Use of Acetylacetone to Prepare a Prodrug of Cycloserine

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Several derivatives of cycloserine (1) were prepared and it was found that (*R*)-4-[(1-methyl-3-oxo-1-butenyl)-amino]-3-isoxazolidinone (11), the condensation product of acetylacetone and cycloserine (1), was an efficacious prodrug of increased stability under aqueous conditions.

One of the most common problems of a medicinal chemist is to design a prodrug of an active structure in an attempt to obtain some advantage such as greater chemical stability, better transport, or lower toxicity.[1,2] Recently, we needed such a prodrug of the antibiotic cycloserine (1) to use in combination with the recently disclosed synthetic antibiotic 3-fluoro-D-alanine-2-*d*.[3] Our primary objective in this case was to reduce the known instability of cycloserine with respect to the formation of dimer **2**. This dimerization takes place particularly well in concentrated aqueous media and can even take place in the solid state.[4] Preventing this dimerization would therefore increase the stability of cycloserine both on the shelf and in physiological media.

Since the formation of dimer **2** requires a reaction of the amino group of cycloserine, functionalization of this amino group is the easiest and most direct approach to retarding dimer formation (Scheme I). This approach has been previously attempted, but it was found that "analogues in which the amino group is alkylated or acylated are inactive",[5] and the conclusion has been drawn that a free amino group is required for biological activity.[5] The only type of amino modification compatible with activity was found to be Schiff bases,[6,7] and their activities are attributed to hydrolysis to cycloserine.[7]

We have prepared a number of new cycloserine analogues in which the amino group is functionalized and have tested them for chemical stability vis a vis cycloserine. Table I exemplifies typical conditions of heat and humidity that were used in these stability studies. Derivatives which had stability superior to cycloserine under such conditions were then tested for their in vitro antibacterial activity and their ability to liberate cycloserine in vivo, as tabulated and described in Table II. Our results largely substantiated previously reported work[7] on such derivatives of cycloserine. Several derivatives, including various types of Schiff bases, proved of insufficient chemical sta-

Scheme I

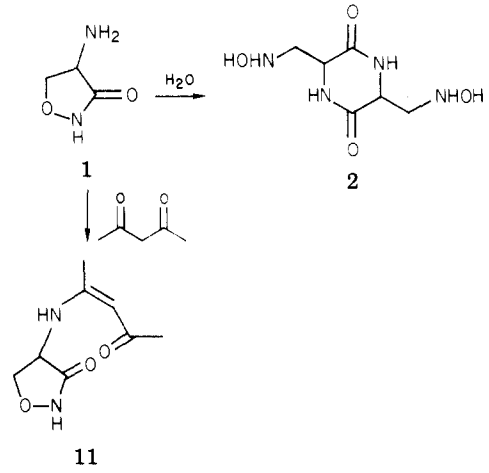

Table I. Comparative Stability of 1 and 11 to Moisture

no.	conditions	assay method	% remaining
1[a]	14 h, 35 °C, 100% humidity	optical rotation	74
11	14 h, 35 °C, 100% humidity	optical rotation	99
1[a]	12 h, 35 °C, 100% humidity	colorimetric[b]	72
1[c]	1 h, 70 °C, 98% humidity	colorimetric[b]	22
11	1 h, 70 °C, 98% humidity	colorimetric[b]	94
11	3 days, room temp, stirring in acetyl acetone	optical rotation	100

[a] Unpurified commercial cycloserine purchased from Nutritional Biochemical. Particle size appears to have some effect on the rate of decomposition of 1 and 11, but 11 was always more stable to moist conditions. [b] L. R. Jones, *Anal. Chem.*, 28, 39 (1956). [c] Cycloserine purified by the method of C. H. Stammer, A. W. Wilson, C. F. Spencer, F. W. Bachelor, F. W. Holly, and K. Folkers, *J. Am. Chem. Soc.*, 79, 3226 (1957).

