mL) containing 30 mg of 10% Pd/C was hydrogenated in a Parr apparatus at 45 psi for 5 h. The solution was filtered and evaporated, and the residue was recrystallized from CH_3CN containing a trace of MeOH. Yield of two crops 122 mg (71%): foams 250 °C; EIMS (20 eV), m/e 456 (M^+, 20%); IR cm^{-1} 1719, 1705, 1664, 1649; R_f 0.33 (EMA 95:5:2); $[\alpha]^{25}_D$ −132° (c 0.9, MeOH). Anal. ($C_{25}H_{33}N_2O_6Cl \cdot H_2O$) C, H, N.

Model Kinetic Studies. To a rapidly stirred solution (2.1 μM) of the naltrexamine derivative (Table I) in 0.025 M NaH_2PO_4 buffer (0.24 mL) of different pH values (7.4, 6.1, 5.5, 5.0, 3.0, or 2.4) was quickly added 10 μL of 0.65 M cysteine in same phosphate buffer. Rapid stirring was continued at 25 °C for a specific time interval (0, 10, 15, 30, or 60 s), and then the reaction mixture was quenched by acidification to pH 1. The amount of compound remaining was determined by using reversed-phase HPLC (25 × 0.46 cm Ultrasphere ODS, 5 μ; isocratic MeOH–KH_2PO_4 buffer, pH 4.4 (65:35) at 1.3 mL/min; UV, 254 nm) using an internal standard. Buffer solutions were purged with nitrogen before use, and each compound was assessed separately for hydrolytic decomposition, which was found not to be significant under the experimental conditions employed. Four time intervals were examined in duplicate for each compound, and a half-life estimate was calculated from the slope of a linear regression of the log amount remaining vs. time.

Pharmacology. The GPI and MVD assays were prepared and performed according to the methods of Rang[31] and Henderson,[32] as described previously.[29]

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Registry No. 2, 72782-05-9; 2·HCl, 72786-10-8; 3·HCl, 91409-37-9; 4, 91409-38-0; 5, 91409-40-4; 5·HCl, 91409-39-1; 6, 91409-41-5; 7, 91409-42-6; 8, 91409-44-8; 8·HCl, 91409-43-7; 9·HCl, 91409-45-9; 10·CH_3SO_3H, 91423-93-7; 11, 91409-47-1; 11·HCl, 91409-46-0; 12, 91409-49-3; 12·HBr, 91409-48-2; 13, 91409-50-6; 14, 91409-52-8; 14·HCl, 91409-51-7; 16, 67025-97-2; 18, 83514-37-8; HOSu, 6066-82-6; HOBt, 2592-95-2; CH_2=C(Cl)COCl, 21369-76-6; *trans*-3-(methoxycarbonyl)acryloyl chloride, 17081-97-9; *trans*-3-acetylacrylic acid, 14300-75-5; sodium *trans*-3-acetylacrylic acid, 91409-53-9; sodium 2-chloroacrylic acid, 32997-86-7; propiolic acid, 471-25-0; 6β-(*cis*-3-carboxyacrylamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan, 91409-54-0; maleimidoacetic acid HOSu ester, 55750-61-3; *N*-[(*o*-nitrophenyl)dithioacetoxy]succinimide, 91409-55-1; 1-[(chloromercuri)acetoxy]benzotriazole, 91409-56-2; benzoylformic acid, 611-73-4; benzoylformic acid chloride, 25726-04-9; *N*-(bromoacetoxy)succinimide, 42014-51-7; benzoyl chloride, 98-88-4.

(31) Rang, H. B. *Br. J. Pharmacol.* **1964**, *22*, 356.

(32) Henderson, G.; Hughes, J.; Kosterlitz, H. N. *Br. J. Pharmacol.* **1972**, *46*, 764.

Latent Inhibitors of Aldehyde Dehydrogenase as Alcohol Deterrent Agents

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A series of compounds structurally related to pargyline (*N*-methyl-*N*-propargylbenzylamine, 4) were synthesized with the propargyl group replaced by a cyclopropyl, allyl, or 2,2,2-trichloroethyl group and, additionally in several cases, with the methyl group replaced by H. The rationale for their preparation was based on the expectation that, like pargyline, which gives rise to propiolaldehyde, oxidative metabolism of the above compounds by the hepatic cytochrome P-450 enzymes would lead to the generation in vivo of the aldehyde dehydrogenase (AlDH) inhibitors, cyclopropanone, acrolein, or chloral. These compounds were evaluated for inhibition of liver AlDH in vivo by measuring the elevation of ethanol-derived blood acetaldehyde in rats and in vitro by the rate of oxidation of acetaldehyde by intact and osmotically disrupted liver mitochondria. Administration of *N*-methyl-*N*-cyclopropylbenzylamine (5) and its nor-methyl analogue (8) to rats raised blood acetaldehyde levels significantly over controls at 2 h. This effect was more pronounced at 9 h, with blood acetaldehyde levels reaching 19 to 27 times control values and approaching the values induced by pargyline. Other compounds elicited significant elevations in ethanol-derived blood acetaldehyde only at 9 h. We suggest that latent inhibitors of AlDH such as 5 or 8 might be useful as alcohol deterrent agents.

