standard incubation solution complemented with 1% bovine serum albumin, 1 mM calcium, and 5 mM theophylline, containing about 1 mg of protein/mL, and samples (1 mL) were incubated at 37 °C for 30 min. Amylase activity was determined by the method of Ceska et al.[28] using the Phadebas reagent. Amylase release was measured as the difference of amylase activity with and without secretagogues at the end of the incubation. Results were expressed as percent of maximal stimulation obtained with CCK-8 (which represents ca. 35–40% of the total amylase contained in the acini).

Binding Studies. Binding of ^{125}I-BH-CCK-9 to Pancreatic Acini. Binding of ^{125}I-BH-CCK-9 on pancreatic acini was performed as described by Jensen et al.[20] The specific activities of the various preparations used in our experiments were 1000–1300 Ci/mmol. Acini from three pancreata were suspended in 100 mL of standard incubation solution complemented with 1% bovine serum albumin. Samples (1 mL containing about 1 mg of protein) were incubated with the appropriate peptide concentrations plus 50 pM ^{125}I-BH-CCK-9 for 30 min at 37 °C. After centrifugation and washings, the radioactivity associated with the acinar pellet was measured. Nonsaturable binding of ^{125}I-BH-CCK-9 was determined as the amount of radioactivity associated with the acini when the incubation contained 1 μM CCK-8. All values reported in this paper are for saturable binding, i.e., total binding minus nonsaturable binding. Nonsaturable binding has always been less than 15% of the total binding.

Binding of ^{125}I-BH-CCK-9 to Guinea Pig Brain Membranes. Binding of ^{125}I-BH-CCK-9 to brain membranes was performed according to Pelaprat et al.[21] The buffer used was 50 mM Tris·HCl, 5 mM $MgCl_2$, 0.1 mg/mL bacitracin, pH 7.4 (Tris/$MgCl_2$/bacitracin buffer). Displacement experiments were performed by incubation of 1 mL of guinea pig brain membranes (about 0.5 mg of protein) in the presence of 15 pM ^{125}I-BH-CCK-9 (or ^{125}I-BH-CCK-8) during 40 min at 25 °C. In the presence of various concentrations of the analogue to be tested, in a total volume of 1 mL. Nonspecific binding was determined in the presence of 1 μM CCK-8.

Supplementary Material Available: Tables containing ^{1}H NMR data of the pseudopeptide derivatives of the C-terminal heptapeptide of cholecystokinin (3 pages). Ordering information is given on any current masthead page.

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β-Substituted Cysteines as Sequestering Agents for Ethanol-Derived Acetaldehyde in Vivo

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A series of β-mono- and β,β-disubstituted cysteines were evaluated in rats as sequestering agents for metabolically generated acetaldehyde (AcH) during the oxidation of ethanol in vivo and compared against D-(−)-penicillamine. Both *threo*- (**5**) and *erythro*-β-phenyl-DL-cysteine (**6**) reduced ethanol-derived blood AcH by ca. 40% and 60%, respectively, whereas the corresponding β-methyl-DL-cysteines (**3** and **4**) and the α-substituted α-methyl-DL-cysteine (**8**) had no effect. β,β-Tetramethylene-DL-cysteine (**7**), however, was as effective as D-(−)-penicillamine in sequestering AcH in vivo, reducing blood AcH after ethanol to 20% of maximal values. Thus, bulky β-substitution or, better, β,β-disubstitution on cysteine is required for such activity. ^{14}C-Labeled 2(*RS*),5,5-trimethylthiazolidine-4(*S*)-carboxylic acid (**1**) prepared by the condensation of D-(−)-penicillamine with [1,2-^{14}C]acetaldehyde was found to be relatively stable in vivo, giving rise to <6% $^{14}CO_2$ excretion in the expired air and the recovery of 65.5% of the administered dose in the urine as unchanged **1**.

It has been reported that chronic alcoholics exhibit elevated levels of blood acetaldehyde (AcH) after consuming alcohol compared to normal individuals,[1] while male offsprings of chronic alcoholic parents also show a tendency to high ethanol-derived blood AcH levels.[2] The "vicious cycle" of acetaldehydemia and hepatotoxicity has been etiologically linked to AcH,[3] the first product of ethanol metabolism. Much discussion has centered on the possibilities for AcH-mediated mechanisms in alcohol dependence,[4] although the data are yet inconclusive. It is known that AcH triggers the release of catecholamines from adrenergic neurons[5] and inhibits hepatic[6] and myocardial[7] protein biosynthesis. AcH also interferes with mitochondrial function in the liver[8] and the heart,[9] and circulating AcH can combine with biogenic amines in vivo to form tetrahydroisoquinolines[10] and can interfere with biogenic amine metabolism to give rise to the formation of tetrahydropapaverolines,[11] some of which show alcohol-addicting liabilities when instilled directly into the

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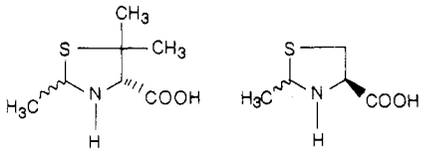
ventricles of rat brain.[12] Ethanol-derived AcH also binds to erythrocyte and plasma proteins, leading to the formation of antibodies against AcH–protein adducts.[13] More recent studies on AcH binding to tissues, biogenic amines, and enzyme cofactors have been described in a monograph.[14]