(1) A. A. Sinkula, *Ann. Rep. Med. Chem.*, **10**, 306 (1975).
(2) N. J. Harper, *Progr. Drug Res.*, **4**, 221 (1962).
(3) (a) F. M. Kahan and H. Kropp, 15th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., Sept. 24–26, 1975, Abstract 100; (b) H. Kropp, F. M. Kahan, and H. B. Woodruff, *ibid.*, Abstract 101; (c) J. Kollonitsch, L. Barash, N. P. Jensen, F. M. Kahan, S. Marburg, L. Perkins, S. M. Miller, and T. Y. Shen, *ibid.*, Abstract 102; (d) F. M. Kahan, H. Kropp, H. R. Onishi, and D. P. Jacobus, *ibid.*, Abstract 103.
(4) See F. A. Lassen and C. H. Stammer, *J. Org. Chem.*, **36**, 2631 (1968), for a discussion of the dimerization.
(5) F. C. Neuhaus in "Antibiotics, 1, Mechanism of Action", D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, Berlin and New York, 1967, p 55.
(6) S. Bianchi, E. Felder, and U. Tiepolo, *Farmico, Ed. Prat.*, **20**, 366 (1965).
(7) See ref 5, p 56.

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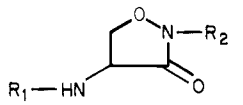
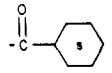
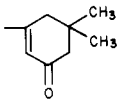
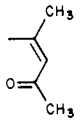
Table II. Antibacterial Activity and Urinary Recovery of Cycloserine Analogues



D-4-amino-3-isoxazolidinone
D-cycloserine

			antibacterial act.:[a] zone of inhibn diameter, nm		disposition in the mouse: urinary recovery, % of dose[b]	
	R_1	R_2	*E. coli*	*Staph. aureus*	bioassay	chem assay
3	$-C(=O)NHCH_3$	-H	22	25	<1.0	16.0
4	$-C(=O)CH_2Cl$	$-C(=O)CH_2Cl$	<6	<6	1.1	43.0[c]
5	$-C(=O)CH_2Cl$	-H	<6	<6	1.0	70.0
6	$-C(=O)OC_2H_5$	-H	<6	<6	<1.0	33.0
7	$-C(=O)NHCH_3$	$-C(=O)NHCH_3$	<6	<6	<1.0	<1.0[c]
8		-H	<6	<6	1.0	53.0
9	$-C(=O)NHC(=O)CCl_3$	-H	14	<6	<1.0	55.0
10		-H	<6	<6	<1.0	55.0
11		-H	39	36	93.0	64.0
1	-H	-H	39	36	64.0	45.0

[a] Antibacterial activities are reported as zones of inhibition in the disk diffusion assay. Paper disks (6.0-mm diameter) impregnated with 200 μg of the test drug were placed onto nutrient agar plates (2-mm deep) seeded with two selected bacterial strains, *Escherichia coli* no. 2017 or *Staphylococcus aureus* no. 2949 (10^4 cfu/cm^2), and inhibitory zones were measured following overnight incubation at 37 °C. [b] Urinary recovery studies were performed in groups of five female albino mice, CD_1 strain, weighing 19–21 g. The test compounds were administered orally in aqueous solution at a dose rate calculated for each derivative to contain 25 mg of cycloserine nucleus/kg body weight, and urine specimens were collected in chilled recepticles over an 8-h period. Urine was assayed both for its content of free cycloserine, using a bioassay, and for total cycloserine content employing the chemical assay described by Jones (see ref *b*, Table I). In the chemical assay, the compound under evaluation was employed to calibrate the standard response curve. The bioassay was conducted by application of samples in Oxford-penicillin assay cups to nutrient agar layers incorporating *Staphylococcus aureus* no. 2949 as the tester strain. Standard solutions of cycloserine prepared in normal mouse urine were used to calibrate the relation between zone diameter and antibiotic content of specimens. [c] By virtue of their derivatization on the ring nitrogen, structures 4 and 7 should not give a Jones colorimetric test. Structure 4, however, did give such a test, indicating that it is not stable to the conditions of the Jones assay.