The biochemical mechanism underlying the multitude of reported alcohol-drug interactions giving rise to the disulfiram–ethanol reaction (DER) is generally believed to be due to inhibition of one or more of the hepatic aldehyde dehydrogenase (AlDH) isozymes.[1] Our interest in drugs that produce a clinical DER is based on the expectation that understanding of these mechanisms at the molecular level should greatly aid in the design of alcohol deterrent agents for the treatment of alcoholism. Indeed, such deterrent drugs as disulfiram and citrated calcium carbimide were discovered accidentally when they were found to be incompatible with alcohol ingestion.[2]

Of the several known potent inhibitors of AlDH only disulfiram and chloral hydrate appear to be direct acting, while the others require prior metabolism to produce the active inhibitors. Thus, the mechanism of AlDH inhibition by disulfiram is by direct interaction with the SH group at the active site of the enzyme to form initially a covalently linked mixed disulfide, followed by intraenzyme displacement of diethyl dithiocarbamate by a second proximate SH group.[3] Cyanamide, the active component in citrated calcium carbiimide, requires activation by liver

(1) (a) Kitson, T. M. *J. Stud. Alcohol* **1976**, *38*, 96. (b) Fried, R. *Subst. Alcohol Actions/Misuse* **1980**, *1*, 5.

(2) (a) Hald, J.; Jacobsen, E.; Larsen, V. *Acta Pharmacol. Toxicol.* **1948** *4*, 285. (b) Ferguson, J. K. W. *Can. Med. Assoc. J.* **1956**, *74*, 793.

(3) (a) Vallari, R. C.; Pietruszko, R. *Science* **1982**, *216*, 637. (b) Kitson, T. M. *Biochem. J.* **1983**, *213*, 551.

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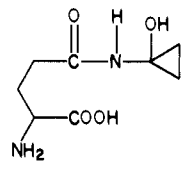
mitochondria,[4] and although the molecular species responsible for the inhibition of AlDH by this compound has not yet been identified or characterized, it is clear that cyanamide is a *latent* and not a *direct* inhibitor of AlDH.

The DER elicited by coprine (1), the active principle found in the mushroom species *Coprinus atramentarius*,[5] has been known to be delayed even to the day *following* the ingestion of this mushroom species,[5a] suggesting that a metabolic product might be involved. It has now been established that coprine is hydrolyzed in vivo to the aminohydrin of cyclopropanone (2) and then to cyclopropanone hydrate (3), and these metabolites—or cyclopropanone itself—are the active species that inhibit AlDH.[6]



1

2, X = NH_2
3, X = OH

Finally, we have shown that pargyline (4), an inhibitor of hepatic mitochondrial AlDH when administered in vivo to mice and rats,[7] undergoes N-depropargylation by the liver microsomal enzymes to produce the highly reactive α,β-unsaturated aldehyde propiolaldehyde, which irreversibly inhibits the enzyme and elevates blood acetaldehyde, a consequence of this inhibition.[8] Other compounds that also release propiolaldehyde by metabolism have likewise been shown to be latent inhibitors of AlDH.[9]

In an effort to produce substances with latent action that release different AlDH inhibitors by design, we prepared a number of compounds with structures related to pargyline (4) and evaluated their activity in rats in vivo by measuring the elevation of circulating blood acetaldehyde after administration of ethanol. These are the cyclopropyl analogue **5**, the allyl analogue **6**, and the 2,2,2-trichloroethyl analogue **7**. We envisioned that dealkylation of the R′ side chain by the microsomal enzymes of liver would liberate, respectively, cyclopropanone from **5**, acrolein from **6**, and chloral from **7**. The hydrates of cyclopropanone[6] and chloral[10] are known inhibitors of AlDH, while acrolein would be expected to behave like propiolaldehyde on the basis of structural similarities. Although N-demethylation and N-debenzylation reactions are competitive, eliminating the methyl substituent as in the nor-methyl analogues **8** and **9** or replacing this group with a second R′ group as in **10** could favor the anticipated metabolic generation of the above species. Also included in this series were the saturated side-chain *n*-propyl analogue **11**, which mimicks **4** and **6**, and the branched-chain isopropyl analogue **12**, which mimicks the cyclopropyl compound **5**.[11]

