$[\alpha]^{25}_D$ -78.5° (c 0.8, MeOH). Anal. ($C_{28}H_{39}N_3O_6$) C, H, N.

Inhibition Studies. The ability of compounds 1-8 to inhibit renin was measured by determining the inhibitory constant (K_i) of each compound. Activated human amniotic prorenin served as the source of renin for these studies. The procedures for the purification and pepsin activation of human amniotic prorenin have been described previously.[11] The K_i of each compound was determined through the use of Dixon plots.[12] Data for these plots were obtained by measuring the reaction velocities of renin at two concentrations of porcine angiotensinogen (0.1 and 0.2 μM) in the presence of varying concentrations of each inhibitor ([1] = 0.25-1.25 mM; [2] = 0.1-0.35 mM; [3], [4], [6], and [7] = 0.3-0.9 mM; [5] and [8] = 0.5-1.5 mM).

The enzymatic assay was carried out by incubating 50 μL of a 250-fold dilution of the renin preparation with 50 μL of angiotensinogen and 10 μL of inhibitor at 37 °C for 30 min. The renin and substrate solutions were made up in 0.1 M sodium phosphate buffer (pH 7.0), 10 mM EDTA, while the inhibitor solutions were made up in a 1:1 mixture of MeOH and H_2O. At the end of the incubation period, the enzymatic reaction was stopped by placing the incubation mixtures on ice and diluting them with 0.8 mL of 0.1 M Tris-acetate buffer, pH 7.4. The angiotensin I produced was measured by radioimmunoassay using the procedure described below.

Reaction velocities were expressed as the number of micromoles of angiotensin I generated per milliliter per hour. Three determinations were made for each inhibitor concentration at each substrate level. A plot of $1/V$ vs. inhibitor concentration was made for each of the compounds tested. The lines were calculated by linear regression analysis. The $-[I]$ value at the intersection of the two substrate lines and the horizontal line at the height of $1/V_{max}$ gave the K_i of the compound (Table I).

The V_{max} of the renin preparation used was determined by measuring the reaction velocity of the preparation at various substrate concentrations (0.25-2 μM) and then plotting the results in a Lineweaver-Burk plot of $1/V$ vs. $1/S$.

Radioimmunoassay of Angiotensin I. A modification of the method described by Haber et al.[17] was used in this study. Samples (50 μL) of the incubation mixture to be assayed were mixed with the 50 μL of [^{125}I]angtioensin I (6500 cpm) in 0.1 M Tris-acetate buffer, pH 7.4, containing bovine serum albumin (4 mg/mL). Rabbit antiserum (100 μL) was added, and the mixture was allowed to equilibrate at 4 °C for 18 h. After this time, the mixture was treated with 0.8 mL of a charcoal suspension containing 4 g/L of charcoal and 0.39 g/L of dextran (average M_r 80 700) in 0.1 M Tris-acetate buffer, pH 7.4. The mixtures were thoroughly mixed and then centrifuged at 7000 rpm for 10 min. The supernatants were decanted and counted in a Packard 5230 gamma scintillation counter. Known amounts of angiotensin I varying from 5 to 250 pg were treated in a similar manner to produce a standard curve.

Acknowledgment. This study was supported in part by a grant from the Graduate School of the University of Minnesota.

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Metabolic Depropargylation and Its Relationship to Aldehyde Dehydrogenase Inhibition in Vivo

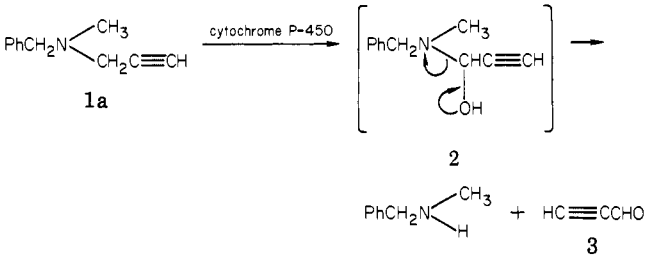
Frances N. Shirota, Eugene G. DeMaster, James A. Elberling, and Herbert T. Nagasawa*

Medical Research Laboratories, Veterans Administration Medical Center, and the Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55417. *Received January 17, 1980*

