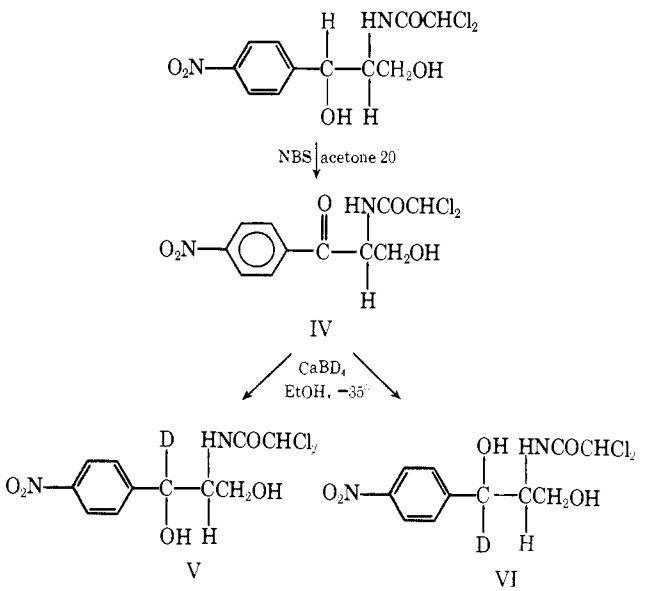
hibited irreversibly by being bound covalently to the remaining radical III in a subsequent step.

The purpose of the different substituents Y in this connection can be pictured as that of analytical feelers which provide information about the electronic changes occurring at the site during the kinetically determining step of drug action. However, it would be hazardous to use eq 1 alone to diagnose the reaction mechanism of chloramphenicol and its derivatives, because Y has only a transmitter function and is not directly involved in bond breaking.

In addition, the interpretation of reaction mechanisms based on correlation studies must be handled with caution, because of the interrelationship between various substitutent constants. For example, Cammarata[10] recently has pointed out that a high intercorrelation exists between σ^2 and E_R and therefore an E_R radical correlation as depicted above does not necessarily imply a radical mechanism of action.

Therefore, in order to test the above hypothesis Hansch, *et al.*,[6] proposed to prepare and to test the activity of α-deuteriochloramphenicol. The ^2H analog should have a lower activity if the rate-determining step is a ^2H radical transfer.

We followed this idea and began synthesis using pure D-*threo*-chloramphenicol as starting material. D-*threo*-α-deuteriochloramphenicol (V) was prepared according to the following scheme.



D-*threo*-Chloramphenicol was oxidized with NBS to the D-ketone IV. $Ca(BD_4)_2$ reduction of the ketone led to a mixture of D-*threo*-(V) and D-*erythro*-α-deuteriochloramphenicol(VI). Repeated recrystallizations from H_2O resulted in enriched content of the D-*threo* isomer. After 5 recrystallization steps a product was obtained, which had the same melting point as chloramphenicol itself. A glc study according to the elegant method of Margosis[11] proved the absence of any erythro impurities in this sample. However, one also has to consider the possibility that some of the D-ketone was racemized during the reduction with $Ca(BD_4)_2$. This would lead to a mixture of D- and L-*threo*-chloramphenicol which could not be resolved by glc.[11] Unfortunately D- and L-*threo*-chloramphenicol form racemic solid solns with the racemate having the same melting point as the pure enantiomers.[12] However, optical rotation measurements are an unambiguous tool to determine the D-threo/L-threo ratio. It was shown[13,14] that no significant isotope effect in optical rotation can be expected upon deuteration, when the frequency of the rotated light is outside of any of the molecular absorption bands and outside the range of normal vibrational frequencies. These prerequisites are fulfilled for the chloramphenicol samples when the frequency of the Na D line is used. Therefore it is legitimate to assume identical specific rotation values for chloramphenicol and its deuterated derivative. Our deuterated chloramphenicol sample shows a specific rotation of $[\alpha]^{25}D$ + 17.0° (*c* 5.0 g/100 ml, EtOH). The reported value for D-*threo*-chloramphenicol is $[\alpha]^{25}D$ + 19.0° (*c* 5.0 g/100 ml, EtOH),[15] which we could confirm in our system. Therefore, a D-threo content of 94.7% can be calcd for our deuterated sample. A low-voltage mass spectrum established the isotopic purity of the chloramphenicol-d_1 as d_0 10, d_1 90%.