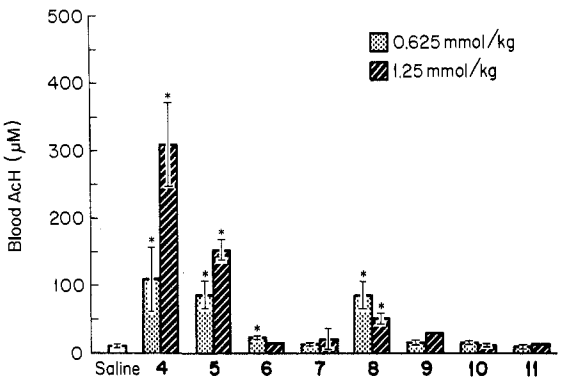


Figure 1. Elevation of blood AcH in rats 2 h after 0.625 and 1.25 mmol/kg doses of some cyclopropyl, allyl, and 2,2,2-trichloromethyl analogues of pargyline (4). The complete protocols are described in the Experimental Section. Insert bars are ± SEM and an asterisk denotes $p < 0.005$ relative to controls (Student's *t* test). For compounds **6**, **9**, and **11**, there was only one surviving animal each at the higher dose.

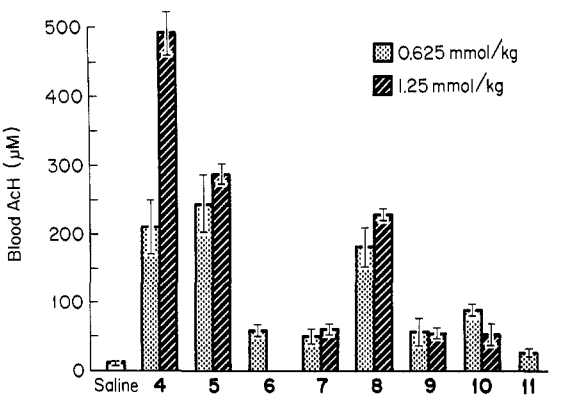
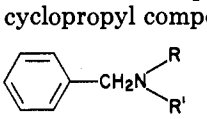


Figure 2. Elevation of blood AcH in rats 9 h after 0.625 and 1.25 mmol/kg doses of the same compounds described in Figure 1. There were no survivors at 9 h for compounds **6** and **11** at the higher dose. Inset bars are ±SEM and all values were significantly higher than saline controls ($p < 0.001$).



	R	R′
4	CH_3	$CH_2C{\equiv}CH$
5	CH_3	c-C_3H_5
6	CH_3	$CH_2CH{=}CH_2$
7	CH_3	CH_2CCl_3
8	H	c-C_3H_5
9	H	$CH_2CH{=}CH_2$
10	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$
11	CH_3	$CH_2CH_2CH_3$
12	CH_3	$CH(CH_3)_2$

Results

Compound **5** was prepared by Eschweiler–Clarke methylation of *N*-cyclopropylbenzylamine (**8**). All other target compounds and intermediates were purchased from commercial sources or were prepared according to pub-

(4) (a) DeMaster, E. G.; Kaplan, E.; Shirota, F. N.; Nagasawa, H. T. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 1333. (b) DeMaster, E. G.; Nagasawa, H. T.; Shirota, F. N. *Pharmacol. Biochem. Behav.* **1983**, *18*, 273.

(5) (a) Reynolds, W. A.; Lowe, F. H. *N. Engl. J. Med.* **1965**, *272*, 630. (b) Hatfield, G. M. *Lloydia* **1975**, *38*, 489. (c) Lindberg, P.; Bergman, R.; Wickberg, B. *J. Chem. Soc., Chem. Commun.* **1975**, 546. (d) Lindberg, P.; Bergman, R.; Wickberg, B. *J. Chem. Soc., Perkin Trans. 1* **1977**, 684.

(6) (a) Tottmar, O.; Lindberg, P. *Acta Pharmacol. Toxicol.* **1977**, *40*, 476. (b) Wiseman, J. S.; Abeles, R. H. *Biochemistry* **1979**, *18*, 427. (c) Wiseman, J. S.; Tayrien, G.; Abeles, R. H. *Biochemistry* **1980**, *19*, 4222.

(7) (a) Cohen, G.; MacNamee, D.; Dembiec, D. *Biochem. Pharmacol.* **1975**, *24*, 313. (b) Dembiec, D.; MacNamee, D.; Cohen, G. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 332. (c) Lebsack, M. E.; Anderson, A. D. *Res. Commun. Chem. Pathol. Pharmacol.* **1979**, *26*, 263.

(8) Shirota, F. N.; DeMaster, E. G.; Nagasawa, H. T. *J. Med. Chem.* **1979**, *22*, 463.

(9) Shirota, F. N.; DeMaster, E. G.; Elberling, J. A.; Nagasawa, H. T. *J. Med. Chem.* **1980**, *23*, 669.