The relationship between metabolic depropargylation in vitro to inhibition of the low K_m aldehyde dehydrogenase (AlDH) of rat liver mitochondria in vivo was determined for a number of compounds bearing a propargyl substituent on nitrogen or oxygen. Only those compounds which enzymatically released the highly reactive α,β-acetylenic aldehyde, propiolaldehyde, when incubated in vitro with phenobarbital-induced rat liver microsomes, e.g., tripropargylamine (**4**), pargyline (**1a**), and *N*-propargylbenzylamine (**1b**), significantly elevated blood acetaldehyde levels when administered in vivo. Mitochondrial AlDH activity in these animals was correspondingly reduced to ≤20% that of control animals. Compounds that did not inhibit mitochondrial AlDH activity to this degree did not produce significant levels of propiolaldehyde when incubated with microsomes. Thus, for this series of compounds, metabolic depropargylation is a requirement for AlDH inhibitory activity in vivo.

The pargyline-ethanol reaction observed in vivo[1a,b] mimics the disulfiram-ethanol interaction[2] in the underlying biochemical-pharmacological mechanism, viz., the inhibition of the enzyme aldehyde dehydrogenase (AlDH). The consequence of this AlDH inhibition is the elevation of blood acetaldehyde (AcH) to levels that can trigger a catecholamine response.[3] Unlike disulfiram which is a direct inhibitor of this enzyme and is therefore effective both in vivo and in vitro, pargyline requires metabolic activation before inhibition can occur.[1b] We have identified this metabolic process as an N-depropargylation reaction mediated by the hepatic cytochrome P-450 enzymes.[4] The result of this N-depropargylation of pargyline is the formation of propiolaldehyde, a highly reactive α,β-acetylenic aldehyde which irreversibly inhibits the low

Scheme I



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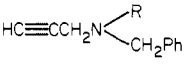
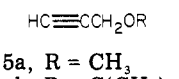
K_m AlDH of liver mitochondria.[1b,4] This isozyme is responsible for the conversion of the bulk of the AcH generated from ethanol to acetate.[5]

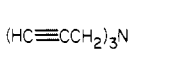
The cytochrome P-450 catalyzed depropargylation of **1a** is believed to take place by initial oxidation of the propargyl side chain at a position α to both the heteroatom and the acetylenic bonds to give the carbinolamine **2**, followed by dissociation to *N*-methylbenzylamine and propiolaldehyde **3**,[4] the latter being the reactive species that inhibits AlDH (Scheme I). Extension of this scheme to some model compounds bearing the propargyl group linked either to nitrogen or oxygen, viz., *N*-propargylbenzylamine (**1b**, which is also a metabolite of **1a**[6]), tri-



1a, R = CH_3
b, R = H



5a, R = CH_3
b, R = $C(CH_3)_3$



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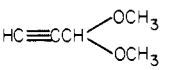
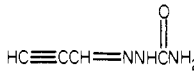


6

propargylamine (**4**), and the methyl and *tert*-butyl ethers of propargyl alcohol (**5a** and **5b**, respectively), would predict the generation of propiolaldehyde in the same manner, while oxidative *demethylation* of even a single methyl group of propiolaldehyde dimethyl acetal (**6**) would be expected to liberate the free aldehyde by an analogous but not identical mechanism.

In order to explore the generality of propiolaldehyde liberation by metabolic depropargylation, the above compounds were evaluated for their ability to (a) inhibit the low K_m AlDH of rat liver mitochondria when administered in vivo and to (b) elevate ethanol-derived blood AcH, a direct consequence of (a). These in vivo effects were further correlated with the liberation of propiolaldehyde on incubation of the compounds in vitro with liver microsomes from phenobarbital-treated and -untreated rats in the presence of an NADPH-generating system.

Chemistry. Propiolaldehyde (**3**) was prepared by modification of the synthetic procedure of Sauer,[7] which avoided the reported sudden uncontrollable exothermic reaction.[8] To maintain control of the reaction at all times, the addition procedure was reversed, i.e., the aqueous propargyl alcohol solution was slowly added to the sulfuric acid at a rate that kept the temperature between 5 and 10 °C. Chromic acid oxidation of the alcohol afforded **3**. Since the propiolaldehyde liberated from microsomal systems on incubation of the test compounds was to be trapped and isolated as its semicarbazone for characterization, it was necessary to prepare authentic propiolaldehyde semicarbazone (**7**)[4] and to rigorously rule out alternative structures such as the allene (**8**) or the cyclized pyrazole (**9**), both isomeric with **7**. The phenylhydrazone, *p*-nitrophenylhydrazone, and 2,4-dinitrophenylhydrazone of **3** are known to cyclize to pyrazole derivatives;[9] however,



