TABLE II
EFFECT OF TWO BENZOTHIAZOLE DERIVATIVES ON MICE INFECTED WITH VARIOUS INFLUENZA STRAINS

	2-Aminobenzothiazole			2-Amino-4-chlorobenzothiazole·HBr			Aminoadamantane·HCl		
Influenza strain	Dose mg/animal (ip)	% survivors treated group	% survivors control group	Dose mg/animal (ip)	% survivors treated group	% survivors control group	Dose mg/animal (ip)	% survivors treated group	% survivors control group
A2/Stockholm/10/63	0.1	30	7	0.1	20	20	1.0	70	8
A2/Stockholm/10/63	0.3	0	7	0.3	50	20			
A2/Stockholm/10/63	0.5	70	7	0.5	90	20	1.0	30	20
A2/Stockholm/10/63	0.5	60	7	0.5	100	20			
A2/Stockholm/10/63	0.5	50	20						
A2/Stockholm/10/63	1.5	60	7	0.5	60	8			
A2/Stockholm/10/63	1.5	80	47	0.5	25	20			
A2/Japan/57	0.5	10	13	0.5	30	13	1.0	50	13
A2/England/64	0.5	60	87	0.5	100	87	1.0	90	87
A2/Singapore/57	0.5	40	60	0.5	60	60	1.0	60	60
A2/Taiwan/64	0.5	10	33	0.5	0	33	1.0	70	33
A2/Hongkong/1/68	0.5	90	17	0.5	90	17	1.0	70	8

2-aminobenzothiazole it would appear that the antiviral effect is not improved by increasing the dose level above 0.5 mg per animal.

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Cycloserine Derivatives[1]

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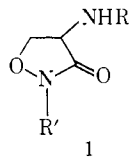
The known[3] biological activity of D-cycloserine (**1a**), a broad spectrum antibiotic, led use to examine derivatives of this compound for antimalarial activity.



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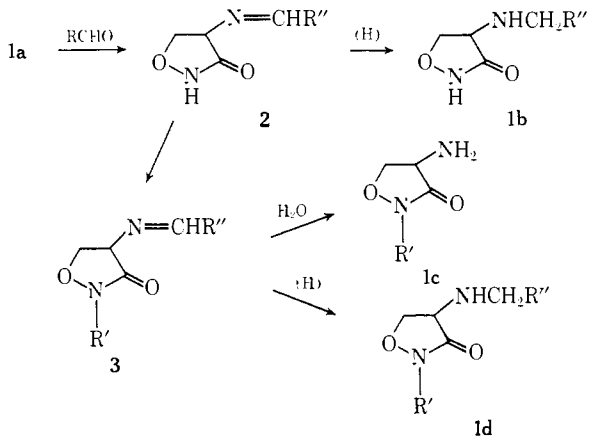
a, R = R′ = H
b, R = aralkyl; R′ = H
c, R = H; R′ = aralkyl
d, R = R′ = aralkyl

We have previously[4] reported the synthesis of several derivatives of cycloserine having general structures **1b** (R = 2-hydroxy-5-chlorobenzyl) and **1c** (R′ = Ph_3C). The pathway by which this was done is shown in Scheme I. In this report, several new Schiff bases (**2**) are described which can be reduced with $NaBH_4$ to

SCHEME I



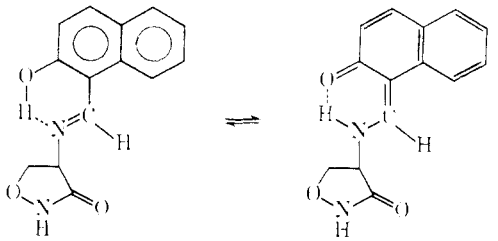
type **1b** or alkylated giving **3** which is further hydrolyzed or reduced to **1c** or **1d**.