bility to warrant biological testing. The derivatives **3–10**, which were chemically more stable than cycloserine and probably require enzymatic cleavage to release cycloserine, were not appreciably active in vitro or capable of releasing cycloserine in vivo. The exception to these findings is the derivative (*R*)-4-[(1-methyl-3-oxo-1-butenyl)amino]-3-isoxazolidinone (**11**),[8] which is the condensation product of cycloserine and acetylacetone.

The condensation of acetylacetone and cycloserine results in the formation of an enamine structure depicted in Figure 1. While it is known[9] that enamines of β-dicarbonyl compounds are stabilized relative to the enamines of monocarbonyl compounds, the suggestion[10] that hydrogen bonding is important in the stability of a structure like **11** is not supported by the comparative stabilities of **11** and derivative **10**. The derivative **10** cannot form a six-member hydrogen-bonded conformation like **11** and yet, by the measures reported in Table II, derivative **10**

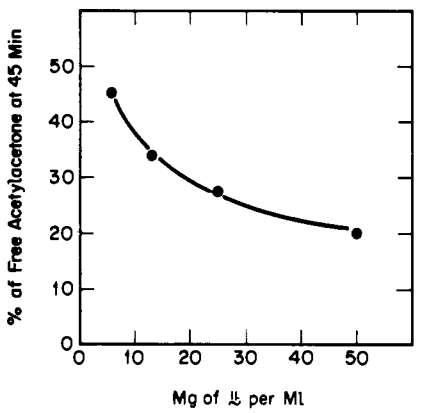


Figure 1. Compound **11** was dissolved in 0.5 M $KDPO_4$ in D_2O containing 1%, w/w, sodium 2,2-dimethyl-α-silapentane-5-sulfonate (DSS), and the pH was adjusted to 7.1 ± 0.05 with concentrated DCl. The solution was immediately transferred to a Varian A-60-A NMR spectrometer set at 37 °C and comparative intensity measurements were made of the two close methyl peaks of structure **11** at 2.0–2.1 ppm relative to DSS and the single methyl peak at 2.25 ppm due to free acetylactone.

(8) The sodium salt of **11**, first prepared by S. M. Miller of these laboratories, has been assigned the designation MK-642 and the nonproprietary name pentizidone sodium.
(9) H. O. House, "Modern Synthetic Reactions", W. A. Benjamin, Menlo Park, Calif., 1972, p 577.
(10) E. Dane, F. Drees, P. Konrad, and T. Dockner, *Angew. Chem., Int. Ed. Engl.*, 1, 6587 (1962).
(11) Further study (see ref 3d) has shown **11** to have a higher initial rate of renal clearance than **1**, a property considered desirable for its intended use in combination with 2-deuterio-3-fluoro-D-alanine.

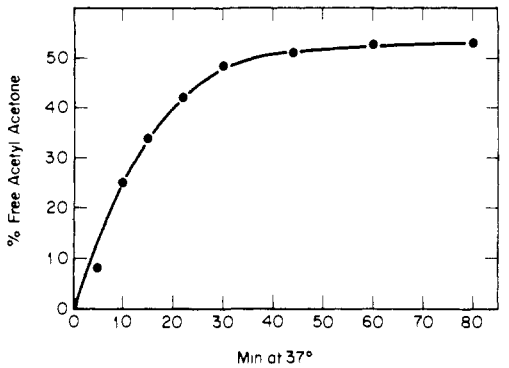


Figure 2. 11, 31.3 mg, was dissolved in 5.0 mL of 0.5 M $KDPO_4$ in D_2O, and the pH was adjusted to 7.0 with concentrated DCl. A portion of the solution was then transferred immediately (4 min after initial dissolving of 11) to a Varian A-60-A spectrometer set at 37 °C and measurements were made as described in the legend of Figure 1.

