85250-33-5; **13c**, 85250-34-6; **14a**, 85250-29-9; **14b**, 85281-33-0; **14c**, 85250-35-7; **14c**·HCl, 85250-36-8; Boc-Cys(Acm)-OBzl, 79396-91-1; Boc-Ala-OH, 15761-38-3; Boc-D-Ala-OH, 7764-95-6; H-Cys(Acm)-OBzl·HCl, 79396-92-2; H-Ala-OBzl·*p*-Tos-OH, 42854-62-6; Boc-Pro-OH, 15761-39-4; Boc-Glu(OBzl)-OH, 13574-13-5; Boc-Leu-OH, 13139-15-6; Cbz-Ala-OH, 1142-20-7; prothrombin, 9001-26-7; vitamin K dependent carboxylase, 64641-76-5.

Supplementary Material Available: Full ^{13}C NMR data for compounds **9a,b** and **3c** in Me_2SO-d_6 (Table II) and $CDCl_3$ (Table III) (2 pages). Ordering information is given on any current masthead page.

Inhibition by Carboxamides and Sulfoxides of Liver Alcohol Dehydrogenase and Ethanol Metabolism

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Sulfoxides and amides were tested as inhibitors of liver alcohol dehydrogenase and ethanol metabolism in rats. With both series of compounds, increasing the hydrophobicity resulted in better inhibition, and introduction of polar groups reduced inhibition. Of the cyclic sulfoxides, tetramethylene sulfoxide was the best inhibitor as compared to the tri- and pentamethylene analogue and other compounds, and it may be a transition-state analogue. The most promising compounds, tetramethylene sulfoxide and isovaleramide, were essentially uncompetitive inhibitors of purified horse and rat liver alcohol dehydrogenases with respect to ethanol as substrate. These compounds also were uncompetitive inhibitors in vivo, which is advantageous since the inhibition is not overcome at higher concentrations of ethanol, as it is with competitive inhibitors, such as pyrazole. The uncompetitive inhibition constants for tetramethylene sulfoxide and isovaleramide for rat liver alcohol dehydrogenase were 200 and 20 μM, respectively, in vitro, whereas in vivo the values were 340 and 180 μmol/kg. The differences in the values may be due to metabolism or distribution of the compounds. Further studies will be required to determine if isovaleramide or tetramethylene sulfoxide is suitable for therapeutic purposes.

Liver alcohol dehydrogenase (EC 1.1.1.1) catalyzes the first step in alcohol metabolism and would be a rational target for inhibiting alcohol metabolism.[1,2] Inhibitors of the dehydrogenase would be useful for studying the metabolism of alcohols[3] and for therapeutically preventing poisoning by methanol[4,5] and ethylene glycol.[6,7]

Pyrazole and its 4-substituted derivatives are effective competitive inhibitors of alcohol dehydrogenation in vitro and in vivo[8] since they bind tightly to the alcohol dehydrogenase–NAD^+ complex.[9] Pyrazole itself is toxic in doses that are required to significantly inhibit alcohol metabolism in vivo,[10] but 4-methylpyrazole is less toxic and more effective as an inhibitor of alcohol dehydrogenase and may be useful for treatment of humans.[5,11] However, 4-alkylpyrazoles depress the central nervous system in rats and mice.[8]

Other organic compounds, such as fatty acid amides[12-16] and aromatic amides,[17] have been tested as inhibitors of the enzyme. *n*-Butyraldoxime and other oximes are about as effective as pyrazole in rats,[18] but in man the oxime induced a reaction like that with disulfiram, in which blood acetaldehyde levels increased after ingestion of ethanol.[19] *n*-Butyramide is almost as effective as pyrazole in blocking ethanol oxidation in rats and is less toxic.[18] Thus, it is worthwhile to test other carboxamides to find potent, nontoxic inhibitors of alcohol dehydrogenase.

Dimethyl sulfoxide forms a highly fluorescent ternary complex[20] with the enzyme and reduced nicotinamide adenine dinucleotide and is an inhibitor in vitro of alcohol dehydrogenase.[21] The three-dimensional structure of the complex has been determined by X-ray crystallography to high resolution.[22] Dimethyl sulfoxide (Me_2SO) is used

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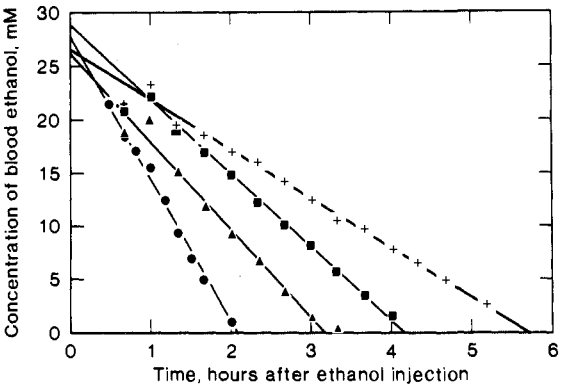


Figure 1. Inhibition of ethanol metabolism by tetramethylene sulfoxide in vivo. Male Sprague–Dawley rats were given ip injections of tetramethylene sulfoxide (0.1 M) in saline at doses of 0.0 (●), 0.25 (▲), 0.5 (■), or 1.0 (+) mmol/kg of body weight. Ten minutes later, ethanol (3.26 M) was injected ip at a dose of 19.6 mmol/kg of body weight. Blood samples were taken at intervals timed from the ethanol injection and analyzed by gas chromatography.

as a solvent for some experimental drugs, for treatment of rheumatoid arthritis and scleroderma,[23] and as a cryoprotective agent.[24] Patients given Me_2SO showed intolerance to alcoholic beverages,[25] and Me_2SO prolonged significantly ethanol-induced loss of righting reflex in mice.[21] A few other sulfoxides have pharmacological activities.[26-29]

Carboxamides and dimethyl sulfoxide are uncompetitive inhibitors of alcohol dehydrogenase, with respect to activity with varied concentrations of alcohol,[13,21,30] and thus offer the important advantage over competitive inhibitors, such as pyrazole, that high concentrations of the alcohol do not eliminate the inhibitory effect. Therefore, we have evaluated some amides and sulfoxides for their inhibitory activity on alcohol dehydrogenation in vitro and in rats.