Of greatest chemical interest is the considerable variation in the ease of racemization of Schiff bases **2** as a function of the aldehyde used. Earlier work[4b] showed that when 5-chlorosalicylaldehyde was employed, the Schiff base was optically quite stable[4a] in solution but was completely racemized during conversion into its 2-trityl derivative **3**, R′ = Ph_3C, or its 2-Me derivative[5] **3**, R′ = Me. These 2-alkylations were carried out in the presence of K_2CO_3 which apparently catalyzed the racemization of the products by abstraction of a proton from the asymmetric center.[4b] Polarimetric measurements on a solution of the Schiff base Na salt in dimethoxyethane showed that the rate of racemization more than doubled after the addition of the alkyl halide. Hydrolysis of both of these Schiff bases gave the racemic 2-alkylated cycloserines in good yield. $NaBH_4$ reduction of 5-chlorosalicylidene-D-cycloserine, however, gave[4b] an optically active derivative **1b**, R = 5-chloro-2-hydroxybenzyl. The 5-nitrosalicylidene Schiff base **2**, R = 5-nitrosalicyl, had the same optical properties, *i.e.*, alkylation gave an optically inactive 2-derivative while reduction gave an active *N*-5-nitro-2-hydroxybenzyl-D-cycloserine.

In an attempt to synthesize an optically active 2-tritylcycloserine, the Schiff base **2**, R″ = 2-hydroxy-

(1) Supported in part by Contract DA-49-193-MD-2993 from the U. S. Army Medical Research and Development Command.
(2) To whom inquiries should be addressed.
(3) Francis C. Neuhaus, in "Antibiotics," Vol. I, D. Gottlieb and P. L. Shaw, Ed., Springer-Verlag, Heidelburg, 1967, Chapter 2.
(4) (a) C. H. Stammer and J. D. McKinney, *J. Org. Chem.*, **30**, 3436 (1965); (b) R. A. Payne and C. H. Stammer, *ibid.*, **33**, 2421 (1968).
(5) Ph.D. Thesis of James D. McKinney, University of Georgia, Athens, Georgia, 1968.

1-naphthyl, was prepared. According to reports by Dudek and coworkers,[6] Shiff bases of this type (**4**) exist to a large extent in the enamine keto form, **4b**. It



4a **4b**

appeared to us that the optical stability of this kind of Schiff base should be greater than that of the others prepared, since tautomerism *away* from the asymmetric center is favored. This compound (**4**) was racemized to the extent of less than 10% when kept in boiling EtOH for 24 hr and could be tritylated in the presence of K_2CO_3 to form optically active 2-trityl Schiff base, **3**. R″ = 2-hydroxy-1-naphthyl; R′ = Ph_3C. Surprisingly, however, hydrolysis of this product, gave only the racemic 2-trityl-DL-cycloserine which we had previously prepared. No further work toward an optically active 2-alkylcycloserine using Schiff bases has been carried out.

N-Acyl derivatives of cycloserine are different from the Schiff bases in their susceptibility to racemization. *N*-Acetyl-D-cycloserine[7a] was 2-methylated by both Hidy, *et al.*,[7a] and Milne, *et al.*,[7b] using CH_2N_2, but neither of these workers reported the optical properties of their products. In our hands, methylation of *N*-acetyl-D-cycloserine with MeI–K_2CO_3 gave an oily product which had the expected spectral properties,[7b] but was devoid of optical rotation. It appeared that *N*-acylcycloserines were also racemized during 2-alkylation under these conditions. *N*-Carbobenzyloxy-D-cycloserine, however, was alkylated with $PhCH_2Cl$, Ph_2CHBr, and 1-bromo-4-chlorobutane in the presence of K_2CO_3 to form optically active and apparently optically pure products. The Cbz group apparently influenced greatly the susceptibility of the ring system to racemization. Only the 2-benzyl compound was successfully deblocked to the corresponding amine, 2-benzyl-D-cycloserine. The optical activity of the 2-benzyl derivative indicated that 2-alkylcycloserines are not intrinsically optically unstable as possibly indicated by the formation of the racemic 2-trityl compound from the optically active Schiff base **3**, R″ = 2-hydroxy-1-naphthyl; R′ = Ph_3C. None of the compounds reported herein showed significant antimalarial activity.

Experimental Section[8]

2-Alkylation of the Isoxazolidone Ring. 2-Methyl-*N*-5-chlorosalicylidene-DL-cycloserine.—To a solution of 2.40 g (10 mmoles) of *N*-5-chlorosalicylidene-D-cycloserine[4a] in 45 ml of dry Me_2CO (dried over molecular sieves, Type 4A) was added 1.60 g (11.5 mmoles) of anhyd K_2CO_3 followed by 3.5 ml (56 mmoles) of MeI. The resulting suspension was refluxed for 4.5 hr. The suspension was filtered and the filtrate was evapd to dryness *in vacuo*. The crude residue was triturated with 25 ml of Et_2O and cooled and the crude yellow product was collected on a filter, weight 2.44 g (96%), mp 141–145°. An anal. sample was obtained by recrystallization from MeOH, mp 146–149°. *Anal.* ($C_{11}H_{11}ClN_2O_3$) C, H, N, Cl.