appears to be less susceptible to hydrolysis than **11**. In fact, for the present purposes, **10** is among those derivatives which are chemically too stable in physiological media to release cycloserine in a nonenzymatic fashion. While the degree to which analogue **11** dissociates into free cycloserine and acetylacetone is a complex function of several factors such as concentration, time, pH, and temperature, Figures 1 and 2 summarize some data on this dissociation with respect to concentration and time at 37 °C and a physicological pH. Concentrations were chosen which were practical for examination by NMR spectroscopy. Using this method, the relative intensity of the methyl groups in structure **11** could be compared to the methyl groups in free acetylacetone. At the concentrations examined, equilibrium was reached in 50 min or less and was unchanged for 24 h, although the intensity of all methyl groups decreased with time due to deuterium exchange.

In the present study, the unique degree of chemical stability afforded by analogue **11** appears to be optimal for a prodrug which releases cycloserine in a nonenzymatic fashion.[11] 11## As can be seen in Table I, the derivative **11** is considerably more stable to heat and humidity than cycloserine but, as reported in Table II, is not only active in vitro but actually gives superior urinary recovery of cycloserine as measured by bioactivity.

Since acetylacetone will react with a great variety of amino groups, the opportunity to prepare derivatives with other drugs which contain an amino function is quite extensive. Such derivatives may also exhibit stability and/or pharmacokinetic advantages. Although less nucleophilic amines may require acid catalysis for reaction with acetylacetone, it was found that simply stirring cycloserine as a suspension in acetylacetone for a day or more at room temperature resulted in a good yield of pure **11**. These mild conditions gave material whose optical activity was undiminished by resubmission to the reaction conditions for 3 more days (see Table I).

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. All IR and NMR spectra are consistent with assigned structures. NMR spectra were obtained with a Varian A-60A spectrometer. Elemental analyses are indicated by symbols of the elements and all results were within ±0.4% of the theoretical values.

2-(Chloroacetyl)-4-(chloroacetamido)-3-isoxazolidinone (3). A mixture of 7.00 g (59 mmol) of **1**, 4.0 mL (67 mmol) of methyl isocyanate, and 150 mL of THF was stirred for 5 days. The resultant precipitate was collected and recrystallized six times from MeOH to give 1.25 g (11.5%) of pure **3**, mp 174 °C dec. Anal. ($C_5H_9N_3O_3$) C, H, N.

2-(Chloroacetyl)-4-(chloroacetamido)-3-isoxazolidinone (4) and 4-(Chloroacetamido)-3-isoxazolidinone (5). A solution of 1.00 g (9.8 mmol) of **1** in 5 mL of 1 N NaOH was stirred and cooled to 5 °C before 1.1 g (10 mmol) of chloroacetyl chloride was added dropwise during a 5-min period. At the same time 5 mL of 1 N NaOH was added dropwise to maintain a basic pH. After 15 h at 7 °C, 250 mg of crude **4** was collected and recrystallized from *i*-PrOH to give 173 mg (7%) of pure **4**, mp 155–156 °C. Anal. ($C_7H_8Cl_2N_2O_4$) C, H, Cl, N. The aqueous filtrate of crude **4** was brought to pH 4 with 2.5 N HCl and lyophilized. The lyophilate was triturated with 3 mL of H_2O, and 157 mg of crude **5** was collected. The crude product was extracted with 10 mL of boiling *i*-PrOH. This extract was concentrated and then diluted with Et_2O to cloudiness. After several hours, 75 mg (4.3%) of pure **5** was collected, mp 146–148 °C. Anal. ($C_5H_7ClN_2O_3$) C, H, N.