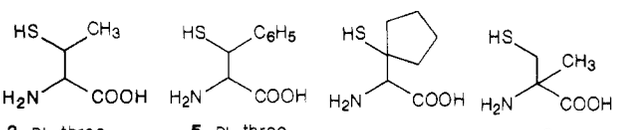
It would seem prudent, therefore, to seek pharmacologic agents that can sequester this circulating AcH derived from ethanol metabolism and divert it to urinary excretion pathways. We had shown earlier that D-(−)-penicillamine, a β,β-dialkyl-substituted β-mercapto-α-amino acid, was an excellent sequestering agent for ethanol-derived blood AcH in vivo.[15] This is a consequence of the nonenzymatic condensation of metabolically generated AcH with the administered D-(−)-penicillamine to yield a substituted thiazolidine-4-carboxylic acid (**1**), which is excreted in the urine.[15] On the other hand, L-cysteine, the naturally occurring sulfhydryl amino acid of protein origin, was found to be totally ineffective in this regard,[15c] due to its rapid catabolism and the ready dissociation in vivo of the thiazolidine-4-carboxylic acid **2** presumably formed by its condensation with AcH.[16]



1 **2**

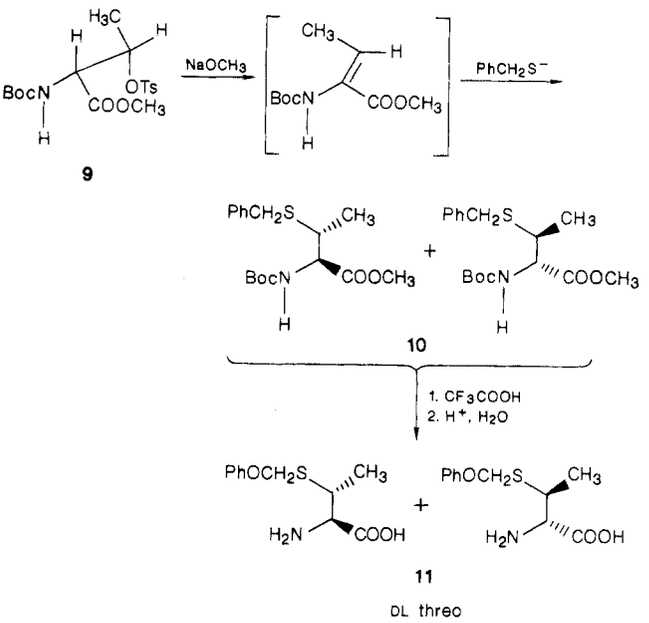
Structure–activity studies suggested that only 1,2-vicinal or 1,3 amino thiols, including N-terminal cysteinyl dipeptides such as L-cysteinyl-L-valine, were capable of reacting with AcH at physiological pH and temperature in vitro.[17] However, except for D-(−)-penicillamine, the above were uniformly poor AcH-sequestering agents in vivo. Indeed, the results of this study suggested that additional β-substitution on a β-mercapto-α-amino acid was required for in vivo activity.

Accordingly, the threo and erythro isomers of β-methyl-DL-cysteine (**3** and **4**, respectivcely) and β-phenyl-DL-cysteine (**5** and **6**, respectively), as well as β,β-tetramethylene-DL-cysteine (**7**), were synthesized for evaluation in rats as aldehyde-sequestration agents. α-Methyl-DL-cysteine (**8**) was also included to compare the effect of α-alkyl vs. β-alkyl substitution on the propensity of these cysteine analogues to sequester AcH.



3, DL threo
4, DL erythro
5, DL threo
6, DL erythro
7
8

Scheme I



9
10
11
DL threo

We also present evidence that the efficacy of D-(−)-penicillamine in sequestering AcH is related to the relative stability in vivo of its AcH condensation product, viz., 2(*RS*),5,5-trimethylthiazolidine-4(*S*)-carboxylic acid (**1**). This contrasts with the behavior of the condensation product of L-cysteine and AcH, viz., 2(*RS*)-methylthiazolidine-4(*R*)-carboxylic acid (**2**), which readily dissociates in vivo to give catabolic products.[16]

Chemistry

erythro-β-Methyl-DL-cysteine (**4**) was obtained by debenzylation of *erythro*-*S*-benzyl-β-methyl-DL-cysteine with Na in liquid NH_3.[18] It was purified as the Hg(II) complex and isolated as its hydrochloride. The synthesis of *threo*-β-methyl-DL-cysteine (**3**) followed a different route, involving a β-elimination/trans addition mechanism with racemization (Scheme I). Thus, attempted direct displacement of the tosyl group of *N*-Boc-*O*-tosyl-D-threonine methyl ester (**9**) with α-toluenethiol in the presence of sodium methoxide gave *N*-Boc-*S*-benzyl-β-methylcysteine methyl ester (**10**), which, however, was devoid of optical activity. Correspondingly, racemic *S*-benzyl-β-methylcysteine (**11**) was obtained after deblocking of the Boc group with CF_3COOH and hydrolysis of the ester group. That this product was exclusively the threo isomer—readily rationalized according to Scheme I—was shown by benzoylation to the known *threo*-*N*-benzoyl-*S*-benzyl-β-methyl-DL-cysteine (**12**), mp 142–144 °C.[18] Debenzylation of **11** gave *threo*-β-methyl-DL-cysteine (**3**), which was also isolated as the hydrochloride.

