UV analysis at 270 nm of the acetates **4f** dissolved in 10 mL of methanol after TLC separation in system B yielded cis/trans ratios of 75.1:24.9 (**3c** as starting material) and 64.7:35.3 (**3d** as starting material), respectively.

Acid Treatment of 4c-trans. **4c-trans** (0.4 mg) was dissolved in 0.05 N HCl (1 mL) and stirred for 25 min at room temperature. After neutralization with $KHCO_3$, an aliquot was chromatographed in acetone:chloroform (2:1) together with reference compounds; **4c-trans**, R_f 0.11; **4c-cis**, R_f 0.35. No trace of the cis compound was detected. It should be noted that the TLC system used gives a lower R_f for the trans isomer **4c** while the trans compound in the acetate series **4e** has the higher R_f value. An analogous reversal of chromatographic mobilities of a related series of compounds was noted by Bachur and co-workers.[30]

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Registry No. **1b**, 4055-40-7; **1c**, 50-07-7; **1d**, 18209-14-8; **3a**, 26909-37-5; **3b**, 26909-43-3; **3c**, 61070-23-3; **3d**, 92762-77-1; **4d-trans**, 92842-90-5; **4d-cis**, 92842-91-6; **4e-trans**, 92762-78-2; **4e-cis**, 92934-66-2; **4f-trans**, 92842-92-7; **4f-cis**, 92842-93-8; mitomycin, 1404-00-8.

Inhibition of Liver Alcohol Dehydrogenase and Ethanol Metabolism by 3-Substituted Thiolane 1-Oxides

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3-Substituted thiolane 1-oxides (methyl, *n*-butyl, *n*-hexyl, and phenyl) were prepared and tested as inhibitors of horse, monkey, and rat liver alcohol dehydrogenases and of ethanol metabolism in rats. These compounds inhibit alcohol oxidation in an uncompetitive manner with respect to ethanol as a varied substrate. Lengthening the alkyl substituent increased the inhibitory potency because of tighter binding in the hydrophobic substrate binding pocket of the alcohol dehydrogenases. Thus, the 3-hexyl derivative was the most potent inhibitor of the purified rat liver alcohol dehydrogenase, with a K_{ii} value of 0.13 μM. The 3-butyl derivative was the best inhibitor of ethanol metabolism in rats, with a K_{ii} value of 11 μmol/kg. The acute toxicity in mice of the butyl derivative was 1.4 mmol/kg. Since high concentrations of alcohol do not prevent the inhibitory effects of these compounds, they may be particularly useful for preventing poisoning by methanol or ethylene glycol.

Alcohols, such as methanol, ethanol, and ethylene glycol, are metabolized in man primarily through liver alcohol dehydrogenase.[1] At least in rats, the enzyme activity is a major rate-limiting factor in ethanol metabolism.[2-4] Thus, specific inhibitors of the dehydrogenase would be useful for studying the physiological role of the enzyme and perhaps for therapeutically preventing poisoning by methanol[5,6] and ethylene glycol.[7,8] These compounds become more toxic when they are oxidized to the corresponding acids.[7,9] The utility of 4-methylpyrazole as an inhibitor of alcohol dehydrogenase and of methanol and ethylene glycol metabolism has been demonstrated in monkeys,[6,10] but it is a competitive inhibitor of alcohol dehydrogenase and may not be the most efficacious compound to use in man.[11]

We prepared and evaluated a series of sulfoxides as inhibitors of alcohol dehydrogenase in vitro and in vivo and found that thiolane 1-oxide (tetramethylene sulfoxide) was an exceptionally potent inhibitor.[12] This compound is an uncompetitive inhibitor of ethanol metabolism in rats, which is advantageous as compared to competitive inhibitors in that the inhibition is not prevented when the concentration of alcohol is increased to levels that saturate the alcohol dehydrogenase. Since the active site of horse liver alcohol dehydrogenase has a large hydrophobic pocket,[13,14] alkyl or aryl substitutents at the 3-position of the five-membered ring in thiolane 1-oxide should enhance the inhibitory potency.