2-Benzyl-*N*-5-chlorosalicylidene-DL-cycloserine was prepared in 38% yield by the 2-alkylation procedure previously described. An anal. sample was crystallized from MeOH, mp 154–155°. *Anal.* ($C_{17}H_{15}O_3N_2Cl$) C, H, N, Cl.

Reduction of Cycloserine Schiff Bases. 2-Triphenylmethyl-*N*-(2-hydroxy-5-chlorobenzyl)-DL-cycloserine.—A solution of 2.4 g (5 mmoles) of 2-triphenylmethyl-*N*-5-chlorosalicylidene-DL-cycloserine[4b] in 150 ml of abs EtOH was stirred at room temp for 1.5 hr with 500 mg of $NaBH_4$. The solution was acidified to pH 5 with AcOH and the solvent was evapd under vacuum. The residue was extracted twice with 50-ml portions of Et_2O and the combined extract was washed with $NaHCO_3$ solution, dried, and evapd. The residue was triturated with hexane and filtered; wt 2.3 g (95%), mp 79–82°. An anal. sample was crystallized from hexane–Et_2O (1:1), mp 83–85°. *Anal.* ($C_{29}H_{25}ClN_2O_3$) C, H, N.

Schiff Base Synthesis. *N*-(2-Hydroxy-1-naphthylidene)-D-cycloserine.—A solution of 5.2 g (30 mmoles) of 2-hydroxy-1-naphthaldehyde in a mixture of 600 ml of abs EtOH and 60 ml of MeOH was stirred with 3.0 g (30 mmoles) of cycloserine for 3 hr at room temp. The solution was filtered and the solvent was evapd giving a residue which was triturated with Et_2O and filtered. The crude product weighed 6.2 g (89%), mp 172–174°; crystallized from MeOH, mp 174–175°. *Anal.* ($C_{13}H_{12}N_2O_3$) C, H, N.

2-Triphenylmethyl-*N*-(2-hydroxy-1-naphthylidene)-D-cycloserine was prepared in 76% yield by the 2-alkylation procedure previously described. An anal. sample was crystallized from an Me_2CO–H_2O, mp 204–206°. $[\alpha]^{25}D$ +203° (*c*, 3.5 in DMF). *Anal.* ($C_{33}H_{26}N_2O_4$) C, H, N.

***N*-(2-Hydroxy-1-naphthylmethyl)-DL-cycloserine** was prepared in 72% yield by the reduction procedure previously described, mp 142–145°. An anal. sample was crystallized from DMF–H_2O, mp 154–155°. *Anal.* ($C_{14}H_{14}N_2O_3$) C, H, N.

2-Triphenylmethyl-*N*-(2-hydroxy-1-naphthylmethyl)-DL-cycloserine was prepared in 72% yield by the reduction procedure previously described, mp 115–122°. An anal. sample was crystallized from EtOH–H_2O, mp 131–134°. *Anal.* ($C_{33}H_{28}N_2O_3$) C, H, N.

***N*-(5-Nitrosalicylidene)-D-cycloserine** was prepared in 75% yield by the general procedure for Schiff base synthesis previously described, mp 159–161°. An anal. sample was crystallized from MeOH, mp 159–61°. $[\alpha]^{25}D$ +236° (*c*, 1.2 in DMF). *Anal.* ($C_{10}H_9N_3O_5$) C, H, N.

2-Triphenylmethyl-*N*-(5-nitrosalicylidene)-DL-cycloserine was prepared in 72% yield by the general procedure for 2-alkylation previously described, mp 136–138°. An anal. sample was crystallized from MeOH, mp 138–140°. *Anal.* ($C_{31}H_{25}N_3O_5$) C, H, N.

***N*-(2-Hydroxy-5-nitrobenzyl)-D-cycloserine** was prepared in 96% yield by the general reduction procedure previously described, mp 110–124°. An anal. sample was crystallized from EtOH–H_2O, mp 137–142°, $[\alpha]^{26}D$ +24° (*c*, 2.6 in DMF). *Anal.* ($C_{10}H_{11}N_3O_5$) C, H, N.