(10) Nakanishi, S.; Shiohara, E.; Tsukada, M.; Yamazaki, H.; Nishiguchi, K. *Arukora Kenkyu* **1979**, *14*, 369.

(11) These experiments were suggested by a referee to complement the saline controls.

Table I. Liver Mitochondrial AlDH Activity 2 h after Administration of **5** to **8** to Rats

compd administered	dose, mmol/kg	N	mitochondrial AlDH act.,[a] nmol AcH oxid min^{-1} (mg of protein)$^{-1}$	remaining act., %
control (saline)		13	11.20 ± 0.48	100
5	1.25	5	3.48 ± 0.16	31
8	1.25	5	2.68 ± 0.15	24
4 (pargyline)	1.25	14	1.48 ± 0.10	13

[a] Mean ± SEM.

lished methods; any procedural deviations of significance are described in the Experimental Section.

The elevations of ethanol-derived blood acetaldehyde in rats—a consequence of the inhibition of hepatic AlDH—2 h after 0.625 and 1.25 mmol/kg doses of compounds **4–10** are shown in Figure 1. It can be seen that except for pargyline (**4**), which served as a positive control, and the cyclopropyl analogues **5** and **8**, the other compounds did not elicit increases in blood acetaldehyde at 2 h. The isopropyl analogue **12** produced convulsions at doses ≥ 0.5 mmol/kg and the animals died before ethanol could be administered. When measured at 9 h, blood acetaldehyde was significantly elevated over controls for every compound listed in Figure 2. The allyl compound **6** as well as the *n*-propyl analogue **11** was toxic at the higher dose (1.25 mmol/kg), and no data are available for this dose since there were no survivors at the time of ethanol administration at 8 h. Blood acetaldehyde elevation was surprisingly independent of dose except for pargyline, and the more important parameter appeared to be the time of observation, with significant elevations occurring at 9 h, a latency in action possibly relfecting metabolic intervention.

That the target enzyme—the low K_m liver mitochondrial AlDH—was in fact inhibited by administration of **5** or **8** was shown by isolating the liver mitochondria from these animals and comparing their AlDH activities to those from control animals receiving saline (Table I). The mitochondrial AlDH from animals treated with **5** and **8** were inhibited 70% at 2 h, and for pargyline, this inhibition was 87%. The selection of **5** and **8** was based on the results of Figure 1, and this enzyme-inhibition data corroborate the in vivo effects of these compounds on blood acetaldehyde elevation.

When incubated with intact rat liver mitochondria in vitro, the cyclopropyl (**5**) and allyl (**6**) analogues of pargyline (**4**) were essentially without effect on the activity of AlDH, as was the monoallyl analogue **9** (Table II). However, the putative in vivo metabolites, acrolein and chloral hydrate, were excellent inhibitors of the mitochondrial enzyme, as was propiolaldehyde, our positive control. Cyclopropanone hydrate (**3**) was not included here since Wiseman et al.[6c] have adequately characterized this inhibition by elegant kinetic studies using the mouse liver and yeast enzymes. Although the inhibition of AlDH by cyclopropanone is known to be reversible, we observed inhibition with compounds **5** and **8** (Table I). The $t_{1/2}$ for the dissociation of this enzyme–inhibitor complex is reported to be 230 min at 25 °C,[6c] and since we isolated the liver mitochondria at reduced temperature (0 °C) and the samples were maintained at this temperature until the AlDH activity was assayed, minimal dissociation of the enzyme–inhibitor complex would be expected. The 2,2,2-trichloroethyl analogue **7** also inhibited the mitochondrial enzyme in a dose-related manner, both with

Table II. Effect of the Cyclopropyl (**5**), Allyl (**6**), and 2,2,2-Trichloroethyl (**7**) Analogues of Pargyline (**4**) and Their Putative Metabolites on Rat Liver Mitochondrial AlDH Activity in Vitro

compd tested	concn, mM	mitochondrial AlDH act.,[a] % of control ± SE
	A. intact mitochondria	
4	0.2	107.0 ± 0.5
	1.0	100.0 ± 7.2
5	0.2	92.3 ± 2.3
	1.0	84.9 ± 2.4
6	0.2	108.5 ± 1.0
	1.0	105.8 ± 2.8
9	0.2	97.9 ± 3.4
	1.0	105.3 ± 4.0
10	0.2	93.6 ± 0.9
	1.0	16.1 ± 0.3
7	0.2	31.3 ± 3.7
	1.0	22.3 ± 3.6
$HC{\equiv}CCHO$	0.2	3.9 ± 5.7
$H_2C{=}CHCHO$	0.2	7.0 ± 2.2
$CCl_3CHO{\cdot}H_2O$	0.2	37.9 ± 0.5
	1.0	19.5 ± 0.6
	B. disrupted mitochondria	
6	0.2	99.3 ± 1.0
	1.0	98.5 ± 0.1
9	0.2	99.3 ± 1.1
	1.0	98.9 ± 0.7
10	0.2	94.7 ± 1.0
	1.0	87.0 ± 3.1
7	0.2	59.8 ± 3.5
	1.0	40.5 ± 1.5
$H_2C{=}CHCHO$	0.2	5.5 ± 2.4