Biological Results and Discussion

Table I shows that increasing the size of the alkyl substituent increases the inhibitory potency of the thiolane

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Table I. Kinetic Constants for Inhibition by 3-Substituted Thiolane 1-Oxides of Liver Alcohol Dehydrogenase in Vitro and in Vivo

no.	R	liver alcohol dehydrogenase:[a] K_{ii} ± SE, μM horse	monkey	rat	inhibn of ethanol elimination in rats:[b] K_{ii} ± SE, μmol/kg	toxicity in mice:[c] LD_{50}, mmol/kg
7a	H	19 ± 1	1600 ± 100	130 ± 8	340 ± 40[d] (26)	45 (44–47) (22)
7b	CH_3	7.5 ± 0.3	460 ± 50	19 ± 2	64 ± 9 (8)	1.3 (1.2–1.4) (39)
7c	C_4H_9	0.63 ± 0.04	2.3 ± 0.2	1.1 ± 0.1	11 ± 2 (10)	1.4 (1.2–1.5) (23)
7d	C_6H_{13}	0.19 ± 0.02	0.35 ± 0.02	0.13 ± 0.02	65 ± 7 (9)	0.77 (0.6–1.4) (24)
7e	C_6H_5	0.36 ± 0.04	1.5 ± 0.1	1.1 ± 0.1	87 ± 37 (6)	4.0 (3.5–4.0) (10)

[a] The procedures are described in the Experimental Section. The intercept inhibition constants (K_{ii}) against varied concentrations of ethanol as substrate were determined with partially purified monkey or rat enzymes under approximately physiological conditions, pH 7.3 and 37 °C, whereas the crystallized horse enzyme was tested at pH 7 and 25 °C. The standard errors are a measure of fit of the data to the inhibition equation. Each in vitro result is from one or two experiments with 32 points each. With ethanol as the varied substrate, the average K_m values were 760 ± 80 μM for horse liver, 120 ± 40 μM for monkey liver; and 860 ± 40 μM for rat liver alcohol dehydrogenase. [b] The K_{ii} was determined with varied concentrations of inhibitor and ethanol by determination of blood alcohol concentrations. The numbers of animals used are given in parentheses. [c] 95% confidence limits and number of animals used are in parentheses. [d] Reference 12.

1-oxides as inhibitors of horse, monkey, and rat liver alcohol dehydrogenases. In all cases, the sulfoxides were uncompetitive inhibitors with respect to varied concentrations of ethanol, and the K_{ii} values are related to the dissociation constants for the enzyme–NADH–sulfoxide complex.[12] The magnitudes of the inhibition constants differ among the species, apparently because of slightly different substrate-binding-site structures. Within each species of enzyme, additional methylene units improve the inhibitory potency, as has been observed for other inhibitors of the enzyme,[16-18] but not with the same incremental improvements.

Addition of 3-substituents also improves the effectiveness of the compound for inhibiting ethanol metabolism in rats (Figure 1). As described recently, elimination of ethanol cannot be completely inhibited even with saturating levels of the inhibitors, since some alcohol is eliminated by excretion or other metabolic pathways.[4] Nevertheless, if the data in Figure 1 are analyzed on the assumption that ethanol oxidation by alcohol dehydrogenase accounts for 85% of the rate of elimination, the data can be explained by a simple equation for uncompetitive inhibition. The inhibition constants are summarized in Table I. These results show that the 3-butyl derivative is the most potent inhibitor, with shorter (methyl) or longer (hexyl) substituents being less effective. A similar effect was observed in studies on the inhibition by a series of 4-substituted pyrazoles of ethanol oxidation by isolated rat hepatocytes and for inhibition by a series of amides and alkylformamides of ethanol metabolism in rats.[17,19] Apparently, the longer alkyl chains bind to membranes or dissolve in adipose tissue so that the concentration of the inhibitor in the aqueous phase is less than would be expected from the dose of inhibitor given. It may be noted also that the inhibition constants for the in vivo studies are always larger than the inhibition constant determined for the isolated rat liver alcohol dehydrogenase. This difference is not due to the different units being used (moles/liter in vitro; moles/kilogram in vivo) since the animal is about 80% water as measured from the distribution of ethanol.[20] Distribution or metabolism of the inhibitor probably reduce the effective concentration of free inhibitor.