Benzyloxycarbonylation of D-cycloserine.—A solution of 5.1 g (50 mmoles) of D-cycloserine in 50 ml of 1 *N* NaOH (50 mmoles) was chilled to 5° and a total of 50 ml of 1 *N* NaOH and 8.5 g (50 mmoles) of benzyloxycarbonyl chloride was added alternately in about 5 equal portions over a period of 30 min with stirring and cooling in an ice bath. After stirring for 15 hr in ice, the solution was extracted with Et_2O, and the aq layer was cooled and acidified to pH 4 with 4 *N* HCl. The crystalline benzyloxycarbonyl-D-cycloserine was filtered, dried, and recrystd from EtOAc; yield 8 g (72%), mp 140–142°, $[\alpha]^{25}D$ +45.7° (*c*, 1 in MeOH). *Anal.* ($C_{11}H_{12}N_2O_4$) C, H, N.

***N*-Benzyloxycarbonyl-2-benzyl-D-cycloserine** was prepared in 52% yield by the 2-alkylation procedure previously described except that 1 equiv of KI was added to catalyze the reaction mp 144–145°, $[\alpha]^{22}D$ +56.5° (*c*, 1 in MeOH). *Anal.* ($C_{18}H_{18}N_2O_4$) C, H, N.

2-Benzyl-D-cycloserine Hydrobromide.—*N*-Benzyloxycarbonyl-2-benzyl-D-cycloserine (0.5 g) was dissolved in 2.5 ml of

(6) G. O. Dudek and E. P. Dudek, *J. Amer. Chem. Soc.*, **88**, 2407 (1966).

(7) (a) P. H. Hidy, E. B. Hodge, V. V. Young, R. L. Harned, G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Stavely, A. Pohland, H. Boaz, and H. R. Sullivan, *ibid.*, **77**, 2345 (1955); (b) G. W. A. Milne and L. A. Cohen, *Tetrahedron*, **23**, 65 (1967).

(8) Spectral data were in accord with the assigned structures. Where analyses are indicated by symbols of the elements the results were within ±0.4% of the calcd values.

HOAc, the solution was cooled to 10°, and 2.5 ml of 4 *N* HBr–HOAc was added. The mixture was allowed to stand for 20 min at 10–15°. Excess HBr was removed *in vacuo* and the product was pptd by addition of 100 ml of dry Et_2O. The crude product was filtered, washed with Et_2O, and crystallized from MeOH–Et_2O; yield 250 mg (60%), mp 184–5°, $[\alpha]^{23}D$ +45.3° (*c*, 1 in MeOH). *Anal.* ($C_{10}H_{13}BrN_2O_2$) C, H, N, Br.

***N*-Benzyloxycarbonyl-2-[4-chlorobutyl]-D-cycloserine.**—To a solution of 1.2 g (5 mmoles) of *N*-benzyloxycarbonyl-D-cycloserine in 20 ml of $CHCl_3$ was added 1.41 ml of Et_3N (10 mmoles) and 1.96 g (10 mmoles) of 1-bromo-4-chlorobutane. After stirring overnight at room temp the mixture was evapd to dryness. The residue was dissolved in EtOAc and washed successively with 5% K_2CO_3 solution and H_2O, then dried, and evapd. The residue was crystallized from Et_2O giving 1 g (60%); mp 74–75°, $[\alpha]^{23}D$ +38.3° (*c*, 2 in MeOH). *Anal.* ($C_{15}H_{19}ClN_2O_4$) C, H, N, Cl.

***N*-Benzyloxycarbonyl-2-benzhydryl-D-cycloserine** was prepared in 75% yield by the procedure described above; mp 118–19°, $[\alpha]^{23}D$ +50.1° (*c*, 2 in MeOH). *Anal.* ($C_{23}H_{22}N_2O_4$) C, H, N.