Biological Results and Discussion

The inhibitory potency of the compounds with purified horse liver alcohol dehydrogenase was determined for comparison to other known inhibitors and to predict their action in vivo. As indicated in Table I, most of the amides and sulfoxides were uncompetitive inhibitors of the horse liver enzyme with respect to its activity on ethanol, and the relevant inhibition constants are reported in Table I. Liver alcohol dehydrogenase clearly prefers to bind hydrophobic amides or sulfoxides rather than analogous compounds containing additional heteroatoms, such as hydroxy, amino, or keto functions. These observations are consistent with previous observations made with liver alcohol dehydrogenase.[12,13,17] It has been shown previously that *n*-substituted amides have reduced affinity for the enzyme,[17] and a similar effect is seen with the sulfoxides; the ethyl sulfoxides have reduced affinity compared to the methyl sulfoxides. On the other hand, the cyclic sulfoxides, such as tri-, tetra-, and pentamethylene sulfoxides have

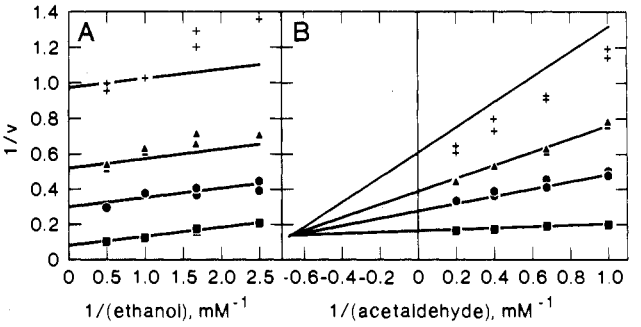
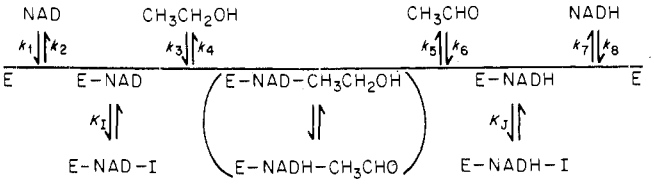


Figure 2. Inhibition of purified horse liver alcohol dehydrogenase by tetramethylene sulfoxide in vitro. Double-reciprocal plots for tetramethylene sulfoxide with (A) ethanol as substrate or (B) acetaldehyde as substrate, at four different concentrations of the inhibitor: 0 (■), 0.05 (●), 0.1 (▲), and 0.2 (+) mM.

Scheme I



very high affinity. Apparently, the enzyme can accommodate the larger alkyl substitutents in ring form better than in the acyclic form. It may be noted also that the enzyme is very active with cyclohexanol, even though it is much less active with 2-propanol.[31] Tetramethylene sulfoxide is a significantly better inhibitor than either the tri- or pentamethylene sulfoxides. Since the five-membered ring in tetramethylene sulfoxide may distort the approximately tetrahedral geometry expected for a sulfoxide, it is possible that the tetramethylene sulfoxide is a transition-state analogue, which has a geometry intermediate between an alcohol and an aldehyde.

The inhibitory potency of the amides and sulfoxides was surveyed by determining the rate of ethanol metabolism in rats given a dose of 1 mmol/kg of the compound. Figure 1 shows the results of such an experiment with tetramethylene sulfoxide, which significantly inhibits the zero-order elimination of ethanol. (The dependence of inhibition on the concentration of inhibitor will be discussed later.) Table I summarizes the results of the survey of the effects of the compounds on metabolism of ethanol in rats. The extent of inhibition of ethanol elimination is only roughly correlated (coefficient of 0.72 for 20 compounds) with the inhibition constants determined in vitro. Isovaleramide, 3,3-dimethylbutyramide, methyl hexyl sulfoxide, and tetramethylene sulfoxide were the most potent inhibitors in vitro and in vivo. One amide and one sulfoxide were chosen for further study.

The inhibition of purified horse liver alcohol dehydrogenase was studied in vitro. As shown in Figure 2, tetramethylene sulfoxide is essentially an uncompetitive inhibitor vs. varied concentrations of ethanol. On the other hand, there are slight slope effects evident in Figure 2A, and the data could also be fitted fairly well to the equation for noncompetitive inhibition. The inhibition pattern of tetramethylene sulfoxide against varied concentrations of acetaldehyde is clearly noncompetitive (Figure 2B). Since horse liver alcohol dehydrogenase essentially has an ordered Bi-Bi mechanism (which can be diagrammed as shown in Scheme I), the patterns of inhibition by tetramethylene sulfoxide are readily explained by the as-

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Table I. Survey of Amides and Sulfoxides as Inhibitors of Horse Liver Alcohol Dehydrogenase and of Ethanol Metabolism in Rats[a]