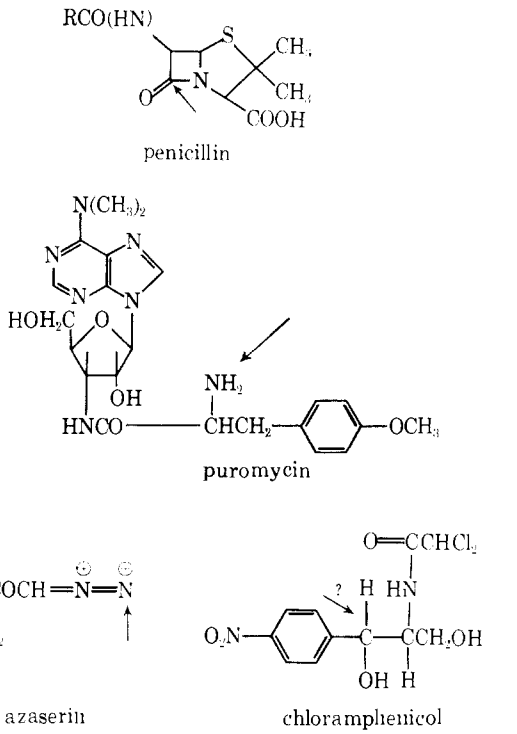


Figure 1.—Chemical structures of four antibiotics. The arrows mark the points of chemical attack at the receptor site.

In Figure 2 the nmr spectra of chloramphenicol and its α-deuterio derivative are compared. The characteristic doublet for the α-H atom at 5.2 ppm is lacking in the deuterated sample.

The comparative study on the inhibitory action of chloramphenicol and the deuterated derivative was performed by Garrett and Heman-Ackah using micro-

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(15) "Handbook of Chemistry and Physics," The Chemical Rubber Co., Cleveland, Ohio, 46th ed, C-248.