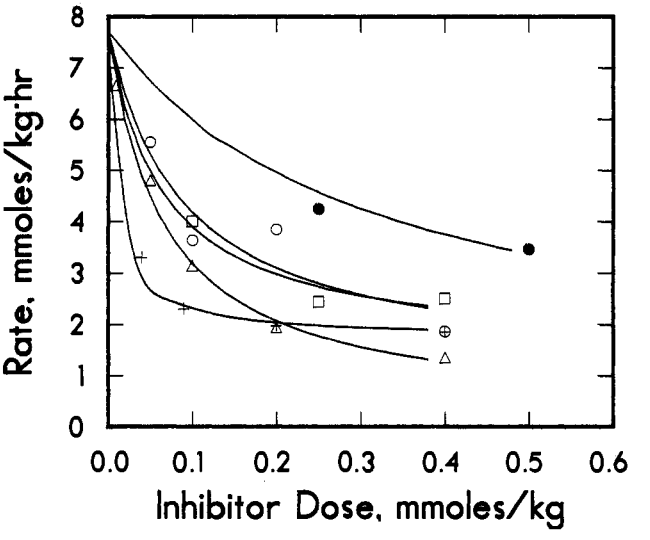

Figure 1. Inhibition of ethanol elimination by 3-substituted thiolane 1-oxides in rats. The elimination of a dose of ethanol of 19.6 mmol/kg of body weight was determined after the rats were given the indicated doses of thiolane 1-oxides: 3-H (●), 3-methyl (□), 3-butyl (+), 3-hexyl (△); 3-phenyl (○). Each set of data was fitted with a nonlinear least-squares program to the equation $v = V_L + V_m/(1 + I/K_{ii})$, where V_L represents the rate of elimination that is not inhibitable and the other term represents the rate of metabolism reduced by the presence of the uncompetitive inhibitor on the assumption that the alcohol dehydrogenase is saturated with ethanol. The value of V_L was 1.2 ± 0.6 mmol/kg·h.

The acute toxicities of the compounds in mice are summarized in Table I. These results confirm the reported toxicity of thiolane 1-oxide and show that substituents in the 3-position increase toxicity by at least an order of magnitude. Comparison of the K_{ii} for inhibition of ethanol metabolism with the LD_{50} suggests again that the 3-butyl derivative probably would be the best compound for inhibiting metabolism of alcohols.

We should emphasize that these uncompetitive inhibitors offer an important advantage over competitive inhibitors. The equation for uncompetitive inhibition reduces to $v/V = K_{ii}/(K_{ii} + I)$ at saturating levels of the alcohol. In contrast, with a competitive inhibitor, such as pyrazole, the equation reduces to $v/V = K_{is}A/(K_{is}A + K_mI)$, where I and A are concentrations of inhibitor and alcohol. Thus, the doses required to achieve the desired inhibition of alcohol metabolism can be calculated simply for the uncompetitive inhibitors.

We think that these compounds should be useful for studies on the metabolism of various alcohols and for

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Table II. Physical and Spectral Properties of Compounds 3-7