compound	in vitro, horse[b]		in vivo, rat[c]
	K_i ± SE, mM	type of inhibn	% inhibn
3-hydroxypropionamide	8.5 ± 0.9	UC	15
4-hydroxybutyramide	6.8 ± 0.4	UC	5
5-hydroxyvaleramide	4.4 ± 0.5	UC	9
6-hydroxycaproamide	1.5 ± 0.1	UC	24
2-(2-hydroxyethyl)glycolamide	5.8 ± 0.9	UC	none
2,2'-thiobis(acetamide)	36 ± 18	UC	7
2-bromo-2-methylpropionamide			5
3-chloropropionamide	5.3 ± 1.0	UC	15
heptafluorobutyramide	2.0 ± 0.3	UC	none
methoxyacetamide	0.60 ± 0.01	UC	18
nonanoamide			15
cyclopropanecarboxamide	0.79 ± 0.05	UC	29
cyclohexanecarboxamide	0.25 ± 0.04	NC	none
isobutyramide	0.22 ± 0.02	UC	36
3,3-dimethylbutyramide	0.15 ± 0.01	UC	71
isovaleramide	0.020 ± 0.001	UC	78
isobutyric acid hydrazide	2.9 ± 0.4	C	
δ-valerolactam	13 ± 7	C	
thiourea	42 ± 11	C	
thiosemicarbazide	1.9 ± 0.2	C	
ethyl methyl sulfoxide	0.50 ± 0.05	UC	16
diethyl sulfoxide	1.1 ± 0.09	UC	
methyl isobutyl sulfoxide	0.34 ± 0.03	UC	14
methyl isoamyl sulfoxide	0.43 ± 0.04	UC	19
methyl hexyl sulfoxide	0.047 ± 0.004	UC	67
methyl tetradecyl sulfoxide	0.010 ± 0.002	UC	15
trimethylene sulfoxide	0.15 ± 0.01	UC	25
tetramethylene sulfoxide	0.019 ± 0.001	UC	63
pentamethylene sulfoxide	0.48 ± 0.04	UC	21
1,4-oxathiane 4-oxide	10.6 ± 0.9	UC	
thiomorpholine *S*-oxide	120 ± 40	C	
1-thiacyclohexan-4-one 1-oxide	9.7 ± 1.3	C	
trans-1,4-dithiane 1,4-dioxide	41 ± 11	UC	
cis-1,4-dithiane 1,4-dioxide	140 ± 80	UC	
methyl phenyl sulfoxide	0.20 ± 0.01	UC	19
ethyl phenyl sulfoxide	12.5 ± 1.5	UC	
methyl benzyl sulfoxide	5.0 ± 0.5	UC	13
phenyl vinyl sulfoxide	0.5 ± 0.1	NC	

[a] The experimental procedures are described under Experimental Section. Each value is the result of one experiment. [b] The compounds were tested as inhibitors against varied concentrations of ethanol. When uncompetitive (UC) inhibition was obtained, the intercept inhibition constant (K_{ii}) is reported. In order to compare this value to the numbers reported for competitive inhibition against varied concentrations of acetaldehyde as substrate, the value of K_{ii} should be divided by 1.7 (see text). When competitive inhibition (C) was obtained, the slope inhibition constant (K_{is}) is reported. No correction is required in this case. In the two examples of noncompetitive inhibition (NC), the K_{ii} value is reported. [c] A fresh rat was used for each study. The dose of ethanol was 19.6 mmol/kg, and the dose of inhibitor was 1.0 mmol/kg. The rate of ethanol metabolism was 7.4 ± 1.0 mmol kg^{-1} h^{-1} for control animals, and the average value of r for controls was 0.83 ± 0.09 L of body water per kilogram of body weight and 0.79 ± 0.07 L/kg for animals with inhibitors.

sumption that the sulfoxide forms dead-end complexes (E–NAD–I, E–NADH–I).[32] If the sulfoxide binds only to the enzyme–NADH complex, it should produce uncompetitive inhibition against varied concentrations of ethanol or competitive inhibition against acetaldehyde as substrate, whereas noncompetitive inhibition against either substrate would occur if the inhibitor binds to both enzyme–coenzyme complexes. The kinetic constants obtained for the studies shown in Figure 2 are recorded in Table II. The slope inhibition constant (K_{is}) obtained with ethanol as the varied substrate is equivalent to the dissociation constant (K_I) for the E–NAD–I complex, and the K_{is} determined with acetaldehyde is equivalent to K_J. Thus, tetramethylene sulfoxide binds most tightly to the enzyme–NADH complex with a K_J value of 11 μM and more weakly to the enzyme–NAD complex with a K_I value of 130 μM.

Similar kinetic studies were performed with the horse liver enzyme and isovaleramide, and the relevant kinetic constants are recorded in Table II. Although the amide is essentially an uncompetitive inhibitor against ethanol and a competitive inhibitor against acetaldehyde, the data for both experiments fit noncompetitive inhibition slightly better. Thus, it appears that the amide binds most tightly to the enzyme–NADH complex and more weakly to the enzyme–NAD^+ complex, as demonstrated also by others.[14,16]

Having determined the intercept inhibition constant against varied concentrations of ethanol (K_{ii}) and the slope inhibition constant for the inhibition against varied concentrations of acetaldehyde (K_{is}), one can calculate a correction factor (K_{ii}/K_{is}) to be applied to the K_{ii} values in order to obtain the true dissociation constant (K_J). This correction factor is derived from the comparison of the equation for noncompetitive inhibition with the rate equation for the ordered Bi-Bi mechanism with dead-end inhibition and is equal to the value of $(k_5 + k_7)/k_5$.[33] Thus, the uncompetitive inhibition constants reported in Table I should be divided by 1.7, if these constants are to be compared to literature values that were determined by direct binding studies or by inhibition against varied

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Table II. Kinetic Constants for Inhibition by Tetramethylene Sulfoxide and Isovaleramide of Liver Alcohol Dehydrogenase in Vitro and in Vivo[a]

enzyme	inhibitor	varied substrate	K_m ± SE, μM	K_{ii} ± SE, μM	K_{is} ± SE, μM	type of inhibn
horse, cryst	$(CH_2)_4SO$	CH_3CH_2OH	620 ± 40	19 ± 1	130 ± 70	UC/NC
horse, cryst	$(CH_2)_4SO$	CH_3CHO	230 ± 40	71 ± 1	11 ± 2	NC
horse, cryst	amide	CH_3CH_2OH	900 ± 50	20 ± 1	160 ± 70	UC/NC
horse, cryst	amide	CH_3CHO	150 ± 30	460 ± 60	11 ± 2	NC[b]
rat, purif	$(CH_2)_4SO$	CH_3CH_2OH	350 ± 40	200 ± 20	230 ± 80	UC/NC
rat, purif	amide	CH_3CH_2OH	510 ± 40	20 ± 1		UC
rat, in vivo	$(CH_2)_4SO$	CH_3CH_2OH	2000 ± 1200[c]	340 ± 40[c]		UC
rat, in vivo	amide	CH_3CH_2OH	1500 ± 1300[c]	180 ± 30[c]		UC