Nitroheterocyclic Antimicrobial Agents. II. 5-Nitro-1,3,4-thiadiazole-2-carboxaldehyde Derivatives

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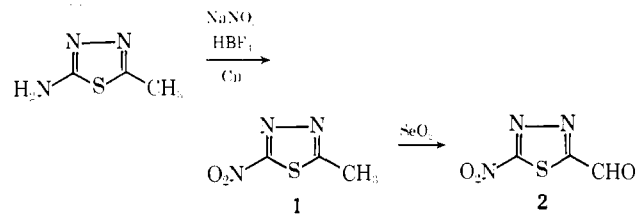
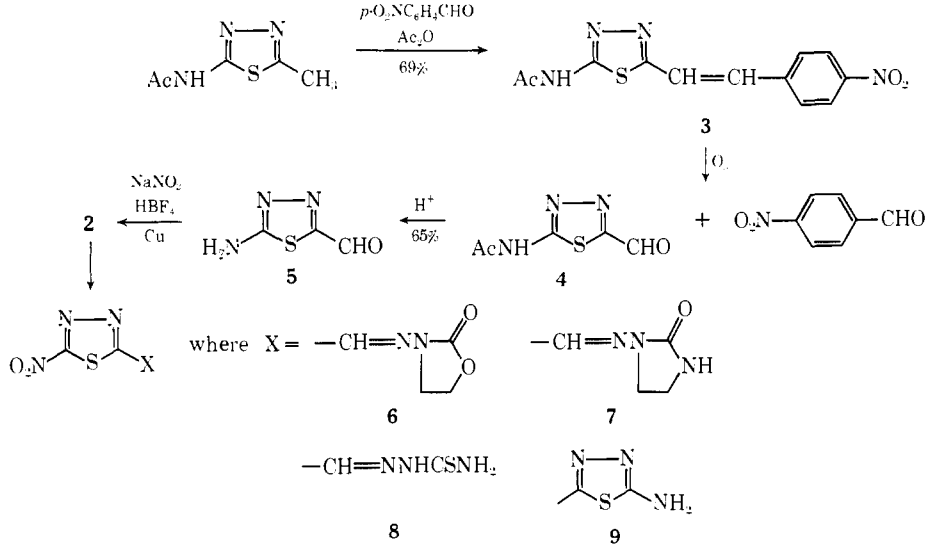
We recently reported the synthesis of 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole,[1] a broad-spectrum antimicrobial agent. Preparation of this compound and related nitroimidazoles arose from a program based on the replacement of the nitrofuryl moiety of antimicrobially active nitrofurans by isosteric nitroheteroaromatic groups. The first series investigated, derivatives of nitrothiazolecarboxaldehydes,[2] exhibited *in vitro* antibacterial and antifungal activity, and several members showed *in vivo* antimicrobial activity. This report deals with the second group of nitroheterocyclic compounds we examined, namely derivatives of 5-nitro-1,3,4-thiadiazole-2-carboxaldehyde.

Chemistry.—Initially, 2-methyl-5-nitro-1,3,4-thiadiazole (**1**) was selected as the primary precursor, and it was prepared from 2-amino-5-methyl-1,3,4-thiadiazole by diazotization and reaction of the diazonium salt with NO_2^- in the presence of Cu. Compound **1**, being unstable under the reaction conditions, did not condense with pyridinecarboxaldehyde in the presence of $ZnCl_2$, Ac_2O, or piperidine. It could be oxidized with SeO_2 in the absence of solvent to afford *ca.* 5% of **2**; however, this method was impractical for our purposes and an alternate route was developed.



1 2

The *p*-nitrobenzylidene derivative **3** was prepared and was ozonized in MeOH to afford **4**, which was hydrolyzed with acid to the aminoaldehyde **5**. The thiadiazolecarboxaldehyde **5** was separated from *p*-nitrobenzaldehyde by acid extraction and converted into **2** by diazotization and displacement with NO_2^- in the presence of Cu. The crude nitroaldehyde was used without purification and overall yields of 6–34% (based on aminoaldehyde **5**) of azomethine derivatives **6–8** were obtained. Ferric ammonium sulfate oxidative cyclization of **8** afforded **9**.



3 4 5 6 7 8 9

Compound **5** was difficult to purify, and microanalyses were unsatisfactory. However, ir and nmr [Me_2CO-d_6; τ 1.83 (s, 2 H, NH_2), −0.04 (s, 1 H, CHO)] supported its structure. The aldehyde **4** was also separable from *p*-nitrobenzaldehyde but it contained some starting material (2-acetamido-5-methylthiadiazole), which persisted as a contaminant even after repeated recrystallizations, and thus the microanalysis

(1) G. Berkelhammer and G. Asato, *Science*, **162**, 1146 (1968).

(2) G. Asato, G. Berkelhammer, and E. L. Moon, *J. Med. Chem.*, **12**, 374 (1969).