No.	yield, %	bp/(mp), °C	formula[a]	NMR ($CDCl_3$), δ	IR ν_{max}, cm^{-1} (liquid film)
3c	49	(81-82)[b]	$C_8H_{14}O_4$	0.86 (t, 3 H), 1.0-1.66 (m, 6 H), 2.33-3.0 (m, 3 H)	3077-2632 (bonded OH in COOH), 1704 (C=O)[c]
3d	70	(85-86)	$C_{10}H_{18}O_4$	0.86 (t, 3 H), 1.0-1.39 (m, 10 H), 2.33-2.96 (m, 3 H)	3077-2632 (bonded OH in COOH), 1704 (C=O)[c]
4c	83	88.5-90/0.6 mm	$C_{12}H_{22}O$	0.86 (t, 3 H), 1.05-1.71 (m, 12 H), 2.04-3.0 (m, 3 H), 4.15 (q, 4 H)	1724 (C=O)
4d	88	107-108/0.8 mm[d]	$C_{14}H_{26}O_4$	0.86 (t, 3 H), 1.03-1.69 (m, 16 H), 2.0-2.86 (m, 3 H), 4.06 (q, 4 H)[f]	1739 (C=O)
4e	87	126-128/0.95 mm[e]	$C_{14}H_{18}O_4$	1.13 (t, 6 H), 2.53 (d, 2 H), 3.14 (d, 1 H), 4.1 (q, OCH_2), 7.29 (s, aromatic protons)	1724 (C=O)
5c	82	112-114/0.5 mm	$C_8H_{18}O_2$	0.86 (t, 3 H), 1.26 (s, 6 H), 1.39-1.73 (m, 3 H), 3.36-3.79 (m, OCH_2), 4.36 (s, OH)	3279 (OH)
5d	83	113-117/0.4 mm	$C_{10}H_{22}O_2$	0.87 (t, 3 H), 1.05-1.77 (m, 12 H), 1.77-2.07 (m, 1 H), 3.08-3.84 (br, OCH_2) 5.01 (s, OH)[f]	3279 (OH)
5e	69	(65-66)	$C_{10}H_{14}O_2$	1.69-2.13 (m, 2 H), 2.94 (s, OH and CH), 3.43-3.83 (m, 4 H) 7.26 (s, 5 H)	3279-3175 (OH), 1600 (benzene ring)[c]
6b	31	135-136[g]	$C_5H_{10}S$	1.1 (d, 3 H), 1.43-3.1 (m, 7 H)	
6c	45	197-200	$C_8H_{16}S$	0.89 (t, 3 H), 1.07-1.79 (m, 6 H), 1.79-4.0 (m, 7 H)	2907-2857 (CH_2), 1449 (CH_2)
6d	21	79-81/0.7 mm	$C_{10}H_{20}S$[h]	0.86 (t, 3 H), 1.06-1.59 (m, 10 H), 1.6-3.9 (m, 7 H)	2924-2857 (CH_2), 1460 (CH_2)
6e	25	86-88/0.85 mm	$C_{10}H_{12}S$	1.66-4.16 (m, 7 H), 7.26 (s, 5 H)	3030 (aromatic CH), 2940-2860 (CH in alkanes), 1610 (benzene ring)
7b	51	96-97/10 mm	$C_5H_{10}SO \cdot 0.3H_2O$[f]	1.19 (t, 3 H), 1.53-3.59 (m, 7 H)	1026 (S=O)
7c	62	95/0.52 mm	$C_8H_{16}SO$	0.89 (t, 3 H), 1.09-1.69 (m, 6 H), 2.0-3.63 (m, 7 H)	1024 (S=O)
7d	82	133-134/1 mm	$C_{10}H_{20}SO$	0.87 (t, 3 H), 1.07-1.83 (m, 10 H), 1.83-3.7 (m, 7 H)	1026 (S=O)
7e	56	175-177/0.2 mm	$C_{10}H_{12}SO$	1.66-4.33 (m, 7 H), 7.29 (d, 5 H)	1070 (S=O), 1615 (benzene ring)

[a] Analyses for C, H, and S were within 0.4%. [b] Lit.[21] mp 81 °C. [c] IR ν_{max} (KBr). [d] Lit.[22] bp 102-105 °C/0.5 mm. [e] Lit.[23] bp 166 °C/13 mm. [f] NMR (CCl_4). [g] Lit.[24] bp 138.2 °C. [h] S: calcd, 18.60; found, 17.90. [i] S: calcd, 25.94; found, 25.08.