[a] The procedures are described under Experimental Section. Each row of in vitro results is from one experiment with 32 points. The data for the tetramethylene sulfoxide in vivo are shown in Figure 3B, which included 26 rats. The results for isovaleramide in vivo were obtained similarly with 20 rats given 0, 0.25, 0.5, and 1.0 mmol of amide per kilogram. The standard errors are a measure of the fit of the data to the inhibition equation. [b] Although the data fit the equation for noncompetitive inhibition slightly better than the equation for competitive inhibition, the effect of the inhibitor on the slopes was much larger than the effect on the intercepts, as reflected by the 40-fold smaller value for K_{is} as compared to K_{ii}. Relatively high concentrations of acetaldehyde (0.4-2.0 mM) and isovaleramide (0-0.4 mM) were required to demonstrate an intercept effect; with lower concentrations the pattern can appear to be competitive. As discussed in the text in conjunction with Scheme I, the noncompetitive pattern indicates that the amide binds tightly to the enzyme-NADH complex and more weakly to the enzyme-NAD^+ complex. [c] In micromoles per kilogram rather than micromoles per liter; the r value was about 0.8 L/kg.

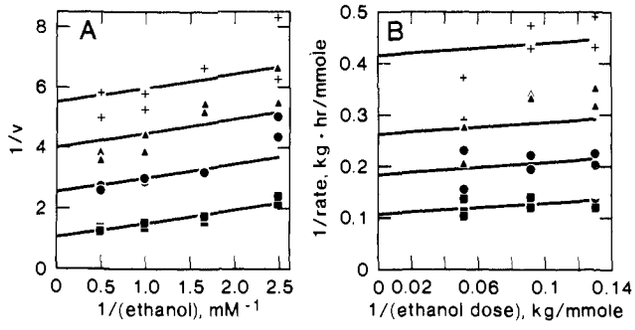

Figure 3. Inhibition by tetramethylene sulfoxide of rat liver alcohol dehydrogenase in vitro and in vivo. (A) Inhibition of purified rat liver enzyme. The conditions are described under Experimental Section. The concentrations of inhibitor were as follows: 0 (■), 0.2 (●), 0.4 (▲), and 0.6 (+) mM. (B) Inhibition of ethanol metabolism. Male Sprague–Dawley rats were given ip injections of tetramethylene sulfoxide (0.1 M) at doses of 0.0 (■), 0.25 (●), 0.5 (▲), or 1.0 (+) mmol/kg of body weight. Ten minutes later, ethanol (3.26 M) was injected ip at doses of 7.69, 10.9, or 19.6 mmol/kg of body weight. Blood samples were taken at intervals timed from the ethanol injection and analyzed for ethanol concentration by gas chromatography.

concentrations of acetaldehyde.

In order to establish a basis for comparing the in vitro studies with in vivo studies, liver alcohol dehydrogenase was purified from rat liver, and the kinetics of inhibition were studied under conditions that are thought to closely resumble the conditions found in vivo. As shown in Figure 3A, tetramethylene sulfoxide is an uncompetitive inhibitor against varied concentrations of ethanol. There may be a slight slope effect, however, due to the formation of the enzyme–NAD–tetramethylene sulfoxide complex. The magnitudes of the kinetic constants are recorded in Table II. Inhibition of the purified rat liver enzyme by isovaleramide was also uncompetitive (Table II).

The type of inhibition of ethanol metabolism in rats was then determined by measuring the rate of ethanol metabolism with varied doses of ethanol and of the inhibitor. An example of the primary data obtained is shown in Figure 1. The results of these studies with tetramethylene sulfoxide are shown in Figure 3B. It appears that tetramethylene sulfoxide is an uncompetitive inhibitor of ethanol metabolism. The kinetic constants for the sulfoxide and isovaleramide are recorded in Table II.

The similarities of the inhibition of the rat liver enzyme and of the metabolism of ethanol in rats suggest that liver alcohol dehydrogenase is the predominant rate-determining factor in the metabolism of ethanol and therefore a good target for inhibitory drugs.[2,34,35] The kinetic constants in vitro and in vivo are similar, although the Michaelis constant for ethanol in vivo could not be accurately determined because at low concentrations of ethanol the equilibration of ethanol between the liver and blood is not sufficiently rapid.[36] The uncompetitive inhibition constants for tetramethylene sulfoxide are very similar in vitro and in vivo. In contrast, the inhibition constant for isovaleramide in vivo is nine times larger than the constant for inhibition in vitro. This difference is not due to the different units used for the inhibition constants, since the animal is about 80% water. On the other hand, the larger inhibition constants for the amide in vivo can indicate that the amide does not freely equilibrate with the cytoplasm in the liver. If the amide were preferentially absorbed in extrahepatic tissues or to membranes, its free concentration in the liver cytosol would be reduced, and the dose required to give a concentration comparable to that seen in vitro must be higher. Such an effect was observed for long-chain amides or N-substituted formamides.[13] Nevertheless, it is clear that the amide is a very effective inhibitor of ethanol metabolism.