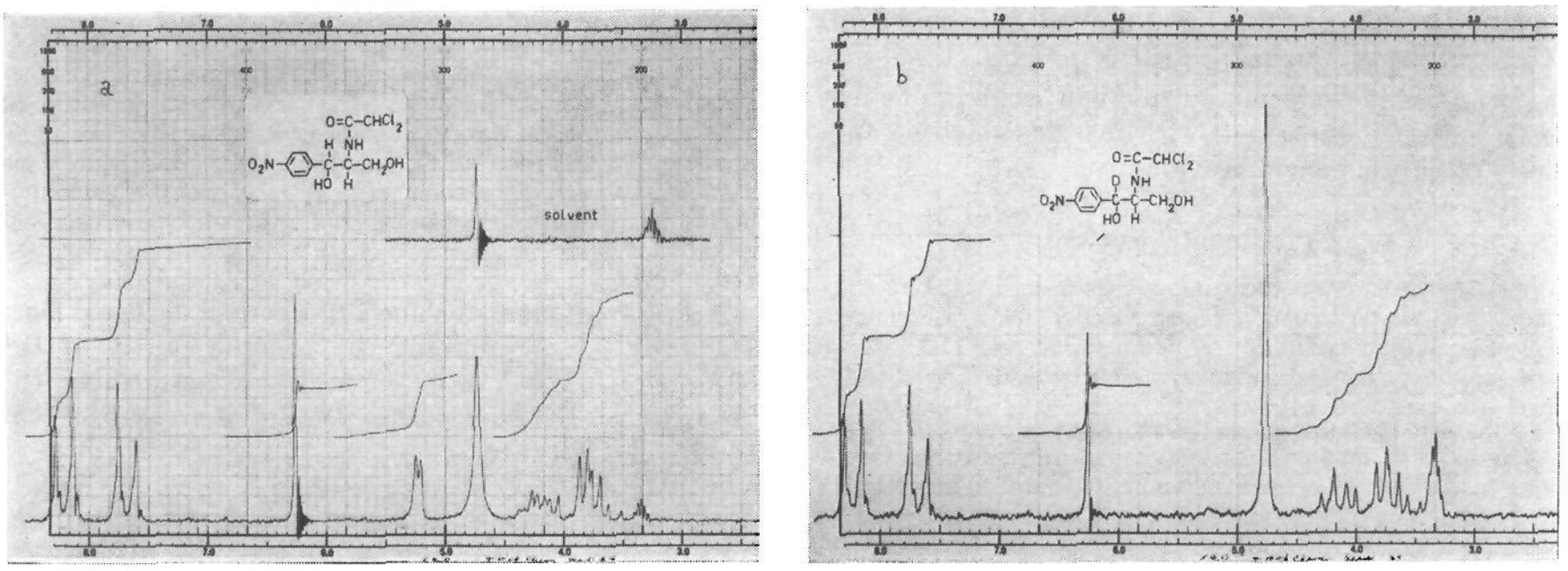

Figure 2.—Nmr spectra of chloramphenicol (a) and its α-deuterated derivative (b). Solvent CD_3OD. Internal standard TMS. Model: Varian A 60.

bial kinetics.+ This method can estimate bacterial growth rates rapidly and precisely, a prerequisite necessary to detect subtle differences in activity.[7]

The apparent growth rate constants (k_{app}) of drug-free and drug-affected cultures (Table I) are obtained

TABLE I

GROWTH RATE CONSTANTS (k_{app}) OF *E. coli* CULTURES AFFECTED WITH VARIOUS CONCENTRATIONS OF DRUGS AT pH 7.05 AND 37.5°

		$k_{app} \times 10^{-4}$ sec^{-1}	
Coding	Concn, μg/ml	Chloramphenicol[a]	α-Deuterio-chloramphenicol
0	0	62.94	62.94
1	0.2	56.72	59.04
2	0.4	50.31	53.65
3	0.6	42.25	47.13
4	0.8	37.17	43.31
5	1.0	30.87	39.42
6	1.2	25.07	33.80

[a] Supplied by Boehringer Mannheim, West Germany.

from the linear portions of the steady-state growth curves. This relationship is expressed as

$$\ln N_t = k_{app}\, t + \ln N_0 \qquad (2)$$

where N_0 is the initial number of organisms/ml and N_t is the number of organisms/ml after time t. The plot of k_{app} *vs.* concn (Figure 3) is linear over the concn ranges studied for the 2 samples of drugs. The specific inhibitory constants k_i for the two samples were obtained from the least-squares slopes of plots of k_{app} *vs.* drug concns in accordance with the expression

$$k_{app} = k_0 - k_i \times C \qquad (3)$$

where k_0 is the apparent growth rate constant of drug-free culture. Values of k_i obtained for D-*threo*-chloramphenicol are $k_i = 31.87 \times 10^{-5}$ ml μg^{-1} sec^{-1} and for its deuterated derivative $k_2 = 24.46 \times 10^{-5}$ ml μg^{-1} sec^{-1}. As is obvious from Figure 3 a significant difference in activity between the 2 samples was found. From the two inhibitory rate constants a k_1/k_2 ratio of 1.30 can be calcd. Taking into account that the