erythro-β-Phenyl-DL-cysteine (**6**) was prepared by hydrolysis of the known *erythro*-β-phenyl-DL-cysteine ethyl ester.[19] For the synthesis of *threo*-β-phenyl-DL-cysteine (**5**) and β,β-tetramethylene-DL-cysteine (**7**), the general procedure developed by Holland and Mamalis [20] starting from the synthon 2-thioxo-5-thiazolidinone (**13**) was modified somewhat (Scheme II). Accordingly, trans-2-oxo-5-phenylthiazolidine-4-carboxylic acid (**16a**) was sol-

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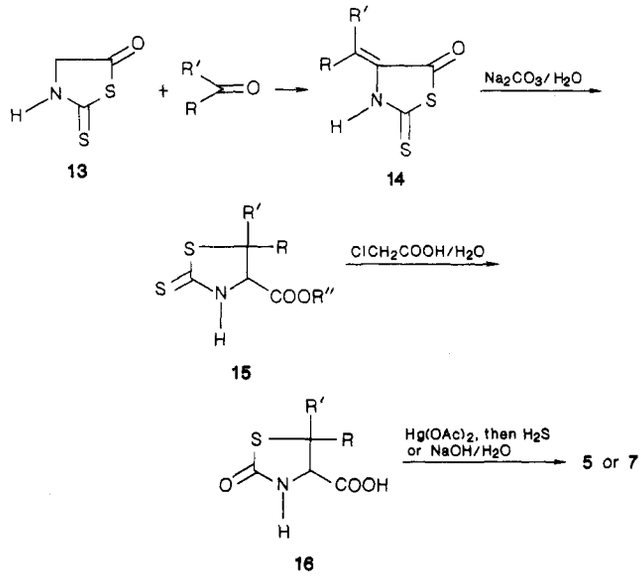
Scheme II[a]



[a] a series: R = H, R′ = C_6H_5, R″ = H. b series: R–R′ = $(CH_2)_4$, R″ = CH_3.

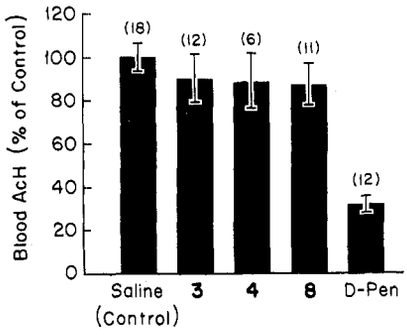


Figure 1. Sequestration of blood AcH in rats by methylcysteines after administration of disulfiram/ethanol. The vertical bar inserts are ±SEM; the *N*'s are indicated in the parentheses. Except for D-(−)-penicillamine (D-pen), none of the methylcysteine values were different from those of controls.

volyzed in the presence of mercuric acetate, and the intermediate mercaptide was decomposed with H_2S to give **5**. Similarly, the synthesis of **7** followed Scheme II. Condensation of cyclopentanone with **13** gave **14b**, which on treatment with sodium methoxide in methanol rearranged to methyl 2-thioxo-5,5-tetramethylenethiazolidine-4-carboxylate (**15b**). Conversion to the corresponding 2-oxo-5,5-tetramethylenethiazolidine-4-carboxylic acid (**16b**) proceeded smoothly with aqueous chloroacetic acid. Since the mercuric acetate catalyzed solvolysis of the 2-oxo derivative (**16b**) gave poor yields of the desired product, **7**, alkaline hydrolysis was effected to give **7** in 54% yield.

The preparation of α-methyl-DL-cysteine (**8**) followed literature procedures,[21] while radioactive 2(*R,S*)[^{14}C-*methyl*-2-^{14}C],5,5-trimethylthiazolidine-4(*S*)-carboxylic acid, [^{14}C]**1**, was prepared by the condensation of D-(−)-penicillamine with [1,2-^{14}C]acetaldehyde following the procedure for the unlabeled compound.[15c]

Biological Results and Discussion

The α- and β-methylcysteines were evaluated as AcH-sequestering agents in rats by using D-(−)-penicillamine as standard, according to our usual test protocol.[17] In this

(21) Connors, T. A.; Ross, W. C. J. *Chem. Ind. (London)* **1958**, 366.