Metabolism of Inhibitors. The studies on the inhibition of ethanol metabolism were essentially initial velocity studies, in which the rate of metabolism of ethanol is measured before the inhibitor itself is metabolized or eliminated from the animal. In order to determine how fast the inhibitors are eliminated, two different doses of the inhibitors were given to rats, and the concentration of the inhibitors in the blood was determined by gas chromatography as described under Experimental Section. It appeared that tetramethylene sulfoxide was eliminated with first-order kinetics with a half-time of 4.5 ± 0.5 h. In contrast, the elimination of isovaleramide or of isobutyramide appeared to occur by a zero-order process, with a rate of 1.1 ± 0.2 mmol kg^{-1} h^{-1}, at concentrations above 2 mM in the blood. (At lower concentrations of the amides,

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the kinetic order of elimination could change, but our methods were not sensitive enough for further studies.) With all three inhibitors, the presence of a moderate dose of ethanol did not affect the elimination kinetics. When the inhibition of ethanol metabolism is being studied, the concentration of inhibitor is declining with time, and one could expect a downward curvature in the ethanol elimination curve. Indeed, this is observed when very high doses of ethanol (65 mmol kg^{-1}) are given and the time course is long, and such data should be analyzed to extract the initial rate of ethanol elimination. With the lower concentrations of ethanol used in the studies reported here, the elimination of ethanol is linear with time (Figure 1), and the initial rate (before much inhibitor is eliminated) is readily determined.

Sulfones or sulfides are possible metabolites of sulfoxides. The major plasma metabolite of tolmesoxide (4,5-dimethoxy-2-methylphenyl methyl sulfoxide, a peripheral vasodilator) is the sulfone.[27] The immunosuppressive agent oxisuran and mesoridazine, the active sulfoxide metabolite of the antipsychotic agent thioridazine, are converted to sulfones.[26] Dimethyl sulfoxide can be reduced to dimethyl sulfide in the cat.[37] Carbophenothion sulfoxide is reduced to carbophenothion in the living rat and by an in vitro system containing rat liver enzyme, reduced nicotinamide adenine dinucleotide, and flavin adenine dinucleotide.[38] Similarly, enzymes in the cestode *Moniezia expansa*, and nematode *Ascaris suum*, and mouse liver have been reported to reduce sulfoxides to thioethers in the absence of oxygen.[39] At this time, we do not know what metabolites are formed from tetramethylene sulfoxide, but the sulfone or sulfide derivatives would probably be inactive as inhibitors of alcohol dehydrogenase. We found that tetramethylene sulfone did not detectably inhibit horse alcohol dehydrogenase at 10 mM concentrations.

Conclusion

These studies show that amides and sulfoxides are potent inhibitors of ethanol metabolism in rats. Since these compounds give essentially uncompetitive inhibition in vivo, they can be effective inhibitors of metabolism, since the extent of inhibition is not reduced as the concentration of alcohol is increased above levels that saturate the alcohol dehydrogenase. For instance, at more than 10 mM ethanol, the relative rate of ethanol metabolism can be expressed by the equation (derived from the equation for uncompetitive inhibition) $v/V = K_{ii}/(K_{ii} + I)$. In contrast, a competitive inhibitor, such as pyrazole, is less effective as the concentration of the alcohol is increased. Nevertheless, these new compounds are not as potent as 4-methylpyrazole, which has a competitive inhibition constant in vivo of about 1 μmol/kg (unpublished experiments). Further studies are required with the sulfoxides and amides in order to find compounds that would bind even more tightly to the enzyme and have suitable pharmacological properties. Tetramethylene sulfoxide has an LD_{50} of 3.5 g/kg[40] and apparently is not toxic at the doses used in our studies. Other sulfoxides, such as ethyl methyl sulfoxides, diisopropyl sulfoxide, and diisoamyl sulfoxide, have LD_{50} values of 3.5, 2.75, and 0.5 g/kg, respectively. Similarly, the amides were not toxic at the doses required for inhibition, and in the case of *n*-butyramide, the LD_{50} is 83 times higher than the dose required to inhibit ethanol oxidation by 50%.[18]

Experimental Section

Materials and Methods. Unless indicated otherwise, the amides, sulfoxides, and starting materials were obtained from Aldrich Chemical Co. Thiourea came from Fischer Scientific Co., and crystalline horse liver alcohol dehydrogenase and coenzyme were purchased from Boehringer Mannheim. NMR spectra were obtained at 60 MHz on a Varian 360L spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standard. Chemical shifts are reported in δ values (parts per million) from Me_4Si. Elemental analyses for C, H, N, and S were carried out by Galbraith Laboratories. Melting points and boiling points are uncorrected.

Chemistry. Organic sulfoxides have attracted increasing attention in recent years as intermediates in various synthetic transformations and as potential medicinal agents. Therefore, a number of synthetic procedures are now available for the oxidation of sulfides to sulfoxides.[41] However, only a few of them produce selective oxidation and good yields under mild conditions with simple reagents. The avoidance of sulfone formation has been of particular interest for our studies with alcohol dehydrogenase, since sulfones bind very weakly to the enzyme. We found that the oxidation with sodium metaperiodate according to the procedure of Leonard and Johnson[42] was specific, convenient, and avoided overoxidation.

All of the compounds used in these studies were characterized by NMR spectra. In the case of methyl-substituted sulfoxides, the methyl protons in CH_3SO absorbed downfield (δ 2.4–2.6) as compared to the CH_3S protons (δ 2.02–2.06). Many of the sulfoxides were hygroscopic and gave relatively low sulfur analysis.

Isobutyl Methyl Sulfoxide. Sodium metaperiodate (11.2 g, 0.0525 mol) was dissolved in 110 mL of water and cooled in an ice bath. Isobutyl methyl sulfide (5.0 g, 0.05 mol) was added and the reaction mixture was stirred at 0 °C overnight. The $NaIO_3$, which precipitated during the reaction, was removed by filtration, and the filtrate was extracted with two 50-mL portions of $CHCl_3$. The extract was dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. Vacuum distillation yielded the main fraction at bp 44–45 °C (0.35–0.4 mm): yield 4.0 g (70%); NMR ($CDCl_3$) δ 1.13 (d, 6 H), 1.76–2.36 (m, 1 H), 2.59 (s, 3 H), 2.5–2.8 (m, 2 H). The compound was hygroscopic. Anal. ($C_5H_{12}SO \cdot 0.6H_2O$) C, H, S.