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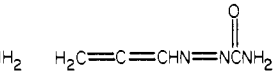

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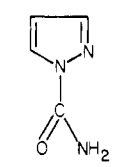

9

the physicochemical properties of **7** were different from **9**, a known compound.[10]

That the product of condensation of propiolaldehyde with semicarbazide was indeed the semicarbazone **7** and not the allenic **8** was adduced by comparison of its infrared and ^1H NMR spectra before and after deuterium exchange. It can be seen that both the amide and terminal acetylenic protons are available for deuterium exchange in **7**, whereas only the amide protons are subject to exchange in **8**. With the exception of the 3050-cm^{-1} band ascribed to the nonexchangeable methine hydrogen, the absorptions[4] due to NH (stretch), ≡CH, and NH_2 (deformation) were all shifted to longer wavelengths on deuterium exchange (Figure 1, Supplementary Material. See paragraph at end of paper regarding supplementary material). The observed $\Delta\nu$ values were in reasonable agreement with the values calculated for X–H → X–D conversions.[11] Moreover, the shift of the acetylenic triple bond absorption from 2080 to 1955 cm^{-1} was compatible with a similar shift reported by Gensler et al.[12] for the acetylenic absorptions for nona-1,4-diyne and hex-1-yne following deuterium exchange of the terminal acetylenic proton(s).

The ^1H NMR spectrum of **7** in Me_2SO-d_6 before deuterium exchange showed a one proton doublet at δ 4.96 coupled to the methine proton at δ 6.56, with a coupling constant of 1.5 Hz (Figure 2, Supplementary Material). The low-field absorption of this acetylenic proton is due to hydrogen bonding with the polar solvent and is diagnostic for a terminal acetylene.[13] A broad three-proton absorption corresponding to the overlap of the primary amide and the methine protons appeared at δ 6.56, while the remaining N-H absorbed as a broad singlet at δ 9.25. Repeated recrystallizations of **7** from D_2O resulted in gradual replacement of the exchangeable hydrogens by deuterium, and concommitant to the disappearance of the acetylenic proton the methine doublet collapsed to a sharp singlet (Figure 2, Supplementary Material). The relative stability in neutral aqueous systems of **7**, a 1,2-addition product of semicarbazide to **3**, compared to the reactivity of **3** itself was somewhat surprising.

The propargyl ethers **5a** and **5b** were prepared by published procedures. Thus, methylation of propargyl alcohol with dimethyl sulfate in the presence of base[14] gave **5a**, while alkylation with isobutylene in acid[15] gave **5b**. Propiolaldehyde dimethyl acetal (3,3-dimethoxy-1-propyne, **6**) was prepared by bromination of acrolein dimethyl acetal

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Table I. Blood AcH Levels After Ethanol and Its Relationship to Mitochondrial AlDH Activity in PB-Treated and -Untreated Rats After Administration of Propargyl and Related Compounds

compd administered (1.25 mmol/kg)	blood AcH, μM ± SE (no. of animals)	T/C	AlDH act., nmol of AcH oxid min^{-1} $(mg\ of\ protein)^{-1}$ ± SE (no. of animals)	% inhibn
saline (controls)				
(a) untreated	11.1 ± 1.3 (11)		10.9 ± 0.53 (11)	
(b) PB treated	7.6 ± 1.0 (10)		9.89 ± 0.63 (10)	
pargyline (1a)				
(a) untreated	224 ± 16 (5)	20.2	1.36 ± 0.07 (5)	88
(b) PB treated	247 ± 9.5 (5)	32.5	0.98 ± 0.09 (5)	90
N-propargylbenzylamine (1b)				
(a) untreated	203 ± 22 (4)	18.3	1.72 ± 0.13 (4)	83
(b) PB treated	382 ± 46 (4)	50.3	1.11 ± 0.11 (4)	89
tripropargylamine (4)				
(a) untreated	15.4 ± 8.2 (4)	1.4	5.37 ± 0.22 (4)	51
(b) PB treated	144 ± 29 (4)	19.0	2.00 ± 0.14 (4)	80
methyl propargyl ether (5a)				
(a) untreated	10.6 ± 1.7 (4)	0.95	10.8 ± 1.76 (4)	1
(b) PB treated	8.3 ± 0.8 (4)	1.1	9.45 ± 0.67 (4)	4
tert-butyl propargyl ether (5b)				
(a) untreated	19.6 ± 4.9 (7)	1.77	12.4 ± 0.95 (7)	
(b) PB treated	13.5 ± 2.6 (4)	1.78	6.56 ± 1.29 (4)	34
propiolaldehyde dimethyl acetal (6)				
(a) untreated	10.6 ± 1.5 (4)	0.95	12.3 ± 0.85 (4)	
(b) PB treated	24.1 ± 8.4 (4)	3.17	5.58 ± 0.54 (4)	44

followed by dehydrobromination with ethanolic KOH.[16]