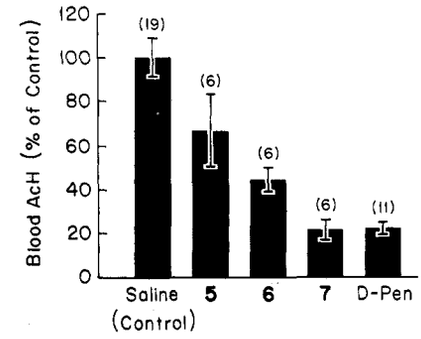


Figure 2. Sequestration of blood AcH in rats after administration of cyanamide/ethanol by *threo*- (**5**) and *erythro*-β-phenyl-DL-cysteine (**6**) and by β,β-tetramethylene-DL-cysteine (**7**). The vertical bar inserts are ±SEM; the *N*'s are indicated in the parentheses. Comparison of the measured data expressed as μM AcH in blood by analysis of variance[29] indicated that all three cysteine analogues **5**–**7** were significantly different from controls at the 95% confidence level. The values for **7** and D-(−)-penicillamine were also significantly different from that for *threo*-β-phenyl-DL-cysteine (**5**).

Table I. Metabolic Disposition of ^{14}C-Labeled **1** in the Rat[a]

	% of administered dose excreted in		
time, h	urine	CO_2	feces
0–2	36.3 ± 5.4	2.01 ± 0.099	
2–4	25.9 ± 3.5	1.70 ± 0.085	
4–8	12.7 ± 2.2	1.36 ± 0.069	
8–16	3.12 ± 2.2	0.48 ± 0.08	
16–24	1.18 ± 0.48	0.28 ± 0.07	
0–24	79.2 ± 2.9[b]	5.83 ± 0.092	1.38 ± 1.5

[a] Average ± SD from two animals. Details of the protocol can be found in the Experimental Section. [b] 82.8% of this urinary radioactivity was accountable as unchanged [^{14}C]**1** as determined by inverse isotope dilution with unlabeled carrier. This corresponds to 65.5% of the administered dose.

set (Figure 1), disulfiram was preadministered to elevate ethanol-derived blood AcH by inhibiting the enzyme aldehyde dehydrogenase (AlDH). It can be seen that neither α-methyl-DL-cysteine (**8**) nor the threo (**3**) or erythro (**4**) isomer of β-methyl-DL-cysteine significantly reduced blood AcH in vivo.

For the remaining cysteine analogues, another AlDH inhibitor, viz., cyanamide, was substituted for disulfiram to raise blood AcH. This substitution does not compromise valid comparisons between the two sets (vide infra). Both the *threo*- and *erythro*-β-phenyl-DL-cysteines (**5** and **6**) were reasonably good sequestering agents for blood AcH, lowering the levels by ca. 40% and 60%, respectively (Figure 2). By far the best AcH-sequestering agent of this series was β,β-tetramethylene-DL-cysteine (**7**). This compound was equally effective as D-(−)-penicillamine itself and reduced blood AcH to 20% of the values found in control animals receiving cyanamide and ethanol only.

Metabolic Disposition of [^{14}C]1. In order to assess the stability in vivo of **1**, the condensation product of D-(−)-penicillamine with AcH,[15a,c] the ^{14}C-labeled compound with the label on the AcH moiety was administered to two rats in separate experiments, and the urine, feces, and expired CO_2 were monitored for radioactivity. It was found (Table I) that less than 6% of the administered dose was recoverable as $^{14}CO_2$, with nearly 80% of this dose being excreted in the urine over 24 h. By inverse isotope dilution with unlabeled carrier, it was determined that 83% of this urinary radioactivity was accountable as unchanged [^{14}C]**1**. Thus, unlike **2**, the condensation product of L-cysteine and AcH, which was rapidly catabolized to CO_2 in the rat, [^{14}C]**1** appears to be relatively stable in vivo. However,

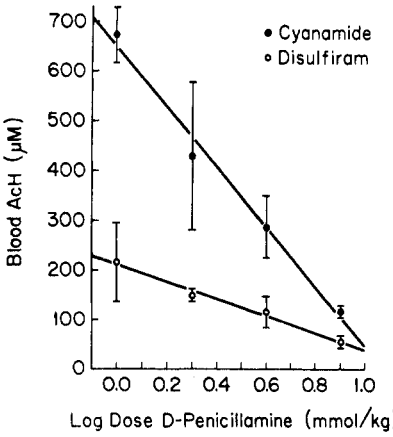


Figure 3. Sequestration of ethanol-derived blood AcH by incremental doses of D-(−)-penicillamine in cyanamide (●) and disulfiram (○) pretreated rats; $N = 3$.

some dissociation and metabolism must have taken place, as reflected by the expiration of finite radioactivity as $^{14}CO_2$ and the less than 100% recovery in the urine as [^{14}C]1 (Table I).

Comparison of Disulfiram and Cyanamide Pretreatment Regimen on AcH Sequestration by D-(−)-Penicillamine. Since the steady-state concentrations of ethanol-derived blood AcH in rats approach the limits of sensitivity of the AcH measurements, it is necessary to impose a metabolic block on the enzyme AlDH in order to raise blood AcH sufficiently to measure its sequestration. Previously, disulfiram had been used as this AlDH inhibitor, but a more potent inhibitor giving rise to higher blood AcH levels after ethanol is available in cyanamide.[22] We therefore opted to substitute cyanamide for disulfiram in the pretreatment regimen.

