Prodrugs of Nitroxyl as Inhibitors of Aldehyde Dehydrogenase

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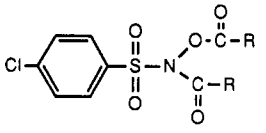
In the preceding paper, analogs of chlorpropamide with an OMe substituent on the sulfonamide nitrogen were shown to inhibit aldehyde dehydrogenase (AlDH), and it was postulated that these compounds were bioactivated by O-demethylation to release nitroxyl (HN=O, nitrosyl hydride), which is an inhibitor of AlDH. Further evidence for the production of nitroxyl from compounds with *O*-acyl instead of OMe on the sulfonamide nitrogen is now presented. Thus, nitrous oxide (N_2O), the end product of nitroxyl dimerization and disproportionation, was found to be generated on alkaline or enzymatic hydrolysis of N,O-diacylated *N*-hydroxyarylsulfonamides. Since the latter compounds strongly inhibit yeast AlDH in vitro after bioactivation by an esterase intrinsic to this enzyme, nitroxyl generated from these compounds must be the common intermediate that inhibits AlDH.

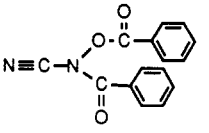
In the preceding paper,[1] it was shown that (arylsulfonyl)ureas and (arylsulfonyl)carbamates with an OMe substituent on the sulfonamide nitrogen exemplified by the general structure I, inhibited aldehyde dehydrogenase (EC 1.2.1.3; AlDH) when administered to rats, as evidenced by the quantum elevation of blood acetaldehyde (AcH) levels when ethanol was given subsequent to I. It was postulated that compounds such as I undergo metabolic O-demethylation in vivo to give compounds represented by II, which disproportionate nonenzymatically to products that solvolyzed to nitroxyl (nitrosyl hydride, HN=O), the putative inhibitor of AlDH (Scheme I). Since nitroxyl has also been shown to be the AlDH inhibitor produced by metabolic activatioin of cyanamide,[2] a clinically prescribed alcohol deterrent agent, this hypothesis that unifies the mechanism of action of two structurally dissimilar classes of compounds that inhibit AlDH in vivo by bioactivation to *identical* molecular species should be deserving of corroborative experimental evidence. Such evidence, albeit deductive, will be presented herewith.

It was shown in the preceding paper that rat liver microsomes can O-demethylate compounds such as I to II in vitro, although II was not isolated and this reaction was not extensive relative to N-demethylation of the aminopyrine used as standard. Since compounds of structure II have not been described in the literature—perhaps because of their instability—and O-demethylation was at best slow in vitro, we sought to block this *N*-hydroxy group in II as the acetate or benzoate ester. This would result in compounds such as III which could liberate II in vivo by esterase rather than oxidative action. We intended to compare the AlDH-inhibitory activities in rats of compounds having structures I and III to gain further insights into the mechanism of bioactivation of these compounds.

Results and Discussion

Attempted selective O-acetylation of the synthon, *N*-hydroxy-4-chlorobenzenesulfonamide, with Ac_2O or AcCl,[3] or selective O-benzoylation with C_6H_5COCl under a variety of conditions gave the N,O-bis-acylated derivatives, **1a** and **1b**, as the only isolable products. Compound **1b** was of special interest because it bore a structural resemblance to the N,O-dibenzoylated derivative **2** of *N*-hydroxycyanamide, the latter the postulated, unstable metabolic oxidation product of cyanamide that decomposes to nitroxyl.[2b] This compound **2** was shown previously to be a good inhibitor of yeast AlDH in vitro,[4] following debenzoylation of the *O*-benzoyl moiety by esterase action intrinsic to this enzyme.[5]





	R
1a	CH_3
1b	C_6H_5

2

An alternative reaction sequence (Scheme II) was used to prepare compounds similar to the general structure III (X = O); viz., **5a** and **5b**. Thus, *N*-(*tert*-butyloxycarbonyl)hydroxylamine (**3**) was O-acetylated, and the sodium salt of this product **4a** was arenesulfonylated with 4-chlorobenzenesulfonyl chloride to give **5a**. The O-benzoylated **5b** was prepared in the same manner by initial O-benzoylation of **3**, followed by arenesulfonylation of the product, **4b**.

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(1) Lee, M. J. C.; Elberling, J. A.; Nagasawa, H. T. N[1]-Hydroxylated derivatives of chlorpropamide and its analogs as inhibitors of aldehyde dehydrogenase in vivo. *J. Med. Chem.*, preceding paper in this issue.