[a] Typical control values for mitochondrial AlDH activity in intact and disrupted rat liver mitochondria were 17.2 ± 0.3 and 28.4 ± 0.9 nmol AcH oxidized min^{-1} (mg of protein)$^{-1}$. With intact mitochondria, the assay is specific for the low K_m AlDH isozyme, whereas, both the low and high K_m isozymes contribute to the measured AlDH activity using disrupted mitochondria and exogenous NAD^+. Separate controls were run for each different drug vehicle used.

intact and osmotically disrupted mitochondria (Table II). Inhibition of AlDH in the latter assures that the inhibition observed in intact mitochondria was not due to inhibitory effects on the reoxidation of intramitochondrial NADH by the respiratory enzymes. This was not the case, however, for the diallyl analogue **10**, which was not inhibitory in the osmotically disrupted mitochondrial system.

Acrolein and propiolaldehyde, when administered intraperitoneally to rats, did not elevate blood acetaldehyde levels when measured at 2 h (1 h after an ethanol dose; data not shown) very likely because of their extreme reactivity and their failure to reach the hepatic mitochondrial sites for AlDH. Administration of the aminohydrin (**2**) or the hydrate (**3**) of cyclopropanone to rats and mice, respectively, has been shown to significantly elevate blood acetaldehyde by two independent groups of investigators,[6a,b] due very likely to the relative metabolic stability of cyclopropanone hydrate. Chloral hydrate slightly elevated blood AcH (17.4 ± 0.85, $p < 0.03$), but others have reported no in vivo activity with this compound.[10]

Discussion

The fact that many of the compounds of this series, viz., **5**, **6**, **8**, **9**, and **10**, significantly elevated ethanol-derived blood acetaldehyde in vivo at 9 h but not at 2 h suggests that they might require bioactivation. These compounds were designed to allow the liver microsomal enzymes to dealkylate the R′ side chain, thereby liberating cyclopropanone, acrolein, or chloral. While we did not attempt to detect their formation in vivo, these putative metabolites were good inhibitors of AlDH in vitro. The aldehyde

products of the competitive dealkylation reactions, viz., formaldehyde and benzaldehyde, are actually substrates for AlDH,[12] lending support to our premise that products of dealkylation of the R' side chain must be involved in the inhibition of AlDH. The 2,2,2-trichloroethyl analogue **7** was anomalous in this regard as it inhibited AlDH of rat liver mitochondria in vitro (Table II) yet elicited only a time dependent increase in blood acetaldehyde in vivo (Figures 1 and 2). It is possible that mitochondrial monoamine oxidase is responsible for its bioactivation. Although **11** showed statistically significant activity at 9 h, the elevation of blood acetaldehyde was only twice that for controls (Figure 2) and therefore not biologically relevant.

We recognize that the allyl compounds **6**, **9**, and **10** can theoretically be metabolized to epoxides or *N*-oxides that may also be active and that the metabolism of **8** by hepatic cytochrome P-450 in vitro can lead to free-radical products that inactivate cytochrome P-450.[13] We are also aware of the potential toxicity of compounds that generate highly reactive products by metabolism. For example, (a) compounds **6** and **9** were toxic at doses > 1 mmol/kg, presumably due to the metabolic generation of acrolein, and (b) we ourselves have shown tht **4** administered in large doses to rats produces centrilobular necrosis of the liver as a consequence of excessive propiolaldehyde generation and hepatic glutathione depletion.[14] Nevertheless, our objective of designing latent inhibitors of AlDH by incorporation into the molecular structure groups that could generate inhibitors of this enzyme by metabolic action appears to have been fulfilled, with the cyclopropyl analogue **5** and **8** being the most promising of this series.

It has long been recognized that certain individuals—particularly of Oriental origin—have a natural aversion to alcohol, i.e., they sustain a mild DER manifested by a flushing syndrome on consumption of only modest quantities of alcoholic beverages, thereby self-imposing sobriety.[15] Recently, it was found that these individuals lack the low K_m hepatic AlDH isozyme[16] —an enzyme that appears to be the same as that inhibited in vivo by disulfiram,[17] pargyline, and cyanamide. The low rate of alcoholism in Orientals compared to that in individuals of European descent[16d] suggests that a rational therapeutic basis for the treatment of chronic alcoholics is to inhibit in these individuals the same enzyme that is genetically deficient in 50% of Orientals, thus mimicking the natural alcohol aversion phenomenon. The present work represents our continuing efforts at ultimately developing such agents.