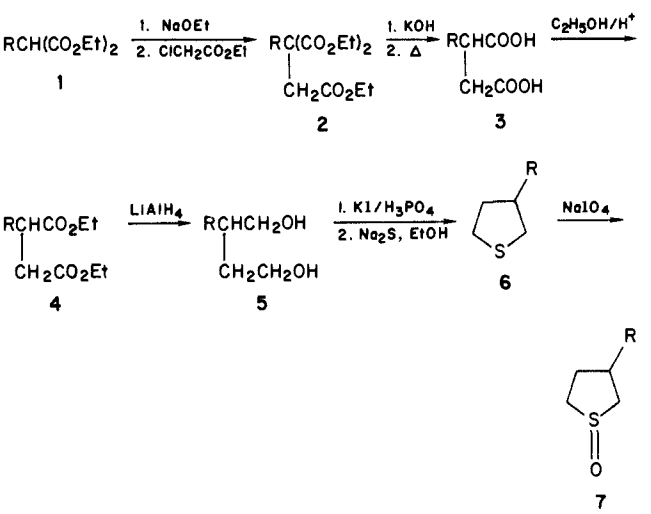
treatment of poisoning due to methanol or ethylene glycol. Further improvements in the potency of the compounds could be achieved by separation of the stereoisomers (out of the four diastereoisomers) that bind most tightly to the enzyme. Inspection of the three-dimensional structure of the horse liver enzyme [13-15] in model building studies suggests that inhibitory potency could be improved further by optimizing the size and shape of the substitutent in the 3-position. Better compounds can be designed for the human liver enzyme once its three-dimensional structure is determined. It is already clear that substituents in the 2-position (except for fluorine) would be detrimental, in general, for tighter binding. Nevertheless, bicyclic or tricyclic compounds, formed by substituents attached to carbons 2 and 3, or 3 and 4, or 2, 3, and 4, could also be better inhibitors.

Experimental Section

Materials and Methods. Unless indicated otherwise, the starting materials were obtained from Aldrich Chemical Co. Crystalline horse liver alcohol dehydrogenase and coenzyme were purchased from Boehringer Mannheim. NMR spectra were recorded at 60 MHz on a Varian 360L spectrometer with methylsilane as internal standard. Chemical shifts are reported in δ values (parts per million) from Me_4Si. IR spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer. Elemental analyses for C, H, and S were carried out by Galbraith Laboratories. Melting points and boiling points are uncorrected.

Chemistry. 3-Substituted thiolane 1-oxides were synthesized as described in Scheme I. Alkylmalonic esters were treated with ethyl chloroacetate in the presence of sodium ethoxide to give the tricarboxylic esters **2**, which were hydrolyzed and decarboxylated to give 2-substituted succinic acids **3**. The dicarboxylic acids were esterified and subsequently reduced to 2-substituted 1,4-diols **5**. The diols were converted to the diiodo compounds with potassium iodide and orthophosphoric acid and then cyclized with sodium sulfide to give 3-substituted thiolanes **6**. Oxidation of **6** with sodium metaperiodate afforded the corresponding 3-substituted thiolane 1-oxides **7**. The experimental details for 3-butylthiolane 1-oxide are given below. The hexylthiolane 1-oxide **7d** was prepared similarly starting from *n*-hexylmalonic ester (**1d**).

Scheme I



a, R = H; b, R = CH_3; c, R = C_4H_9; d, C_6H_{13}; e, R = C_6H_5

The starting material for 3-methylthiolane 1-oxide (**7b**) was 2-methyl-1,4-butanediol (**5b**, Fluka) and for 3-phenylthiolane 1-oxide (**7e**), it was phenylsuccinic acid (**3e**). The physical and spectral characteristics of the compounds are described in Table II. The compounds **7b-e** are a mixture of stereoisomers. We tried to separate 3-phenylthiolane 1-oxide by chromatography on a Baker chiral phase column using hexane–2-propanol as the mobile phase without success.

2-*n*-Butylsuccinic Acid (3c). In a 500-mL three-necked flask fitted with a stirrer, 108 g (0.5 mol) of diethyl *n*-butylmalonate was added dropwise to metallic sodium (13 g, 0.565 mol) in 200 mL of sodium-dried benzene over a period of about 45 min. To this gently refluxing suspension was slowly added ethyl chloroacetate (65 g, 0.53 mol); the reaction mixture was refluxed for 9-10 h and cooled; water was added and the benzene layer separated. The aqueous layer was extracted two to three times with benzene, and the combined extracts were dried over anhydrous $MgSO_4$. The solvent was removed by evaporation and the residue distilled under vacuum to give ethyl 3,3-dicarbethoxyheptanoate: bp

120-124 °C (0.7 mm), yield 117 g (76%).