The following compounds were prepared similarly. Isoamyl methyl sulfoxide: bp 62–63 °C (0.4 mm); NMR ($CDCl_3$) δ 0.94 (d, 3 H), 1.36–1.83 (m, CH_2, CH), 2.53 (s, 3 H), 2.63–2.86 (m, 2 H). Anal. ($C_6H_{14}SO \cdot 0.7H_2O$) C, H; S: calcd, 21.83; found, 21.21. Hexyl methyl sulfoxide: bp 84–85 °C (0.55 mm); NMR ($CDCl_3$) δ 0.89 (t, 3 H), 1.19–1.56 (m, 8 H), 2.56 (s, 3 H) 2.39–2.86 (m, 2 H). Anal. ($C_7H_{16}SO \cdot 0.3H_2O$) C, H; S: calcd, 20.86; found, 19.53. Diethyl sulfoxide: bp 40–41 °C (0.4 mm) [lit.[42] bp 45–47 °C (0.15 mm)]. Trimethylene sulfoxide: bp 43–44 °C (0.36 mm) [lit.[43] bp 91–92 °C (14 mm)]. Pentamethylene sulfoxide: mp 60–62 °C (lit.[44] mp 60–61.5 °C). 1,4-Oxathiane 4-oxide: mp 44–45 °C (lit.[42] mp 46–47.2 °C. 1-Thiocyclohexan-4-one 1-oxide: mp 108–110 °C (lit.[42] mp 109–110 °C). *trans*-1,4-Dithiane 1,4-dioxide: mp 263–264 °C dec (lit.[45] mp 263 °C dec). *cis*-1,4-Dithiane 1,4-dioxide: mp 235–250 °C dec (lit.[45] mp 235–250 °C dec). Methyl phenyl sulfoxide: bp 95 °C (0.53 mm) [lit.[42] bp 83–85 °C (0.1 mm)].

Thiomorpholine *S*-Oxide. To a solution of 22.5 g (0.105 mol) of $NaIO_4$ in 220 mL of water cooled in an ice bath was added 10.0 g (0.05 mol) of thiomorpholine, and the reaction mixture was stirred overnight at 0 °C. The precipitated $NaIO_3$ was removed, and the filtrate was concentrated on a rotary evaporator to a thick

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syrup. On addition of CH_3OH, more white solid ($NaIO_3$) separated, which was removed, and the solvent was evaporated, leaving behind an oily liquid. This was distilled under reduced pressure: yield 3.5 g (30%); bp 120–122 °C (0.6 mm) with slight decomposition; NMR ($CDCl_3$) δ 2.07 (s, 1 H), 2.66–3.1 (m, 4 H), 3.26–3.76 (m, 4 H). Anal. (C_4H_9NSO) C, H, N; S: calcd, 26.89; found, 25.77.

3-Hydroxypropionamide. To 10.5 g (0.146 mol) of 3-hydroxypropionitrile in 15 mL of water were added, with caution, 100 mL of 10% H_2O_2 and then 75 mL of 10% Na_2CO_3. A vigorous reaction with the evolution of gas took place. The reaction mixture was kept at 0 °C for 2 days. The solvent was removed by evaporation, and the viscous liquid obtained was distilled under reduced pressure: bp 152 °C (1.5 mm); NMR (D_2O) δ 2.46 (t, 2 H), 3. 81 (t, 2 H). Anal. ($C_3H_7NO_2$) C, H, N.

4-Hydroxybutyramide. Butyrolactone (44 g, 0.51 mol) was treated with 50 mL of concentrated NH_3 for 48 h. The solvent was removed, and the residue was crystallized from ethanol/acetone: mp 39–44 °C; NMR (D_2O) δ 1.73 (m, 2 H), 2.3 (t, 2 H), 3.63 (t, 2 H). Anal. ($C_4H_9NO_2$) C, H, N.

6-Hydroxycaproamide. This was prepared from ϵ-caprolactone and concentrated NH_3 by the above procedure: mp 40–43 °C; bp 185 °C (2 mm); NMR (D_2O) δ 1.46 (m, 6 H), 2.26 (t, 2 H), 3.59 (t, 2 H). Anal. ($C_6H_{13}NO_2$) C, H, N.

Isovaleramide and 3,3-dimethylbutyramide were prepared from isovaleryl chloride and *tert*-butylacetyl chloride by reaction with concentrated NH_3.[46] 3,3-Dimethylbutyramide: mp 132–133 °C (lit.[47] mp 132 °C); NMR ($CDCl_3$) δ 1.05 (s, 9 H), 2.1 (s, 2 H). Anal. ($C_6H_{13}NO$) C, H, N. Isovaleramide: mp 136 °C (lit.[47] mp 137 °C).