Experimental Section

Spectrophotometers used were as follows: IR, Beckman IR-10; NMR, Varian T-60A ($(CH_3)_4Si$ internal standard). For gas chromatography (GC), a Packard Instrument Model 419 gas chromatograph equipped with a flame ionization detector and an Autolab 6300 digital integrator were used. Pargyline hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO. Abbreviations: AcH = acetaldehyde.

***N*-Cyclopropylbenzylamine (8).** This compound was prepared by catalytic (PtO_2) hydrogenation of *N*-benzylidenecyclopropylamine according to Bumgardner et al.:[18] 81% yield; bp 77.5–79.4 °C (3.0–3.4 mm) [lit.[18] bp 53 °C (0.2 mm)]; sp gr 0.95; NMR ($CDCl_3$) δ 7.23 (s, 5 H, phenyl), 3.80 (s, 2 H, benzyl), centered 2.10 (m, 1 H, CH<), 1.77 (s, 1 H, NH), centered, 0.42 (m, 4 H, CH_2CH_2).

***N*-Methyl-*N*-cyclopropylbenzylamine (5).** A mixture of compound **8** (29.4 g, 0.200 mol), 120 mL of 88% formic acid, and 56 mL of 37% aqueous formaldehyde in 500 mL of absolute ethanol was heated under reflux for 20 h. After removal of most of the ethanol by distillation, the cooled residue was made basic to pH 11 with 6 N NaOH. The resulting emulsion was diluted to 450 mL with H_2O and the mixture was extracted with ether for 12 h in a continuous liquid–liquid extractor. The ether extract was concentrated and the residual oil was distilled at 40–72 °C (1.2–2.3 mm). This crude product was redistilled by using a Teflon spinning-band column to give 23.2 g (72% yield) of **5**: bp 83.8–84.8 °C (8.2–9 mmHg); n^{21}_D 1.5067, sp gr 0.91 [lit.[19] bp 56–57 °C (4 nm); n^{25}_D 1.5061]; NMR ($CDCl_3$) δ 7.20 (s, 5 H, phenyl), 3.60 (s, 2 H, benzyl), 2.20 (s, 3 H, CH_3), centered 1.67 (m, 1 H, CH<) 0.38, 0.47 (2 s, 4 H, CH_2CH_2).

***N*-Allylbenzylamine (9) and *N*,*N*-Diallylbenzylamine (10).** These were prepared by alkylation of benzylamine with allyl bromide as described:[20] bp (for **9**) 97–101 °C (9.4–10.4 mmHg) [lit.[10] bp 63 °C (0.75 mm)]; bp (for **10**) 101–102 °C (4.4 mmHg) [lit.[10] bp 73 °C (1 mm)]. The NMR spectra of **9** and **10** in $CDCl_3$ were essentially identical with the spectra reported for these compounds.

***N*-Methyl-*N*-allylbenzylamine (6).** To a cooled solution of *N*-methylbenzylamine (24.2 g, 0.200 mol) in 200 mL of hexane was added allyl bromide (24.20 g, 0.200 mol) in 200 mL of hexane followed by 100 mL of 2 N aqueous NaOH with stirring, while the temperature was maintained below 20 °C. The mixture was stirred for 6 h and then allowed to stand at room temperature for 42 h. A precipitate that had formed between the layers dissolved on stirring. The hexane layer was decanted and the aqueous phase extracted twice with 30 mL of ether. The combined hexane and ether solution was concentrated and the residue distilled to give 18.2 g of crude distillate boiling at 87–103 °C (18 mmHg). Careful redistillation gave 4.99 g of **6**: bp 74.6–78.4 °C (18 mm) [lit.[21] bp 255–256 °C (760 mm)]; NMR ($CDCl_3$) δ 7.23 (m, 5 H, phenyl), centered 5.93 (m, 1 H, CH=), centered 5.13 (m, 2 H, $=CH_2$), 3.47 (s, 2 H, benzyl), 2.98 (dd, 2 H, J = 6 Hz, NCH_2), 2.0 (s, 3 H, CH_3).

***N*-Methyl-*N*-(2,2,2-trichloroethyl)benzylamine (7).** This is an adaptation of the procedure of Sekiya et al.[22] except that

(12) (a) Eckfeldt, J. H.; Yonetani, T. In "Methods in Enzymology"; Wood, W. H., Ed., Academic Press: New York, 1978; pp 474–479. (b) Eckfeldt, J. H.; Mope, L.; Takio, K.; Yonetani, T. *J. Biol. Chem.* **1976**, *251*, 236.