Results

Effect on Mitochondrial AlDH and Blood AcH in Vivo. Equimolar doses (1.25 mmol/kg) of the model compounds **1b**, 4, **5a**,**b**, and **6** were administered to phenobarbital (PB) treated rats as well as to rats not treated with PB. Following an acute dose of ethanol at 1 h, blood AcH levels and the liver mitochondrial AlDH activities were measured 1 h after ethanol. These results are compared to the results obtained with pargyline (**1a**) in Table I.

PB-untreated rats given pargyline (**1a**) or *N*-propargylbenzylamine (**1b**) had significantly elevated blood AcH compared to control rats that were sham injected with saline and ethanol. Mitochondrial AlDH activities were correspondingly and severely inhibited by 80–90%. There were slight augmentations of the blood AcH levels as well as in the degree of inhibition of AlDH in PB-treated rats. Although tripropargylamine (4) administration to PB-untreated rats did not elevate blood AcH to a significant degree, AlDH was found to be inhibited ~50%. Administration of 4 to PB-treated rats, however, resulted in a tenfold elevation in blood AcH accompanied by a commensurate increase in mitochondrial AlDH inhibition to 80%.

Methyl propargyl ether (**5a**) administration did not inhibit mitochondrial AlDH even in PB-treated rats (Table I). This was also reflected in the blood AcH levels, which were indistinguishable from controls. In PB-treated rats, *tert*-butyl propargyl ether (**5b**) administration produced a 34% inhibition of mitochondrial AlDH. As was the case for 4 in PB-untreated animals, this level of enzyme inhibition was not sufficient to raise blood AcH. Similarly, propiolaldehyde dimethyl acetal (6) administration inhibited mitochondrial AlDH by 44% in PB-treated animals but had no effect in raising blood AcH.

Microsomal Depropargylation in Vitro. The enzymatic formation of propiolaldehyde (3) from **1a**, **1b**, 4, **5a**, **5b**, and **6** on incubation with rat liver microsomes in the presence of an NADPH-generating system is recorded in Table II. The propiolaldehyde formed was trapped in situ as its semicarbazone and subsequently released by treatment of the isolated semicarbazone with acid. The liberated **3** was identified by head-space gas chromatography[17] coupled to mass spectroscopy[4] and quantitated by reference to a standard curve prepared from authentic propiolaldehyde semicarbazone (**7**). The absolute requirement for NADPH for the depropargylation reaction was indicated by the absence of propiolaldehyde formation when glucose 6-phosphate and $NADP^+$ were omitted from the system.

Table II. Generation of Propiolaldehyde (3) in Vitro by Various Propargyl and Related Compounds on Incubation with Liver Microsomes from PB-Treated and -Untreated Rats

compd incubated (3 mM)	propiolaldehyde generated, μmol ± SE[a]
pargyline (1a)	
(a) untreated	0.61 ± 0.01
(b) PB treated	2.58 ± 0.50
N-propargylbenzylamine (1b)	
(a) untreated	0.20 ± 0.03
(b) PB treated	1.16 ± 0.12
tripropargylamine (4)	
(a) untreated	0.25 ± 0.02
(b) PB treated	3.50 ± 0.24
methyl propargyl ether (5a)	
(a) untreated	
(b) PB treated	0.05 ± 0.01
tert-butyl propargyl ether (5b)	
(a) untreated	
(b) PB treated	0.22 ± 0.01
propiolaldehyde dimethyl acetal (6)	
(a) untreated	
(b) PB treated	0.05 ± 0.01

[a] Based on a standard curve using propiolaldehyde semicarbazone as the standard. The incubation system and the method of assay are described under Experimental Section. When $NADP^+$ and glucose 6-phosphate were omitted from the incubation systems, the propiolaldehyde levels were at the lower limits of detectability.