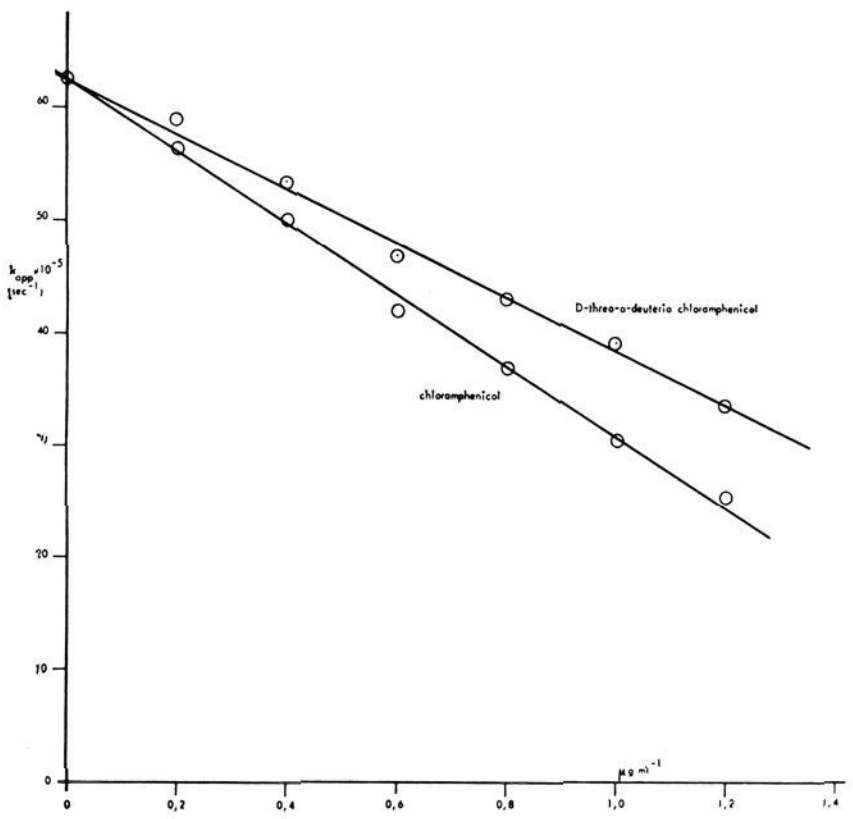

Figure 3.—Plots of apparent generation rate constants for *E. coli* as a function of the concentration of the two chloramphenicol samples.

biologically active configuration is the D-threo[16] a kinetic isotope effect of 1.27 results on the basis of the above mentioned analytical data for the deuterated chloramphenicol sample. Hence, D-*threo*-α-deuteriochloramphenicol has only approximately 80 % of the activity of D-*threo*-chloramphenicol.

The magnitude of this isotope effect is very reasonable in light of the recent study of McMahon and Craig.[17] These authors have shown that the isotope effect for the microsomal oxidation of α-deuterioethylbenzene is 1.8. Of course, identical enzymes are not involved in the 2 systems. However, compared to ethylbenzene the benzylic CH bond in chloramphenicol is more highly activated and this fits nicely with the smaller kinetic isotope effect found for chloramphenicol.

Therefore, this study confirms the prediction by Hansch, *et al.*,[6] of the lower activity of D-*threo*-α-deuteriochloramphenicol as compared to the parent

+ E. R. Garrett and S. M. Heman-Ackah (College of Pharmacy, University of Florida, Gainesville, Fla.). The kindness of Dr. E. R. Garrett and Dr. S. M. Heman-Ackah in performing this study and permitting its results to be included herein is highly appreciated.

(16) R. E. Maxwell and V. S. Nickel, *Antibiot. Chemother.*, **4**, 289 (1954).
(17) R. E. McMahon, H. R. Sullivan, L. C. Craig, and W. E. Pereira, Jr., *Arch. Biochem. Biophys.*, **132**, 575 (1969).

compound. This finding is additional support for the hypothesis that the benzylic CH bond is broken in the kinetically determining step of chloramphenicol action and it is consistent with the idea that the antibiotic blocks an enzyme covalently.

Experimental Section

Optical rotation measurements were performed using an automatic polarimeter (Perkin-Elmer Model 141, Überlingen/Bodensee, West Germany). A Perkin-Elmer Model F 900 was used for the glc method and analysis was performed according to ref 11.

D-α-Dichloroacetylamino-β-hydroxy-p-nitropropiophenone (D-Ketone IV).—40 g of D-*threo*-chloramphenicol was dissolved in 1 l. of Me_2CO and mixed with 100 ml of H_2O and 40 ml of AcOH. To this soln 30 g of NBS was added, and the mixt was allowed to stand for 15 hr at room temp. After evapn of the solvent the product was recrystd from Et_2O, 15.8 g of white needles, mp 124–125°, $[\alpha]^{25}D$ + 20.8° (*c* 2.5 g/100 ml, EtOH).

D-*threo*-α-Deuteriochloramphenicol.—Reduction of IV with $Ca(BD_4)_2$ was carried out in EtOH at −30 to −35° as described according to ref 18 for chloramphenicol itself. The resulting raw material was recrystd 5 times from H_2O. All crystn steps were induced by inoculation with a trace of D-*threo*-chloramphenicol; white needles, mp 150–151°, $[\alpha]^{25}D$ + 17.0° (*c* 5.00 g/100 ml, EtOH).