To assure comparability of the results obtained with either agent, the dose–response curves for D-(−)-penicillamine, a known in vivo AcH-sequestering agent,[15] were constructed for both pretreatment regimens. As shown in Figure 3, blood AcH levels of rats treated with cyanamide or disulfiram followed by ethanol decreased logarithmically with the dose of D-(−)-penicillamine, and because cyanamide was a more potent AlDH inhibitor, the effect was more pronounced with this agent. When the *mean* blood AcH levels from cyanamide-treated rats were plotted against the corresponding values from disulfiram-treated rats, a linear relationship was found with a correlation coefficient of 0.979 (Figure 4). These results demonstrate that cyanamide can in fact replace disulfiram in these assays and that data obtained with one agent can be directly compared to data obtained with the other when compared as "percent of control" values.

Conclusions

On the basis of the structure–activity relationships accumulated to date, it is clear that sequestration of ethanol-derived blood AcH in vivo, the ultimate criterion for activity, requires not only the β-mercapto-α-amino acid structure of cysteine but additional substitution on the β-carbon with a bulky aromatic group such as phenyl or, for optimal activity, geminal substitution with methyl groups or a polymethylene group. This provides further rationale for such analogue syntheses in the future.

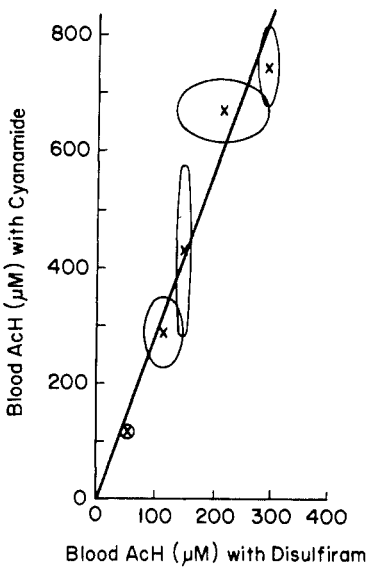


Figure 4. Relationship of blood AcH levels at equimolar doses of D-(−)-penicillamine after pretreatment with cyanamide or disulfiram, followed by ethanol. Doses and N were as in Figure 3, and the ellipses represent ±SEM. The highest point represents data without D-(−)-penicillamine.

The reason for the efficacy of bulky β-substituted cysteines as aldehyde-sequestering agents appears to reside in the stability of the thiazolidine-4-carboxylic acid formed by condensation with AcH, as exemplified by the lack of appreciable metabolism of [^{14}C]1 in vivo (Table I). It would be of interest to ascertain whether the thiazolidinecarboxylic acid derived from **7** and AcH is as stable as **1** in vivo. As prior resolution of **7** into its optical antipodes is necessary in order to carry out such studies, these experiments will be deferred until such time when this is accomplished.

Experimental Section

Melting points were determined on a Mettler FP-2 melting point apparatus, except for the amino acids and their hydrochlorides for which a Mel-Temp capillary melting point apparatus with a digital thermometer was used, and are corrected to reference standards. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. The target amino acids were chromatographed on silica gel GF (Analtech, Inc., Newark, DE) using *n*-BuOH/HOAc/H_2O (4:1:1), and the ratios (R_{Pen}) of these R_f values to that of D-(−)-penicillamine were recorded. The amino acid hydrochlorides were neutralized with 1% $NaHCO_3$ before spotting on the TLC plates. Headspace gas chromatography was carried out on a Packard Model 419 Becker flame-ionization gas chromatograph equipped with an Autolab 6300 digital integrator. Radioactivity was determined in a Packard Model 4640 liquid scintillation spectrometer using Aquasol (New England Nuclear) to dissolve the radioactive substances. Feces (dried) were combusted to CO_2 in a Packard Model BD-306 sample oxidizer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. D-(−)-Penicillamine was purchased from Aldrich Chemical Co., Milwaukee, WI, and cyanamide from Sigma Chemical Co., St. Louis, MO. For the pharmacological evaluations, male rats of Sprague–Dawley descent (Biolabs, Inc., St. Paul, MN) were used. These studies were performed in adherence with guidelines established in the "Guide for the Care and Use of Laboratory Animals" published by the U.S. Department of Health and Human Resources (NIH Publication 85-23, revised 1985). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the research protocol was approved by the Animal Study Subcommittee of the Minneapolis VA Medical Center.

***erythro*-β-Methyl-DL-cysteine (4).** The benzylthio group of *erythro*-*S*-benzyl-β-methyl-DL-cysteine was cleaved with sodium in liquid ammonia,[18] and the product was isolated as the Hg(II)

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complex, followed by removal of the Hg(II) with H_2S. Mercuric acetate was used instead of $HgCl_2$, and it was necessary to reprecipitate the isolated amino acid as the Hg(II) complex to obtain **4** as an analytically pure hydrochloride: mp 171–174 °C dec; 53% yield; R_{Pen} = 0.69. Anal. ($C_4H_{10}NO_2SCl$) C, H, N.