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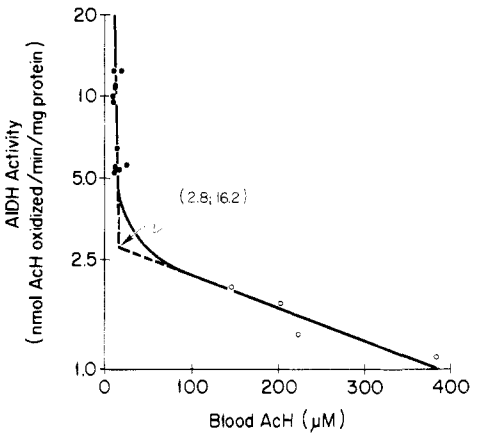

Figure 3. Log-linear plot of the data of Table I showing the experimental relationship of the degree of AlDH activity (inhibition) with blood AcH after ethanol treatment in rats. The linear portions of the curves (● and ○) and the intersect were plotted by computer using a linear-regression program.

While readily detectable levels of **3** were formed from **1a**, **1b**, or **4** with microsomes from animals not pretreated with PB, incubation with microsomes from PB-treated animals resulted in a 4- to 11-fold enhancement in the amounts of **3** formed (Table II). With the propargyl ethers **5a** and **5b**, as with propiolaldehyde dimethyl acetal (**6**), microsomes from PB-treated rats produced relatively insignificant but detectable levels of **3**.

Discussion

The extent of liver microsomal production of propiolaldehyde (**3**) in vitro and the degree of inhibition of the low K_m AlDH of liver mitochondria by compounds potentially capable of releasing **3** by metabolism indicate that **3** is, in fact, responsible for AlDH inhibition in vivo. It was further noted that blood AcH was not elevated significantly until the mitochondrial enzyme was drastically inhibited, i.e., by ≥80%. This is to be expected on the basis that the rate-limiting step in the overall metabolism of ethanol to acetate is the oxidation of ethanol to AcH catalyzed by liver alcohol dehydrogenase.[18] Thus, AcH should not accumulate until the enzyme AlDH is severely inhibited. This is experimentally verified in Figure 3. It can be seen that ethanol-derived blood AcH did not rise above 20 μM until the mitochondrial enzyme activity was reduced to 25% of the control values seen in untreated rats, i.e., to 2.80 nmol of AcH oxidized min^{-1} (mg of protein)$^{-1}$ vs. an average of 10.5 nmol of AcH oxidized min^{-1} (mg of protein)$^{-1}$ in controls.

Whether these results can be explained on the basis of the induction of the cytosolic high K_m ϕ-isozyme by phenobarbital treatment[19] is not clear, since commercially available random-bred animals were used here, and the induction phenomenon is highly genetically controlled. On the other hand, the widely held assumption that the liver AlDH levels are only marginally adequate to cope with the AcH generated from ethanol[20] may not be valid based on the present evidence.

While we fully expected greater metabolic conversion of **5a**, **5b**, and **6** to **3** in vitro, its minimal formation correlated well with the lack of significant elevations in blood AcH and the less than maximal inhibition of AlDH observed in vivo—even with PB-treated animals. The greater inhibition of AlDH by the *tert*-butyl propargyl ether (**5b**) over the methyl propargyl ether (**5a**) may be due to the relative ease of O-demethylation vs. O-depropargylation of the latter, while the direct oxidative removal of the *tert*-butyl group of **5b** by liver microsomes is mechanistically improbable,[21] thus favoring depropargylation.

The significant formation of **3** from **1a**, **1b**, and **4** in vitro and the fact that **3** was also detectable in the microsomal metabolism of the oxygen compounds **5a**, **5b**, and **6** completely discounts the role of metabolites other than **3** in AlDH inhibition in vivo. For example, Lindeke et al.[22] have suggested that acrolein could be formed from **1a** via its *N*-oxide after initial rearrangement of the *N*-oxide to an allenic intermediate. We were unable to detect acrolein after incubation of **1a** with PB-induced rat liver microsomes. Under the conditions of our head-space gas chromatographic assay, synthetic acrolein semicarbazone *added* to the system showed the presence of acrolein in the gas phase after acidification; hence, this compound would have been detectable if present as a metabolite of **1a**.