(13) (a) Hanzlik, R. P.; Tullman, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2048. (b) Macdonald, T. L.; Zirvi, K.; Burka, L. T.; Peyman, P.; Guengerich, F. P. *J. Am. Chem. Soc.* **1982**, *104*, 2050.

(14) DeMaster, E. G.; Sumner, H. W.; Kaplan, E.; Shirota, F. N.; Nagasawa, H. T. *Toxicol. Appl. Pharmacol.* **1982**, *65*, 390.

(15) (a) Wolff, P. H. *Science* **1972**, *175*, 449. (b) Wolff, P. H. *Am. J. Hum. Genet.* **1973**, *25*, 193. (c) Ewing, J. A.; Rouse, B. A.; Pellizzari, E. D. *Am. J. Psychiatry* **1974**, *131*, 206.

(16) (a) Harada, S.; Misawa, S.; Agarwal, D. P.; Goedde, H. W. *Am. J. Hum. Genet.* **1980**, *32*, 8. (b) Teng, Y. S. *Biochem. Genet.* **1981**, *19*, 107. (c) Goedde, H. W.; Agarwal, D. P.; Harada, S. *Enzyme* **1980**, *25*, 281. (d) Goedde, H. W.; Agarwal, D. P.; Harada, S.; Meer-Tackmann, D.; Roufu, D.; Bienzle, U.; Kroeger, A.; Hussein, L. *Am. J. Hum. Genet.* **1983**, *35*, 769. (e) Goedde, H. W.; Harada, S.; Agarwal, D. P. *Hum. Genet.* **1979**, *51*, 311.

(17) Harada et al. (Harada, S.; Agarwal, D. P.; Goedde, H. W. *Subst. Alcohol. Actions/Misuse* **1982**, *3*, 107) have reported that, although disulfiram does not inhibit the low K_m AlDH of liver in vitro, a metabolite of disulfiram, viz., diethylamine, is a good inhibitor of this isozyme (K_i = 0.1 mM). On this basis, they suggest a commonality in the mechanism of the DER and alcohol sensitivity in Orientals. However, when we administered diethylamine to rats (0.625 and 1.25 mmol/kg, ip, n = 3) in experiments similar to that described in Figures 1 and 2, we did not observe any increases in ethanol-derived blood acetaldehyde.

(18) Bumgardner, C. L.; Lawton, E. L.; Carver, J. C. *J. Org. Chem.* **1972**, *37*, 407.

(19) Horrom, B. W.; Martin, W. B. U.S. Patent 3083226 (CL. 260-271), March 26, 1967 (to Abbot Laboratories); *Chem. Abstr.* **1963**, *59*, 9888.

(20) Jones, J. B.; Hysert, D. W. *J. Org. Chem.* **1971**, *49*, 325.

(21) Emde, H.; Schellbach, H. *Arch. Pharm. (Weinheim, Ger.)* **1911**, *249*, 111.

(22) Sekiya, M.; Matsuda, O.; Ito, K. *Chem. Pharmacol. Bull.* **1975**, *23*, 1579.

the product was not distilled, since, in our hands, some decomposition appeared to take place on distillation. A solution of *N*-methyl-*N*-(butoxymethyl)benzylamine[23] (36.0 g, 0.174 mol) and trichloroacetic acid (85.1 g, 0.521 mol) in 175 mL of dioxane was heated with stirring at 65 °C for 8 h. The dioxane was evaporated under reduced pressure and the residue was made basic with 200 mL of 1.25 N NaOH. After extraction with ether (4 × 100 mL), the combined ether extract was dried (Na_2SO_4) and evaporated to give 34.1 g of an amber oil. This product was dissolved in a minimum amount of hexane–methylene chloride (2:1) and percolated through a 4.5 × 25 cm column of silica gel (30–70 mesh) eluting with the same solvent mixture. The residue (21.3 g) from the first 900 mL of eluate which still showed presence of impurities (by TLC on silica gel plates) was redissolved in hexane and chromatographed once again on a 4.5 × 43 cm column of silica gel (30–70 mesh) using hexane as eluant. The first 800 mL of eluate was collected and the solvent evaporated to yield 15.0 g (34% yield) of a colorless oil, n^{21}_D 1.5530 (lit.[22] n^{22}_D 1.5340). The NMR spectrum in $CDCl_3$ was essentially identical with that reported. Anal. ($C_{10}H_{12}NCl_3$) H, N; C: calcd, 47.55; found, 47.00.