***threo*-S-Benzyl-β-methyl-DL-cysteine (11).** To a solution of α-toluenethiol (15.53 g, 0.125 mol) in MeOH (75 mL) cooled to 10 °C was added Na (3.22 g, 0.14 mol) in small pieces with stirring. To the stirred solution was added *N*-Boc-*O*-tosyl-D-threonine methyl ester (**9**)[23] (48.43 g, 0.125 mol), and stirring was continued for 20 h. The reaction mixture was then filtered, and the filtrate was evaporated to dryness. The resulting product (**10**), *which was devoid of optical activity*, was treated with ether (200 mL), and any insoluble material was removed by filtration. After evaporation of the filtrate to dryness, the residue was dissolved in trifluoroacetic acid (100 g), which resulted in visible evolution of CO_2. After 1 h, the solvent was evaporated in vacuo, the residue was dissolved in 12 N HCl (100 mL), and the mixture was heated at 65 °C for 5 h to hydrolyze the ester group. The solvent was again evaporated to dryness, the residue was taken up in H_2O (500 mL), and the solution was neutralized with 6 N NaOH. The precipitate that formed on standing overnight was collected and dissolved in 3 N NH_4OH (300 mL), and the ensuing solution was decolorized (carbon) and evaporated under reduced pressure to give **11** in three crops as white powdery crystals: 6.46 g (23% yield); mp 172–175 °C (lit.[24] mp 171–173 °C). Benzoylation of **11** gave *threo*-*N*-benzoyl-*S*-benzyl-β-methyl-DL-cysteine, mp 142–144 °C, after recrystallization from CH_2Cl_2/hexane (lit.[18] mp 145–147 °C).

***threo*-β-Methyl-DL-cysteine (3).** This compound was obtained by debenzylation of **11** by the method described above for **4**. The yield was 55% as the hydrochloride: mp 151–156 °C dec.; R_{Pen} = 0.94. Anal. ($C_4H_{10}NO_2SCl$) C, H, N.

***erythro*-β-Phenyl-DL-cysteine (6).** A solution of *erythro*-β-phenyl-DL-cysteine ethyl ester hydrochloride[19] (24.76 g, 0.095 mol) in 10% HCl (110 mL) was heated under reflux for 4 h. The reaction mixture was then decolorized (carbon) and neutralized with 6 N NaOH. The gray precipitate that formed was redissolved in boiling H_2O (200 mL), and the solution was then decolorized and concentrated under reduced pressure until the mixture set to a wet slurry. This was swirled in EtOH (100 mL), and the solids were collected. The product was washed with EtOH (2 × 50 mL) and ether (2 × 20 mL) and dried. Recrystallization from H_2O as above gave **6** as a white powder after drying for 18 h under vacuum at 70 °C: 5.75 g (31% yield); mp 187–188 °C dec; R_{Pen} = 1.17. Anal. ($C_9H_{11}NO_2S$) C, H, N.

***threo*-β-Phenyl-DL-cysteine (5).** A solution of mercuric acetate (27.44 g, 0.0861 mol) in H_2O (100 mL) was added to a solution of *trans*-2-oxo-5-phenylthiazolidine-4-carboxylic acid (**16a**)[25] [mp 190–192 °C (lit.[20] 194–195 °C)] (5.50 g, g, 0.025 mol) in boiling H_2O (250 mL). A white solid precipitated, and evolution of CO_2 was evident. The precipitate was collected, washed with H_2O, and suspended in 0.4 N HCl (300 mL). A stream of H_2S was bubbled through the suspension for 20 min; the HgS was then removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in hot EtOH (50 mL), and the solution was concentrated to 25 mL and cooled to give a precipitate of dense crystals. These were collected and washed with ether (50 mL) to give 2.00 g (35% yield) of **5**-HCl, mp 195.8–196.7 °C (lit.[19] mp 204–205 °C). A 0.658-g sample in 4 mL of H_2O was neutralized with 6 N NaOH, and the precipitate that resulted was collected and recrystallized from hot water (15 mL) to give colorless crystals of pure **5**: mp 193–194 °C dec; R_{Pen} = 1.15. Anal. ($C_9H_{11}NO_2S$) C, H, N.

2-Thioxo-4-cyclopentylidene-5-thiazolidinone (14b). To a solution of 2-thioxo-5-thiazolidone (**13**)[26] (11.16 g, 0.084 mol) in warm cyclopentanone (40 mL) was added morpholine (7 drops). After 1 day another addition of morpholine was made. After standing for 14 days, the solid cake that had formed was broken up and collected. The solids were extracted directly on the filter funnel by repeatedly (6×) percolating 50-mL portions of hot THF through the solids. The brown residue (4.0 g) was then suspended in H_2O (20 mL) and the mixture heated under reflux for 5 min. The H_2O-insoluble residue was collected and extracted once again with hot THF (4 × 50 mL) as above. The combined THF extracts (ca. 500 mL) were passed through a 3.6 × 20 cm column of activated alumina (MN W-200, neutral) and eluted with THF. Evaporation of the eluate (800 mL) gave **14b** as yellow crystals: 8.65 g (52% yield); mp 241–242 °C dec; (lit.[27] mp 244–245 °C).