Enzymology. Crystallized horse liver alcohol dehydrogenase was freed from endogenous ethanol by filtration through a column of Sephadex G-50. Inhibition studies were carried out with varied concentrations of ethanol (0.4–2 mM) at 1 mM NAD^+ in 46 mM sodium phosphate buffer, pH 7, at 25 °C. For the reverse reaction, the kinetics were studied with freshly distilled acetaldehyde at varied concentrations (1–5 mM) and 0.1 mM NADH. In each experiment, at least three different concentrations of inhibitor were tested and 32 initial velocities were determined (as in Figures 2 and 3A). A Cary 118C spectrophotometer was used to record the initial velocities of the change in absorbance at 340 nm. The inhibition constants were computed by using Cleland's programs.[48] Data for noncompetitive inhibition were fitted to the equation $v = VS/[K_m(1 + I/K_{is}) + S(1 + I/K_{ii})]$, whereas the equation for uncompetitive inhibition has no K_{is} term, and competitive inhibition gives no K_{ii} term. The equation giving the lowest standard errors and residual variance was concluded to give the best fit. Rat liver alcohol dehydrogenase was partially purified by precipitation with $(NH_4)_2SO_4$ (35–75% saturation), passage through DEAE-cellulose, and chromatography on Sephadex G-100.[49] Inhibition studies were performed as with the horse liver enzyme, except that the buffer was 83 mM potassium phosphate, pH 7.3, and 40 mM KCl, total $\mu = 0.25$, and the temperature was 37.8 °C. The concentration of NAD^+ was 0.5 mM. These conditions are thought to resemble those found in vivo.[34] The concentrations of ethanol were 0.4, 0.6, 1, and 2 mM, and the inhibitors were varied between 0 and 0.6 mM.

Biological Evaluation. The effects of the potential inhibitors on ethanol metabolism in rats were studied by the following procedure. Fed, male Sprague–Dawley rats (175–280 g) were administered intraperitoneal injections of the inhibitor to be tested in a dose of 1.0 mmol/kg of body weight (unless otherwise indicated). The solutions of amides were 0.46 M or lower in physiological saline. Sulfoxides were 0.1 M in physiological saline. Nonanoamide and methyl tetradecyl sulfoxide were suspended at 0.1 mol/L in 2% sodium carboxymethylcellulose. Ten minutes later, ethanol (3.26 M in physiological saline) was administered by intraperitoneal injection at 19.6 mmol/kg of body weight. Blood samples (10 μL) were drawn from the tail at intervals timed from the ethanol injection. The blood was expelled into a 0.5-mL polypropylene centrifuge tube containing 50 μL of 4.0 M *tert*-butyl alcohol and stirred. To this was added 20 μL of 0.14 M $Ba(OH)_2$ and 20 μL of 0.14 M $ZnSO_4 \cdot 7H_2O$.[50] The tube was capped, and the contents were mixed and centrifuged for 1 min at 10000*g* and 4 °C. A sample of the supernate (0.5 μL) was injected into a gas chromatograph (Varian 3740) equipped with flash injectors and either a 80–100 mesh Porapak S or a 80–100 mesh Chromosorb 102 column (6 ft × 2 mm i.d., glass, Supelco) and developed at 150 °C with N_2 carrier gas flow of 30 mL/min. The injector temperature was 180 °C, and the detector temperature was at 190 °C. Ethanol eluted at 2.8–3.0 min, and the internal standard, *tert*-butyl alcohol, eluted at 6.7–7.0 min. Ethanol was also analyzed with a 60/80 Carbopack B/5% Carbowax 20M column (6 ft × 2 mm i.d., glass, Supelco) developed at 80 °C with N_2 carrier gas flow of 20 mL/min (injector at 140 °C, detector at 150 °C) from which ethanol eluted at 2.2–2.3 min and *tert*-butyl alcohol at 4.0–4.5 min. The concentration of ethanol was calculated with a Hewlett-Packard 3388A integrator. The rate of ethanol metabolism was calculated from the blood ethanol elimination curves by Widmark's method.[51]

In order to study the rate of elimination of tetramethylene sulfoxide, fed, male Sprague–Dawley rats (240–290 g) were given injections (ip) of tetramethylene sulfoxide at 10 mmol/kg of body weight, or the sulfoxide at 10 mmol/kg followed 10 min later by ethanol at 19.6 mmol/kg, or the sulfoxide at 5 mmol/kg. The sulfoxide was diluted to a concentration of 1.0 M with physiological saline, and the ethanol was diluted to 3.26 M for injection. Blood samples were taken at intervals timed from the tetramethylene sulfoxide injection, and the concentration of sulfoxide was measured by gas chromatography on a 3 ft × 2 mm i.d. glass column packed with 0.8% THEED on 80/100 Carbopack C (Supelco) operated at 125 °C (detector 180 °C, injector 170 °C) and 40 mL/min N_2 carrier flow. Tetramethylene sulfoxide eluted at 10.7 min, and the internal standard, 1,3-propanediol, eluted at 3.2 min. The elimination of isovaleramide was studied similarly, except that the concentration of the solution of amide injected into the rats was 0.67 M. Isovaleramide eluted in the range of 39–50 min, and the internal standard, 1,3-propanediol, eluted at 5–8 min. The metabolism of isobutyramide was studied similarly, except that a 1 M solution of the amide was injected, and the GC column was operated at 115 °C. Isobutyramide eluted at 7.5 min, and the internal standard, ethylene glycol, eluted at 1.8 min.

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Registry No. EC 1.1.1.1, 9031-72-5; ethanol, 64-17-5; tetramethylene sulfoxide, 1600-44-8; trimethylene sulfoxide, 13153-11-2; pentamethylene sulfoxide, 4988-34-5; isovaleramide, 541-46-8; 3-hydroxypropionamide, 2651-43-6; 4-hydroxybutyramide, 927-60-6; 5-hydroxyvaleramide, 29686-12-2; 6-hydroxycaproamide, 4547-52-8; 2′-(2-hydroxyethyl)glycolamide, 17370-17-1; 2,2′-thiobis(acetamide), 14618-65-6; 2-bromo-2-methylpropionamide, 7462-74-0; 3-chloropropionamide, 5875-24-1; heptafluorobutyramide, 662-50-0; methoxyacetamide, 16332-06-2; nonanamide, 1120-07-6; cyclopropanecarboxamide, 6228-73-5; cyclohexanecarboxamide, 1122-56-1; isobutyramide, 563-83-7; 3,3-dimethylbutyramide, 926-04-5; isobutyric acid hydrazide, 3619-17-8; δ-valerolactam, 675-20-7; thiourea, 62-56-6; thiosemicarbazide, 79-19-6; ethyl methyl sulfoxide, 1669-98-3; diethyl sulfoxide, 70-29-1; methyl isobutyl sulfoxide, 56817-93-7; methyl isoamyl sulfoxide, 55860-10-1; methyl hexyl sulfoxide, 1561-73-5; methyl tetradecyl sulfoxide, 3079-31-0; 1,4-oxathiane 4-oxide, 109-03-5; thiomorpholine *S*-oxide, 39213-13-3; 1-thiacyclohexan-4-one 1-oxide, 17396-36-0; *trans*-1,4-dithiane 1,4-dioxide, 10348-98-8; *cis*-1,4-dithiane 1,4-dioxide, 10348-97-7; methyl phenyl sulfoxide,