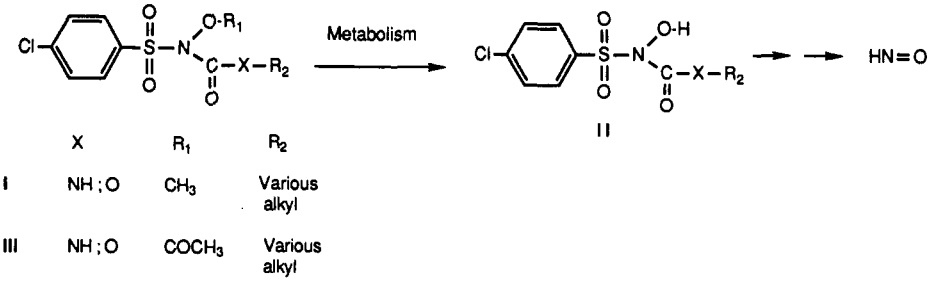
(2) (a) Shirota, F. N.; Demaster, E. G.; Nagasawa, H. T. Cyanide is a product of the catalase-mediated oxidation of the alcohol deterrent agent, cyanamide. *Toxicol. Lett.* **1987**, *37*, 7-12. (b) Nagasawa, H. T.; DeMaster, E. G.; Redfern, B.; Shirota, F. N.; Goon, D. J. W. Evidence for nitroxyl in the catalase-mediated bioactivation of the alcohol deterrent agent cyanamide. *J. Med. Chem.* **1990**, *33*, 3120-3122.

(3) Smith, P. A. S.; Hein, G. E. The alleged role of nitroxyl in certain reactions of aldehydes and alkyl halides. *J. Am. Chem. Soc.* **1960**, *82*, 5731-5740.

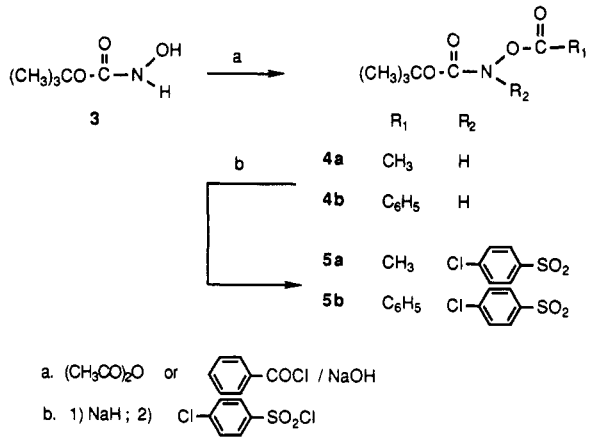
(4) Nagasawa, H. T.; Lee, M. J. C.; Kwon, C.-H.; DeMaster, E. G.; Shirota, F. N. An N-hydroxylated derivative of cyanamide that inhibits yeast aldehyde dehydrogenase. *Alcohol* **1992**, in press.

(5) Dickinson, F. M.; Haywood, G. W. The role of the metal ion in the mechanism of the K^+-activated aldehyde dehydrogenase of *Saccharomyces cerevisiae*. *Biochem. J.* **1987**, *247*, 377-384.

Scheme I



Scheme II



Because **2**, the N,O-dibenzoylated derivative of *N*-hydroxycyanamide, was active in inhibiting yeast AlDH in vitro (vide supra), we had anticipated that **1b** might also inhibit this enzyme, since O-debenzoylation of this compound by the esterase action of yeast AlDH would give an intermediate *N*-hydroxy compound that could eliminate 4-chlorobenzenesulfinic acid, thereby generating nitroxyl. The insolubility of **1b** in our enzymatic assay medium presented a severe obstacle to such in vitro experiments, and we were unable to make a valid comparison. However, the *N,O*-diacetyl derivative **1a** was sufficiently water soluble for evaluation in this system for the inhibition of yeast AlDH. Indeed, **1a** turned out to have comparable potency as **2**, with an IC_{50} of 0.039 mM (Figure 1) compared to an IC_{50} of 0.025 mM for **2**.[4]

That nitroxyl formation is the likely mechanism for this inhibitory action on yeast AlDH by **1a** was indicated by the time-dependent formation of N_2O on incubation of **1a** with yeast AlDH (Figure 2). Similar results were obtained on incubation of **1a**, **5a**, and **5b** with porcine liver esterase. Enzymatic hydrolysis of the acetyl group of **5a** by porcine liver esterase was much more rapid than the hydrolysis of the benzoyl group of **5b**, as reflected by the differential production of N_2O from these two compounds (Figure 2). Moreover, the rate of deacetylation of **1a** appeared to be much slower with yeast AlDH than with the porcine liver esterase, although the specific esterase activities of the two enzymes were not strictly comparable.

Hydrolysis of the acetyl group of **1a**, **5a**, and **5b** with dilute aquoeus NaOH likewise gave rise to time-dependent production of N_2O (Figure 3). As with the enzymatic reaction, the benzoate group of **5b** appeared to be less readily hydrolyzed in this system, and N_2O formation over 250 min was minimal.