2-Oxo-1-thia-3-azaspiro[4.4]nonane-4-carboxylic Acid (16b). A mixture of methyl 2-thioxo-1-thia-3-azaspiro[4.4]nonane-4-carboxylate (**15b**)[27] (17.12 g, 0.074 mol) and chloroacetic acid (34.97 g, 0.370 mol) in H_2O (175 mL) was heated under reflux for 5 h. The mixture yielded a mass of crystals when kept refrigerated overnight, which were collected and washed with cold H_2O (30 mL). Recrystallization from THF/hexane gave **16b** as white crystals: 11.21 g (75% yield); mp 179–181 °C. Anal. ($C_8H_{11}NO_3S$) C, H, N.

β,β-Tetramethylene-DL-cysteine (7). A solution of **16b** (4.00 g, 0.0199 mol) in 6 N NaOH (20 mL) was equally distributed to four polypropylene test tubes with screwcaps. Each tube was flushed with N_2, capped, and maintained at 80 °C in a heating block for 18 h. The cooled solutions were combined and acidified to pH 2 with concentrated HCl. When gas evolution ceased, the pH was adjusted to 6.5 with 6 N NaOH. The precipitate that had formed was sedimented by centrifugation, the aqueous phase was decanted, and the solid residue was dissolved in 45 mL of boiling H_2O. The crystals that had precipitated on chilling (4 h) were collected, washed with EtOH (4 × 10 mL) followed by ether (4 × 10 mL), and dried for 18 h under vacuum at 70 °C to give **7**: 1.89 g (54% yield); mp 201–203 °C dec; R_{Pen} = 1.08. Anal. ($C_7H_{13}NO_2S$) C, H, N.

α-Methyl-DL-cysteine (8). This compound was obtained as its hydrochloride by debenzylation of *S*-benzyl-α-methylcysteine by the method described above for **4**. The yield was 41.2%: mp 186–188 °C dec (lit.[21] mp 190–195 °C); R_{Pen} = 0.83. Anal. ($C_4H_{10}NO_2SCl$) C, H, N.

Sequestration of Ethanol-Derived Blood AcH in Rats by Substituted Cysteines. For the methylcysteines (Figure 1), the procedure was identical with that previously described.[17a] For the remaining substituted cysteines (Figure 2), cyanamide replaced disulfiram as follows: the rats were fasted for 24 h before ip administration of 0.5 mmol/kg of cyanamide dissolved in H_2O, followed 3 h later by D-(−)-penicillamine (in H_2O, 8.0 mmol/kg, ip) or the substituted cysteines (8.0 mmol/kg ip). Compounds **5–7** were administered as pulverized suspensions in 2% (carboxymethyl)cellulose, while compounds **3**, **4**, and **8** were administered as neutralized (pH 7.0) aqueous solutions (all at 1.0 mL/100 g body wt). Ethanol (43.5 mmol/kg as a 20% aqueous solution) was administered ip 1 h after the AcH-sequestering agent, and the animals were sacrificed 1 h subsequent to this for measurement of blood AcH by headspace gas chromatography.[17b] Blood AcH levels of naive rats treated with ethanol alone were 10.4 ± 1.9 (SEM) μM.[17b]

Comparative Sequestration of Blood AcH after Ethanol Administration by Incremental Doses of D-(−)-Penicillamine in Cyanamide- and Disulfiram-Pretreated Rats. The respective pretreatment protocols with disulfiram and cyanamide were as described above. D-Penicillamine was administered at doses of 1.0, 2.0, 4.0, and 8.0 mmol/kg 24 h after disulfiram and 4 h after cyanamide. Subsequent procedures were identical thereafter for the two groups, viz., ethanol was administered 1 h after D-(−)-penicillamine and animal sacrifice followed 1 h after ethanol.

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(25) Prepared from *trans*-**15a**. The rearrangement of **14a** using hot aqueous Na_2CO_3 gave, in our hands, *trans*-**15a** directly without the necessity for further heating with Na_2CO_3. *cis*-**15a** prepared according to Chatterjee et al. (Chatterjee, R.; Cook, A. H.; Heilbron, J.; Levy, A. L. *J. Chem. Soc.* **1948**, 1337) had mp 221–223 °C (lit.[20] mp 230–234 °C dec).