The tricarboxylic ester (30 g, 0.1 mol) was added to potassium hydroxide (25.2 g, 0.45 mol) in an equal volume of water and the reaction mixture was refluxed for 8 h. At the end of the reaction most of the alcohol formed during reaction was removed by distillation. The residue was neutralized with concentrated hydrochloric acid and further heated at 130–140 °C in an oil bath for about 7 h (CO_2 evolution had ceased at this time). Water was added and the aqueous layer was extracted several times with ether. The combined extracts were dried over anhydrous $MgSO_4$ and the solvent was evaporated. The residual viscous liquid was triturated with a little ether and set aside until colorless crystals appeared.

Diethyl *n*-Butylsuccinate (4c). The above acid (20.9 g, 0.12 mol) was refluxed with 16.6 g of absolute alcohol in 50 mL of sodium-dried benzene and concentrated H_2SO_4 (4.3 mL) for 12 h. The reaction mixture was poured over 200 mL of water. The benzene layer was separated, washed with saturated sodium bicarbonate solution and water, and dried over anhydrous $MgSO_4$. The benzene was evaporated and the residue distilled in vacuum.

2-*n*-Butyl-1,4-butanediol (5c). In a 500-mL three-necked flask equipped with a stirrer and a dropping funnel, $LiAlH_4$ (4.6 g, 0.12 mol) and 150 mL of sodium-dried ether were stirred until most of the $LiAlH_4$ dissolved. A solution of diethyl 2-*n*-butylsuccinate (23 g, 0.1 mol) in 75 mL of dry ether was added at such a rate that the ether refluxed gently. At the end of the addition, the reaction mixture was refluxed for 2 h. The excess of $LiAlH_4$ was decomposed by adding 13 mL of ethyl acetate slowly and with stirring. The reaction mixture was poured over 100 mL of 6 N HCl. The mixture was transferred to a separatory funnel and the ether layer separated, washed once with water, and dried over anhydrous $MgSO_4$. Ether was evaporated and the product was distilled under reduced pressure.

3-Butylthiolane (6c). The 2-*n*-butyl-1,4-butanediol was converted into 2-butyl-1,4-diiodobutane by the action of potassium iodide and polyphosphoric acid.[25] The product was light brown, bp 105–109 °C (0.35–0.4 mm), and was used for the preparation of the sulfide as follows. In a 500-mL three-necked flask, equipped with a stirrer, two dropping funnels, and a condenser, 100 mL of 95% ethanol was heated to reflux. In one dropping funnel was placed 2-butyl-1,4-diiodobutane (36.6 g, 0.1mol) in 20 mL of ethanol and in the second funnel was placed 26.5 g (0.11 mol) of $Na_2S·9H_2O$ in about 30 mL of hot water. The reagents were added at approximately the same rate (over about 60 min), and the reaction mixture was refluxed further for 5–6 h. At the completion of the reaction, the mixture was distilled until no sulfide could be detected in the distillate. To this solution was added sufficient 5% aqueous $HgCl_2$ with stirring to obtain all of the sulfide as a white precipitate. The precipitate was collected by filtration and was subjected to steam distillation, until no more oily liquid distilled. The distillate was extracted with ether, and the extract was dried over KOH pellets. Diethyl ether was evaporated to give the sulfide. In the cases of **6d** and **6e**, the sulfides did not distill over with the alcohol, so these were extracted from the reaction mixture in the flask and purified by complex formation with $HgCl_2$ and steam distillation.

3-Butylthiolane 1-Oxide (7c). Sodium metaperiodate (4.1 g, 0.019 mol) was dissolved in 40 mL of water and cooled in an ice bath. 3-Butylthiolane (2.62 g, 0.018 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 40-mL portions of chloroform. The extract was dried over anhydrous $MgSO_4$, the solvent was removed under reduced pressure, and **7c** was obtained by vacuum distillation.