Acknowledgments.—The authors wish to thank Doz. Dr. Prox for determining the isotopic purity of the chloramphenicol-d_1, Dr. Daneck for performing the glc analysis, and Dipl.-Chem. W. R. Ulrich for excellent technical assistance.

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Chemotherapeutic Nitroheterocycles. 7.[1] Substituted 5-Alkylthiomethyl-3-(5-nitro-2-imidazolyl)methyleneamino-2-oxazolidinones[2]

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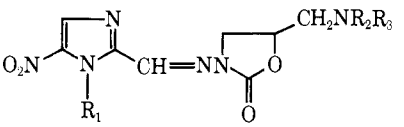
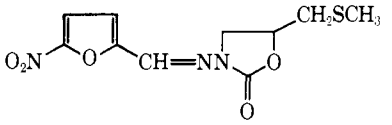
The syntheses of title compds were accomplished by condensation of 1-substituted 5-nitroimidazole-2-carboxaldehydes with 5-alkylthio-3-amino-2-oxazolidinones. The substances were highly active against *Trichomonas vaginalis in vitro* (Table I). 5-*n*-Butylthiomethyl-3-(5-nitro-2-imidazolyl)methyleneamino-2-oxazolidinone (**7d**) was the most effective compd *in vivo* showing ED_{50} 16.5 mg/kg.

In a previous paper[1] the effectiveness of substituted 5-aminomethyl-3-(5-nitro-2-imidazolyl)methyleneamino-2-oxazolidinones (**1**) against *Trichomonas vaginalis* both *in vitro* and *in vivo* was reported. As the antitrichomonal drug nifuratel[3] (**2**) has a similar structure, it was interesting to synthesize substituted 3-(5-nitro-2-imidazolyl)methyleneamino-2-oxazolidinones with 5-alkylthiomethyl side chains (**7**) and to investigate their antimicrobial activity.

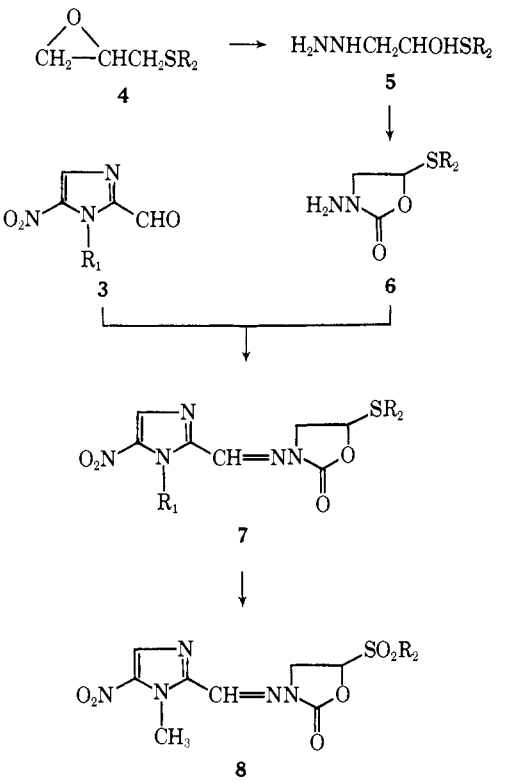


1



2

Chemistry.—5-Alkylthio-3-(5-nitro-1-methyl-2-imidazolyl)methyleneamino-2-oxazolidinones (**7a–7f**, Table I) were synthesized by condensation of 5-nitro-1-methylimidazole-2-carboxaldehydes[1] (**3**, R_1 = CH_3) with 5-alkylthiomethyl-3-amino-2-oxazolidinones (**6**) in MeOH–HCl. These compds were prepared according to known procedures[4] from 1-alkylthio-2,3-epoxypropanes[5] (**4**) *via* ring opening with hydrazine to **5** and reaction with diethyl carbonate. When 5-nitro-1-(2-acetoxyethyl)imidazole-2-carboxaldehyde[1] (**3**, R_1 = $C_2H_4OCOCH_3$) was condensed with **6** (R_2 = *n*-C_4H_9)



4 5 3 6 7 8

(1) Part 6: C. Rufer, H.-J. Kessler, and E. Schröder, *J. Med. Chem.*, **14**, 94 (1971).

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