4-[(Ethoxycarbonyl)amino]-3-isoxazolidinone (6). In a manner similar to the preparation of **4** and **5**, 6.0 g (59 mmol) of **1** was reacted with 7.8 g (72 mmol) of ethyl chloroformate. In a manner similar to the isolation of **5**, 1.0 g (9.8%) of pure **6** was isolated from the *i*-PrOH-soluble portion of the crude product by precipitation with Et_2O and petroleum ether, mp 114–116 °C. Anal. ($C_8H_{10}N_2O_4$) C, H, N.

2-[(Methylamino)carbonyl]-4-[[(methylamino)carbonyl]amino]-3-isoxazolidinone (7). A suspension of 0.50 g (0.49 mmol) of **1** was stirred in 20 mL of dry THF for 5 days with 1.0 mL (16.8 mmol) of methyl isocyanate. The resultant precipitate was collected and washed with Et_2O to give 0.90 g (85%) of pure **7**, mp 159–161 °C. Anal. ($C_7H_{10}N_4O_4$) C, H, N.

3-[(Cyclohexylcarbonyl)amino]-4-isoxazolidinone (8). Two grams (19.6 mmol) of **1** was reacted with 3.6 g (24.5 mmol) of cyclohexanecarbonyl chloride and worked up in a manner similar to the preparation of **5** to give 0.43 g of crude **8**, mp 172–175 °C. Recrystallization from CH_3CN gave 0.31 g (7%) of analytically pure **8**, mp 182–184 °C. Anal. ($C_{10}H_{16}N_2O_5$) C, H, N.

1-(3-Oxo-4-isoxazolidinyl)-3-(trichloroacetyl)urea (9). To a stirred mixture of 1.50 g (1.48 mmol) of **1** in 20 mL of THF was added 3.18 g (16.9 mmol) of trichloroacetyl isocyanate. The mixture was stirred for 2 days, and the THF was removed in vacuo. The residue was crystallized by dissolving in 1:1 MeOH–$CHCl_3$ and precipitating with Et_2O to yield 1.15 g (58%) of **9**, mp 156.5–158.0 °C. Anal. ($C_6H_6N_3O_4Cl_3$) C, H, N, Cl.

4-[(3,3-Dimethyl-5-oxo-1-cyclohexen-1-yl)amino]-3-isoxazolidinone (10). A mixture of 2.74 g (26.9 mmol) of **1** and 3.76 g (26.9) mmol) of dimedone was ground to a fine powder and stirred for 6 days in 65 mL of dry (dried with 3Å molecular sieves) DMF. The resultant clear solution was concentrated at reduced pressure using PhH to remove the last traces of DMF. The residual 6.8 g of solid was dissolved in 50 mL of MeOH, and 500 mL of Et_2O was added. The mixture was immediately filtered and, after standing 15 h, 1.85 g (31%), mp 193.5–195.0 °C, of **10** was collected. Recrystallization from MeOH–Et_2O gave 1.25 g of analytical material, mp 192.5–194.5. Anal. ($C_{11}H_{16}N_2O_3$) C, H, N.

(*R*)-4-[(1-Methyl-3-oxo-1-butenyl)amino]-3-isoxazolidinone (11). A mixture of 1.02 g (10 mmol) of **1** and 10 mL of acetylacetone was stirred for 2 days. Insoluble solid was collected on a filter and washed three times with 2-mL portions of Et_2O and dried at 0.1 mmHg to give 1.27 g (70%) of analytically pure **11** as a colorless solid: mp 146 °C; $[\alpha]^{27}_D$ −158° (1% MeOH); IR (Nujol) 5.85 μm; UV (MeOH) λ_{max} 310 (ε 19 400) (NaOH added), 313 nm (ε 20 900); NMR (Me_2SO) δ 8.0 and 8.15 (3 proton singlets, $-CH_3$). Unlike cycloserine (**1**), **11** is readily soluble in MeOH, EtOH, and DMF and is also fairly soluble in $CHCl_3$, Me_2CO, THF, and dioxane. The solubility of **11** in water is ~5%. Anal. ($C_8H_{12}N_2O_3$) C, H, N.

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