Enzymology. 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. In each experiment, at least three different concentrations of inhibitor were tested and 32 initial velocities were determined. A Cary 118C spectrophotometer was used to record the initial velocities of the change in absorbance at 340 nm. Data for uncompetitive inhibition were fitted to the equation $v = VA/[K_m + A(1 + I/K_{ii})]$ using Cleland's program.[26] Rat and rhesus monkey alcohol dehydrogenases were partially purified, with all steps at 0–5 °C. Centrifuged liver homogenates were dialyzed against 5.0 mM Tris-HCl, pH 8.4, containing 1.0 mM β-mercaptoethanol and passed through DEAE-cellulose (60 cm^3/40 g of liver) equilibrated with the same buffer. The effluent was applied to a N^6-(6-aminohexyl)-5′-adenosine 5′-monophosphate–Agarose (PL-Biochemicals) column (5 cm^3/40 g of liver) and the enzyme was eluted with 0.1 M sodium phosphate buffer, pH 7.5, containing 1 mM β-mercaptoethanol, 0.1 mM NAD^+, and 1.1 mM pyrazole.[27] The active fractions were dialyzed against the pH 7.5 buffer and 20% sucrose in order to concentrate and stabilize the enzyme and to remove NAD^+ and pyrazole. The specific activities of the purified enzymes were 0.5–1.0 unit/mg as assayed at 25 °C.[28] 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 °C. The concentration of NAD^+ was 0.5 mM. These conditions are thought to resemble those found in vivo.[29]

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 0–1.0 mmol/kg of body weight. The sulfoxides were diluted to 0.1 M or less in physiologic saline. Ten minutes later, ethanol (1.96 M in physiologic saline) was administered by ip 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 and deproteinized.[30] A sample of the supernate (0.5 μL) was injected into a gas chromatograph (Varian 3740) equipped with flash injectors and two 60/80 Carbopack B/5% Carbowax 20M columns (6 ft × 2 mm i.d., glass, Supelco), which were developed at 80 °C with N_2 carrier gas flow of 20 mL/min (injector at 160 °C, detector at 180 °C). The concentration of ethanol was calculated by using a Hewlett-Packard 3388A integrator by reference to an internal standard of *tert*-butyl alcohol. The rate of ethanol metabolism was calculated from the blood ethanol elimination curves by Widmark's method.[31] With high concentrations of inhibitor, the elimination showed downward curvature, as the inhibitor was also being eliminated. Therefore the initial velocity of ethanol elimination was calculated from the tangent to the curve after the 60-min absorption phase. The program NONLIN[32] was used to fit the inhibition data (Figure 1).

Toxicity of Sulfoxides. Fed, male Swiss–Webster mice (16–20 g, from Harlow Industries) were administered ip injections of a solution of 0.1–10 M sulfoxide at 0.1 mL/10 g of body weight. The percentage deaths in a 24-h period were converted to probits and the LD_{50} values calculated.[33]

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Registry No. **1d**, 5398-10-7; **3c**, 1457-39-2; **3d**, 5702-91-0; **3e**, 635-51-8; **4c**, 24766-10-7; **4d**, 18755-30-1; **4e**, 34861-81-9; **5b**, 2938-98-9; **5c**, 80676-17-1; **5d**, 18755-31-2; **5e**, 6837-05-4; **6b**, 4740-00-5; **6c**, 1551-24-2; **6d**, 1613-50-9; **6e**, 16766-62-4; **7a**, 1600-44-8; **7b**, 93111-02-5; **7c**, 93111-03-6; **7d**, 29711-02-2; **7e**, 93134-22-6; diethyl *n*-butylmalonate, 133-08-4; ethyl chloroacetate, 105-39-5; ethyl 3,3-dicarbethoxyheptanoate, 67610-46-2; 2-butyl-1,4-diiodobutane, 93111-04-7; alcohol dehydrogenase, 9031-72-5; ethanol, 64-17-5.