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1193-82-4; ethyl phenyl sulfoxide, 4170-80-3; methyl benzyl sulfoxide, 824-86-2; phenyl vinyl sulfoxide, 20451-53-0; isoamyl methyl sulfide, 13286-90-3; hexyl methyl sulfide, 20291-60-5; diethyl sulfide, 352-93-2; trimethylene sulfide, 287-27-4; pentamethylene sulfide, 1613-51-0; 1,4-oxathiane, 15980-15-1; 1-thiacyclohexan-4-one, 1072-72-6; 1,4-dithiane, 505-29-3; methyl phenyl sulfide, 100-68-5; isobutyl methyl sulfide, 5008-69-5; thiomorpholine, 123-90-0; 3-hydroxypropionitrile, 109-78-4; butyrolactone, 96-48-0; ϵ-caprolactone, 502-44-3; isovaleryl chloride, 108-12-3; *tert*-butylacetyl chloride, 7065-46-5.

Notes

Synthesis of Thiazole-4-carboxamide Adenine Dinucleotide. A Powerful Inhibitor of IMP Dehydrogenase

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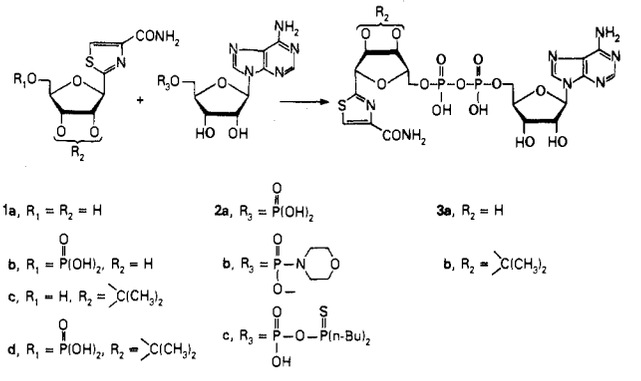
The chemical synthesis of thiazole-4-carboxamide adenine dinucleotide (TAD), previously identified as the active anabolite of the oncolytic 2-β-D-ribofuranosylthiazole-4-carboxamide (TR), has been achieved by three different approaches: (1) incubation of adenosine 5'-monophosphate (AMP) and 2-β-D-ribofuranosylthiazole-4-carboxamide 5'-monophosphate (TRMP) with excess DCC in aqueous pyridine, (2) reaction of adenosine 5'-phosphoromorpholidate with TRMP in pyridine, and (3) reaction of adenosine-5'-phosphoric di-*n*-butylphosphinothioic anhydride with TRMP in the presence of $AgNO_3$. While the first approach produced only traces of TAD, the last two afforded 31 and 16% yields, respectively, of isolated TAD. The synthetic material was indistinguishable from biosynthesized TAD as judged by its HPLC behavior, NMR, UV and mass spectra, enzymatic resistance to alkaline phosphatase and susceptibility to venom phosphodiesterase, IMP dehydrogenase inhibitory activity, and cytotoxicity. TAD and TR were equally effective against murine P388 leukemia when employed at equimolar doses.

2-β-D-Ribofuranosylthiazole-4-carboxamide (TR, **1a**, Scheme I) shows remarkable activity against several murine tumors, including the Lewis lung carcinoma.[1] In a previous communication from this laboratory, it was reported that this novel C-nucleoside was anabolized to an analogue of NAD that was responsible for its potent inhibition of IMP dehydrogenase (IMPD) and consequent depression of guanine nucleotides.[2] The presence of a phosphodiester linkage in the structure of this anabolite was first surmised from its enzymatic resistance to alkaline phosphatase and susceptibility to venom phosphodiesterase. Subsequently, the structure was completely elucidated by ^1H NMR and mass spectral studies.[2] These results were consistent with an analogue of NAD (**3a**) in which the nicotinamide portion had been replaced by thiazole-4-carboxamide. This anabolite, appropriately abbreviated TAD, became an interesting target for chemical synthesis. Our initially reported biochemical synthesis from ATP and the 5'-monophosphate of TR (**1b**, TRMP) in the presence of NAD pyrophosphorylase proved unwieldy for scale up.[2]

The present work discusses three different approaches to the chemical synthesis of TAD. Initially, TRMP (**1b**), synthesized by the modified procedure of Yoshikawa,[3,4] and AMP (**2a**) were reacted in the presence of excess dicyclohexylcarbodiimide (DCC) in aqueous pyridine. This procedure was identical with the one originally used for the synthesis of NAD;[5] however, when it was used only very small yields of TAD were achieved. An improvement over the first method was achieved, then, by selectively activating one nucleotide before the coupling reaction. To this end, AMP (**2a**) was converted to its phosphoromorpholidate derivative **2b**[6,7] and reacted with TRMP (as the tri-*n*-octylamine salt) in pyridine for 2 h at 60 °C. This procedure afforded pure TAD (**3a**) in 9% yield. Lowering the temperature of the reaction to room temperature and stirring the mixture for 6 days increased the yield of TAD to just 12%. Finally, an intermediate temperature of 45 °C produced the highest yields of TAD (31%) after 5 h.

Scheme I



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