***N*-Methyl-*N*-propylbenzylamine (11) and *N*-Methyl-*N*-isopropylbenzylamine (12).** These were produced by reduction of the Schiff bases prepared by condensation of benzaldehyde with *n*-propylamine or isopropylamine, followed by reductive methylation as described above for the synthesis of **5**. For **11**: bp 74–77 °C (5 mmHg) [lit.[24] bp 96–98 °C (15 mm)]; n^{22}_D 1.4945; NMR ($CDCl_3$) δ 7.33 (s, 5 H, phenyl), 3.48 (s, 2 H, benzyl), 2.18 (s 3 H, NCH_3), 2.35, centered 1.52, 0.90 (t, 2 H, J = 6 Hz; m, 2 H; t, 3 H, J = 6 Hz, $CH_2CH_2CH_3$). For **12**: 79–82 °C (5 mmHg); n^{22}_D 1.4979; NMR ($CDCl_3$) δ 7.33 (s, 5 H, phenyl), 3.52 (s, 2 H, benzyl), centered 2.87 (m, 1 H, CH), 2.17 (s, 3 H, NCH_3), 1.07 (d, 6 H, J = 6 Hz, CH_3).

Biological Evaluation. (a) Elevation of Ethanol-Derived Blood AcH in Rats. Solutions of the test compounds for injections (Figures 1 and 2) were made up at two concentrations, viz., 0.125 and 0.0625 M. It was necessary to convert the amines to their hydrochloride forms to achieve solution and the final pH values varied from 1.9 to 5.5. Compound **7** was insoluble in dilute HCl and was therefore administered as a suspension formulated in 2% aqueous (carboxymethyl)cellulose. Male random bred rats of Sprague–Dawley descent (BioLab Corp., St. Paul, MN) were fasted overnight and the test compounds (0.625 or 1.25 mmol/kg, ip) were administered 2 or 9 h before sacrifice. Ethanol (2.0 g/kg, ip) was administered 1 h before sacrifice. Control animals received saline in place of the drug. The rats were stunned with a blow to the head and blood was withdrawn by cardiac puncture. Aliquots (0.2 mL) were placed in 20-mL serum vials containing 1.0 mL of 5 mM sodium azide and 0.8 μmol of *n*-propyl alcohol (the latter as internal standard for GC). The vials were capped, frozen on dry ice and kept frozen at −78 °C until assayed for AcH by head-space gas chromatography.[25] In this procedure, artifactual formation of acetaldehyde from ethanol in blood is inhibited by sodium azide.[26]

(b) Liver Mitochondrial AlDH Activity after Administration of 5 and 8 to Rats (Table I). Two hours after administration of 1.25 mmol/kg, ip, of **5** and **8** (or saline and **4** for controls), the rats were sacrificed and the intact liver mitochondria were prepared and assayed for AlDH activity as described below.

(c) In Vitro Effects of Pargyline Analogues and Their Putative Metabolites on Rat Liver Low K_m Mitochondrial AlDH (Table II). Liver mitochondria were isolated in 0.25 M sucrose–0.1 mM EDTA (pH 7.5) from overnight-fasted male rats weighting 180–250 g. The mitochondria were washed once with 0.25 M sucrose–0.1 mM EDTA and twice with 0.25 M sucrose before use. Proteins were determined by the method of Lowry et al.[27] with bovine serum albumin as the standard.

The activity of the low K_m mitochondrial AlDH isozyme was assayed in intact rat liver mitochondria by measuring the rate of AcH disappearance from a closed incubation system essentially as previously described.[28] This assay system is dependent on intramitochondrial oxidation of NADH and is specific for the low K_m isozyme located in the matrix of the mitochondria. The incubation mixture (1.0 mL) contained 0.25 M sucrose, 5 mM $MgSO_4$, 1.0 mM EDTA, 10 mM KCl, 10 mM sodium phosphate (pH 7.5), and rat liver mitochondria. Following a 5-min preincubation at 38 °C, the reaction was initiated by the addition of AcH (200 nmol) and, after a second 5-min period, was quenched with $HClO_4$ (final concentration, 0.5 N). In Table II, mitochondria were osmotically disrupted by omission of the sucrose from the media and AlDH activity was supported by 1.0 mM exogenous NAD^+.

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(23) Ito, K.; Oba, H.; Sekiya, M. *Chem. Pharmacol. Bull.* **1975**, *20*, 2112.
(24) Buck, J. S.; Baltzly, R. *J. Am. Chem. Soc.* **1944**, *63*, 1964.
(25) Nagasawa, H. T.; Goon, D. J. W.; DeMaster, E. G.; Alexander, C. S. *Life Sci.* **1977**, *20*, 187.
(26) DeMaster, E. G.; Redfern, B.; Weir, E. K.; Pierpont, G. L.; Crouse, L. J. *Alcohol.: Clin. Exp. Res.* **1983**, *1*, 436.
(27) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
(28) DeMaster, E. G.; Nagasawa, H. T. *Res. Commun. Chem. Pathol. Pharmacol.* **1978**, *21*, 497.