Uridine 5′-Diphosphate Glucose Analogues. Inhibitors of Protein Glycosylation That Show Antiviral Activity

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A series of analogues of uridine 5′-diphosphate glucose and uridine 5′-diphosphate glucosamine have been synthesized by reaction of 2,3,4,6-tetra-*O*-benzyl-, 2,3,4,6-tetra-*O*-benzoyl-, 2,3,4,6-tetra-*O*-acetyl-, and 2,3,4,6-tetra-*O*-palmitoyl-α-D-glucopyranose and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose with chlorosulfonyl isocyanate and 2′,3′-*O*-isopropylideneuridine. Isopropylidene and acetyl groups of the resulting 5′-*O*-[[[[(α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine derivatives were removed by reaction with a TFA/water (5:1) mixture and methanolic ammonia, respectively. The 5′-*O*-[[[[(2″,3″,4″,6″-tetra-*O*-benzyl- and 2″,3″,4″,6″-tetra-*O*-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (**13** and **19**) and the corresponding deisopropylidenated derivatives showed antiviral activity as determined by the inhibition of the cytopathic effect induced by HSV-1 replication and by the plaque assay method. Compound **13** inhibited glycosylation of proteins in HSV-1 infected HeLa cells.

Nucleoside diphosphate sugars are intermediates that donate glycosyl residues in the biosynthesis of polysaccharides, glycolipids, glycoproteins, and some components of the bacterial cell wall. Compounds that interfere with protein glycosylation show a variety of biological effects,[1,2] such as inhibition of the replication of enveloped animal viruses.[3-6] For instance, 2-deoxy-D-glucose is a glycosylation inhibitor having antiviral activity,[4-9] which is transformed to UDP-2dGlc (**3**) and GDP-2dGlc (**5**), and as such interferes with the biosynthesis of the dolichol-linked oligosaccharide precursor of the *N*-glycosylated glycoproteins.[10] These interferences are mainly due to the replacement of glucose and mannose by 2-deoxyglucose, i.e., the replacement of UDP-Glc (**1**) and GDP-Man (**4**) by UDP-2dGlc (**3**) and GDP-2dGlc (**5**) (Chart I). The nucleoside antibiotic tunicamycin[11] (**6**) and the related Streptomycete antibiotic streptovirudin[12] are effective protein glycosylation inhibitors that block the enzymes reversibly, translocating phosphoryl-*N*-acetyl-D-glucosamine from UDP-GlcNAc (**2**) to polyprenol phosphate.[2] However, the use of tunicamycin as an antiviral agent is hampered by its high toxicity. The metabolites of 2-deoxyglucose, GDP-2dGlc (**5**), and UDP-2dGlc (**3**), tunicamycin, and streptovirudin are structurally related to the natural nucleoside diphosphate sugars in that all of them have a sugar residue (glucose, *N*-acetylglucosamine 2-deoxyglucose, mannose) linked to the nucleoside moiety (uridine, guanosine) by a five-atom bridge. We hypothesized that this is an essential structural requirement for these compounds to act as substrates or inhibitors of glycosyltransferases and therefore we designed, synthesized, and tested as protein glycosylation inhibitors and as antiviral agents a series of analogues of uridine diphosphate glucose and of uridine diphosphate *N*-acetylglucosamine in which the diphosphate bridge has been replaced by an isosteric $-OCONHSO_2O-$ residue.

Reaction of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**7**) with chlorosulfonyl isocyanate (**8**) in methylene chloride at low temperature and with exclusion of moisture afforded the unstable [[[(chlorosulfonyl)amino]carbonyl]oxy]glucose **9**, which, by in situ reaction with 2′,3′-*O*-isopropylideneuridine gave 5′-*O*-[[[[(2″,3″,4″,6″-tetra-*O*-benzyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (**13**) in 40% yield (Scheme I). Compound **9** was formed by reaction of the isocyanate group, more reactive toward nucleophiles than the chlorosulfonyl group of **8**,[13] with the glucose anomeric hydroxyl group. This was demonstrated by the obtention of the 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl carbamate **10** in 67% yield when the crude reaction mixture of **7** and **8** was left in contact with ambient moisture. Also in support of the assigned structure to **9**, and thus the nature of the indicated five-atom bridge as glucosyl–$OCONHSO_2O$–uridine and not the inverse one glucosyl–OSO_2NHCOO–uridine,

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