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2(*R*,*S*)[^{14}C-*methyl*-2-^{14}C],5,5-Trimethylthiazolidine-4-(*S*)-carboxylic Acid ([^{14}C]1). A solution of D-(−)-penicillamine (149.2 mg, 1.00 mmol) dissolved in 2.0 mL of water in a round-bottomed flask adapted with a short liquid-addition tube with drip tip and stoppered at the other end was cooled in an ice bath. The ampule of [1,2-^{14}C]acetaldehyde of specific activity 9.5 mCi/mmol [4.6 mg (0.1 mmol) dissolved in 1.0 mL of water] was frozen in a dry ice/acetone bath. The ampule was then broken, inverted, and placed into the addition tube. The system was stoppered and tilted at a 45° angle to allow the radioactive solution to drip into the reaction flask as it thawed. After 5 min, the tube was rinsed with acetaldehyde stock solution (1.0 mL, 0.0396 g, 9.00 mmol) made by diluting 0.51 mL of freshly distilled acetaldehyde to 10.0 mL with H_2O. Total acetaldehyde = 0.0442 g, 1.00 mmol. After an additional 5 min, the vials and addition tubes were rinsed with cold water (0.5 mL each) and removed. Stirring was maintained in the cold for 1 h and at room temperature for 1.5 h. The reaction mixture was then concentrated to dryness in vacuo and the solid residue recrystallized from ethyl acetate to give 96.7 mg (55.2% yield) of [^{14}C]1: mp 165–166 °C; specific activity 6.00×10^6 cpm/mg (0.47 mCi/mmol). A second crop of lower specific radioactivity was obtained by addition of 100 mg of unlabeled 1 to the residue after evaporation of the mother liquor above, followed by recrystallization (98 mg; 0.26 mCi/mmol). A repeat procedure yielded a third crop (93 mg, 0.14 mCi/mmol). TLC of crops 1 and 2 in *n*-BuOH/HOAc/H_2O (50:11:25) and *n*-PrOH/H_2O (7:3) showed only single radioactive spots, R_f 0.52 and 0.75, respectively, when scanned with a radiochromatogram scanner.

Metabolic Disposition of [^{14}C]1 in the Rat. The metabolism of 1 by the rat was investigated in two separate experiments using ^{14}C-labeled 1. The injection solution was made up of 700.3 mg of unlabeled carrier 1 and 0.70 mg of [^{14}C]1 in water (5 mL). Administration of this solution at 1.0 mL/100 g body weight represented a dose of 8.0 mmol/kg and provided radioactivity of 5.96×10^6 dpm by actual assay (duplicate 500-μL samples of the injection solution were counted).

A male rat of Sprague–Dawley descent weighing 207 g was fasted overnight. After administration of [^{14}C]1, the animal was placed in an all-glass metabolism cage that allowed the separate collection of urine, feces, and expired air. CO_2 was collected in two successive traps containing cold methoxyethanol/ethanolamine (2:1).[28] Sample collections were made at 2, 4, 8, 16, and 24 h after drug administration. At each collection time, the CO_2-trapping solution was removed from both the primary and secondary traps, the volume recorded, and fresh solution added. After the sides of the metabolism cage were rinsed with a small amount of water, urine volume was recorded and the urine stored over Na_2CO_3 (200 mg). The radioactivity of duplicate samples of urine and the CO_2-trapping solutions was determined in Aquasol by liquid scintillation spectrometry. Feces collected over 24 h were lyophilized and pulverized, and 250-mg samples were combusted to $^{14}CO_2$ for radioactivity determinations.

The 24-h urines were pooled, and carrier 1 (400 mg) was added. The solution was thoroughly mixed, filtered to remove debris, adjusted to 100 mL with water, and assayed for total radioactivity. After acetylation, the acetylated 1 was recrystallized to constant specific radioactivity as described previously for the isolation of radioactive 1 from rat urine.[15c] This experiment was repeated with a second rat weighing 247 g, and the results (averaged from both experiments) are presented in Table I.

Acknowledgment. This work was supported by the Veterans Administration. We thank W. E. Smith for technical assistance with the pharmacological studies and Dr. D. J. W. Goon and Dr. E. G. DeMaster for helpful discussion. J.C.R. was a recipient of the Louise T. Dosdall Fellowship in Science.

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Tricyclic Compounds as Selective Antimuscarinics. 1. Structural Requirements for Selectivity toward the Muscarinic Acetylcholine Receptor in a Series of Pirenzepine and Imipramine Analogues

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Received December 29, 1986

The M_1-selective antiulcer drug pirenzepine (1) is a tricyclic compound with close resemblance to tricyclic psychotropic agents such as imipramine (2). Despite this fact, pirenzepine is devoid of any psychotropic effects, exhibiting measurable antagonistic effects in biochemical assays and receptor binding studies only toward the muscarinic receptor system. To understand how different groups in these tricyclic molecules affect binding affinities, a set of nine compounds structurally related to pirenzepine (1) and imipramine (2) has been selected for analysis, comprising three different tricycles and three different side chains. The compounds were tested for their affinity to the imipramine and muscarinic receptors in homogenized rat cortex tissue. The result of these studies suggests that it is the nature and placement of accessory groups that determine the differences in receptor recognition and the binding process. In the case of pirenzepine (1), preferential binding toward the muscarinic receptor is brought about by the endocyclic amide group, by the positioning of the protonated N atom of the side chain, and to a minor extent by the exocyclic amide group. From these findings a putative model for the explanation of selective binding of pirenzepine (1) to the muscarinic receptor has been derived.

Pirenzepine (1) (Gastrozepin) is the first M_1-selective muscarinic receptor antagonist that has been introduced into ulcer therapy, providing safe and unproblematic treatment of gastritis and duodenal and peptic ulcer.[1] Pirenzepine selectively inhibits vagally stimulated gastric secretion and various neuronal muscarinic responses but has a lower potency for heart and smooth muscle receptors.[2,3] The selectivity found in whole animal pharma-

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