The present results suggest that drugs bearing the propargyl group on nitrogen or oxygen functional groups can and might well be metabolically depropargylated in vivo to propiolaldehyde (**3**), thereby blocking AlDH and provoking a disulfiram–ethanol reaction when ingested together with alcoholic beverages. Indeed, pinazepam[23] and 7-propargyltheophylline[24] are known to be metabolized to depropargylpinazepam and theophylline, respectively, presumably by analogous mechanisms. The possibilities for clinical drug–alcohol reactions here should, therefore, be thoroughly assessed.

Experimental Section

Spectrophotometers used were as follows: IR, Beckmann IR-10; NMR, Varian T60A [$(CH_3)_4Si$ internal standard]. Pargyline hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO. *N*-Propargylbenzylamine, propargyl alcohol, acrolein dimethyl acetal, and tripropargylamine were obtained from Aldrich Chemical Co., Milwaukee, WI. Tripropargylamine [**3**, bp 89–91 °C (35 mm)] and *N*-propargylbenzylamine [**1b**, bp 102–103 °C (9.6 mm)] were fractionally distilled before use. Distillations were performed on a Nester–Faust Teflon annular spinning-band column with an efficiency of 125 theoretical plates unless otherwise specified. The following compounds were synthesized by known procedures without attempting to optimize yields.

Propiolaldehyde (3). Caution: The addition of an aqueous H_2SO_4 solution to aqueous propargyl alcohol[7] might be hazardous![8] The "Organic Syntheses" procedure[7] was modified by reversing the order of addition. In a well-ventilated hood, a solution of 135 mL of concentrated H_2SO_4 in 200 mL of H_2O was cooled to 0 °C in an ice–salt bath. A cold solution of propargyl alcohol (112.1 g, 2.0 mmol) in 240 mL of H_2O was added to the stirred H_2SO_4 solution at a rate slow enough to keep the temperature between 5 and 10 °C. A cold solution of CrO_3 (210 g, 2.1 mol) dissolved in a mixture of 135 mL of H_2SO_4 and 400 mL of H_2O was slowly added under N_2 to the vigorously stirred solution at 45 mmHg over a period of 3 h while maintaining the temperature between 2 and 10 °C. The product was isolated as described by Sauer.[7] Fractional distillation of the crude product through an 8-cm column packed with glass helices gave 13.8 g (12.8% yield) of pure **3**: bp 53.4–54.5 °C (lit.[7] bp 54–57 °C); NMR ($CDCl_3$) δ 9.27 [s, 1 H, CHO], 3.75 (s, 1 H, ≡CH).[25] The sem-

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icarbazone (7) of 3 was prepared as described previously.[4] Its IR and ^1H NMR spectra before and after deuterium exchange appear as Figures 1 and 2, Supplementary Material.

3-Methoxy-1-propyne (5a). Using the procedure described by Reppe,[14] dimethyl sulfate (18.1 g, 0.14 mol) was added dropwise to a solution of propargyl alcohol (13.5 g, 0.21 mol) in 25 mL of 13 N NaOH at 50–55 °C. The crude product, obtained by distillation of the reaction mixture at 55–59 °C, was fractionally distilled to give 2.5 g of **5a**: bp 61–62 °C (lit.[14] 65 °C); NMR ($CDCl_3$) δ 4.07 (d, 2 H, J = 2 Hz, $-CH_2O-$), 3.38 (s, 1 H, $-OCH_3$), 2.47 (t, 1 H, J = 2 Hz, $\equiv CH$).

3-(1,1-Dimethylethoxy)-1-propyne (5b). Gaseous isobutylene was bubbled through a solution of propargyl alcohol (46.6 g, 0.83 mol) containing 1.3 g of concentrated H_2SO_4 at 45–55 °C as described by Mantione[15] until 46.2 g (0.82 mol) was absorbed. Repeated fractional distillation gave **5b**: bp 117 °C (lit.[15] 116–117 °C); NMR ($CDCl_3$) δ 4.05 (d, 2 H, J = 2 Hz, $-CH_2O-$), 2.37 (t, 1 H, J = 2 Hz, $\equiv CH$), 1.27 (s, 9 H, CH_3).

3,3-Dimethoxy-1-propyne (6). The procedure described by Claisen[16] was modified to convert acrolein dimethyl acetal to **6**. Bromine (37.9 g, 0.24 mol) in 30 mL of CH_2Cl_2 was added dropwise to cold (0 °C) acrolein dimethyl acetal (24.3 g, 0.25 mol) over 1.5 h while the reaction temperature was maintained at 0 °C. After removal of the CH_2Cl_2, the residue was heated under reflux in 1.82 N ethanolic KOH (330 mL) for 2 h. The reaction mixture was then poured into 2.5 L of H_2O and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were dried (Na_2SO_4) and the solvent was removed in vacuo. Fractional distillation of the residue gave 3.0 g (13% yield) of **6**: bp 108–110 °C (lit.[16] bp 110 °C); NMR ($CDCl_3$) δ 5.08 (d, 1 H, J = 1.5 Hz, acetal C-H), 3.37 (s, 6 H, CH_3), 2.55 (d, 1 H, J = 1.5 Hz, $\equiv CH$). A considerable amount of partially dehydrobrominated product still remained, but this was not further processed.

Biological Evaluation. (a) Microsomal Depropargylation in Vitro. Male Sprague–Dawley rats were maintained ad libitum on water or on aqueous 0.1% sodium phenobarbital solution in place of their drinking water for 8 days.[26] After an overnight fast, the livers were removed and homogenized (25% w/v) in 0.15 M KCl. The homogenate was centrifuged at 10000g for 10 min, and the supernatant fluid was removed. The microsomes were isolated from the latter by centrifugation at 100000g for 60 min, and the pellet was washed once with 0.15 M KCl, resedimented, and then suspended to a final concentration corresponding to 0.5 g wet weight of liver/mL. The test compounds (**1a**·HCl, **1b**·HCl, **4**·HCl, **5a**, **5b**, or **6**) were incubated at 37 °C in a shaking water bath for 30 min as follows: Each of six flasks contained the test compound (3 mM), sodium phosphate buffer (pH 7.4, 83 mM), $NADP^+$ (2.0 mM), glucose 6-phosphate (2.5 mM), KCl (16.5 mM), $MgCl_2$ (4.0 mM), nicotinamide (8.3 mM), semicarbazide hydrochloride adjusted to pH 7.4 (30 mM), glucose-6-phosphate dehydrogenase (40 units), and microsomes (100000g fraction) from PB-treated or naive rats corresponding to 500 mg wet weight of liver in a final volume of 12 mL. Glucose 6-phosphate and $NADP^+$ were omitted from control incubations. The reaction was initiated by the addition of enzyme and terminated by the dilution of the system with 15 mL of cold 0.1 M phosphate buffer, pH 7.4. The contents of the incubation flasks for each compound were pooled (6 × 12 mL). Propiolaldehyde semicarbazone (**7**) was extracted five times from each incubation set with 200-mL portions of EtOAc. The combined EtOAc extracts were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was dissolved in 10 mL of deionized water. Aliquots (0.20 mL) were placed in 20-mL serum bottles and capped. Samples were frozen on dry ice and kept frozen at −20 °C until assayed. **3** was released by addition through the septum of 0.5 mL of 5 N $HClO_4$ and analyzed by a head-space gas chromatographic technique previously described.[4,17] A standard curve was prepared using known concentrations of authentic **7**. The total propiolaldehyde generated from the combined set of six incubation flasks (Table II) represents the average of three to five GC determinations. Values are reported as the mean plus or minus standard error (SE).

(b) AlDH Activity in Vivo. Male Sprague–Dawley rats were maintained ad libitum on water or on 0.1% sodium phenobarbital solution for 8 days.[26] After an overnight fast, the compounds listed in Table I (1.25 mmol/kg, ip) were administered 2 h before sacrifice, followed by ethanol (2.0 g/kg, ip) an hour later. Control animals received saline in place of the drug. The animals were stunned by a quick blow to the head, and blood was immediately withdrawn by cardiac puncture. Aliquots (0.2 mL) were placed in 20-mL serum vials containing 1.0 mL of 5 mM sodium azide (the latter to minimize the artifactual generation of AcH from ethanol), capped, frozen on dry ice, and kept frozen at −20 °C until assayed for AcH by head-space gas chromatography.[17] Mitochondria were prepared and AlDH activity was assayed as previously described.[1b] Protein concentrations were determined according to Lowry et al.[27] AlDH activity was expressed as nmol of AcH oxidized min^{-1} (mg of protein).$^{-1}$

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Supplementary Material Available: Figures 1 and 2, IR and ^1H NMR spectra of **7** before and after deuterium exchange (2 pages). Ordering information is given on any current masthead page.

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