We envision the following mechanism for the generation of nitroxyl (measured as its end product, N_2O) from compounds **1a**, **5a**, and **5b** (Scheme 3). Enzymatic

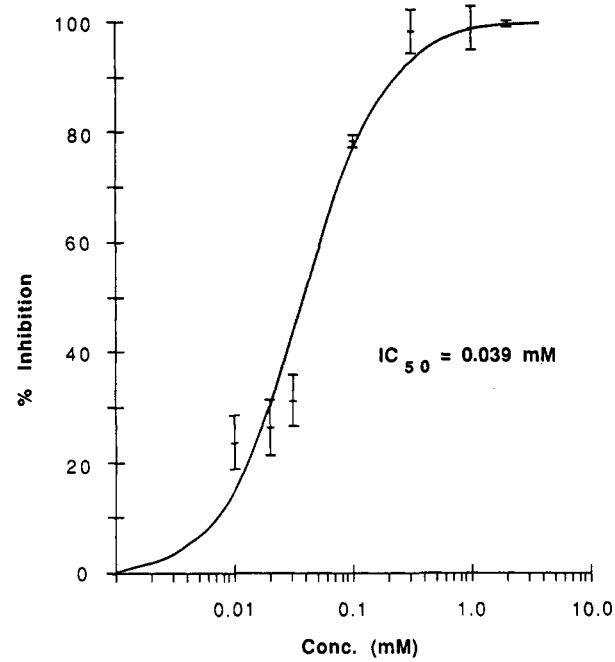


Figure 1. Concentration-dependent inhibition of yeast AlDH in vitro by the *N,O*-diacetyl derivative of 4-chloro-*N*-hydroxybenzenesulfonamide (**1a**). IC_{50} = 50% inhibitory concentration.[12]

hydrolysis of the acetyl group in **1a** or **5a**, or the benzoyl group in **5b**, would give an intermediate N-acylated N-arenesulfonylated hydroxylamine **6** which we believe to be unstable and to disproportionate to 4-chlorobenzenesulfinic acid (**7**) and an acylnitroso compound **8**. The latter, **8**, would be expected to solvolyze readily in aqueous physiological systems to nitroxyl and the corresponding carboxylic acid.[6] The fate of nitroxyl ultimately giving N_2O follows Scheme 3. In support of this mechanism, the action of porcine liver esterase on **5a** was significantly inhibited by bis(*p*-nitrophenyl) phosphate (BNPP), a specific esterase inhibitor[7] (Figure 4).

That acylnitroso compounds such as **8** do in fact solvolyze to nitroxyl was indicated by the rapid concentration dependent production of N_2O from **8a**, prepared in situ by oxidation of *N*-(*tert*-butyloxycarbonyl)hydroxylamine (**3**) with 1,2-diazenedicarboxylic acid bis-(*N',N'*-dimethylpiperazide) diiodide (DIP+2)[8] in aqueous phosphate buffer at pH 7 (Scheme 4). Although quantitative conversion was not achieved, it can be seen that N_2O production was essentially over by 10 min (Figure 5).

(6) Beckwith, A. L. J.; Evans, G. W. Reactions of alkoxy-radicals. Part III. Formation of esters from alkyl nitrites. *J. Chem. Soc.* **1962**, 130–137.
(7) Wick, M. J.; Hanna, P. E. Bioactivation of N-arylhydroxamic acids by rat hepatic N-acetyltransferase. *Biochem. Pharmacol.* **1990**, 991–1003.
(8) Kosower, E. M.; Kanety-Londner, H. Glutathione. 13. Mechanism of thiol oxidation by diazenedicarboxylic acid derivatives. *J. Am. Chem. Soc.* **1976**, *98*, 3001–3007.

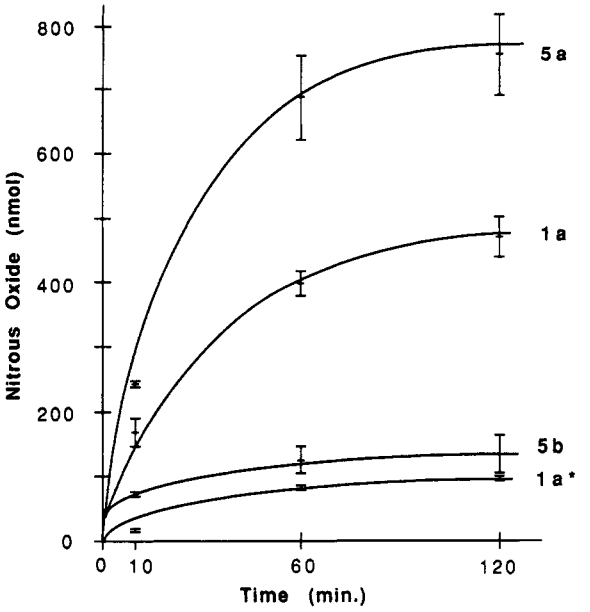


Figure 2. Generation of N_2O by the action of porcine liver esterase on the N,O-diacylated *N*-hydroxyarylsulfonamides, **1a**, **5a**, and **5b**. Generation of N_2O by the action of yeast AlDH on **1a** is indicated by **1a***. The details of the experiment and the determination of N_2O by headspace gas chromatography are described in the Experimental Section. The results are given as mean ± SEM, $n = 3$.

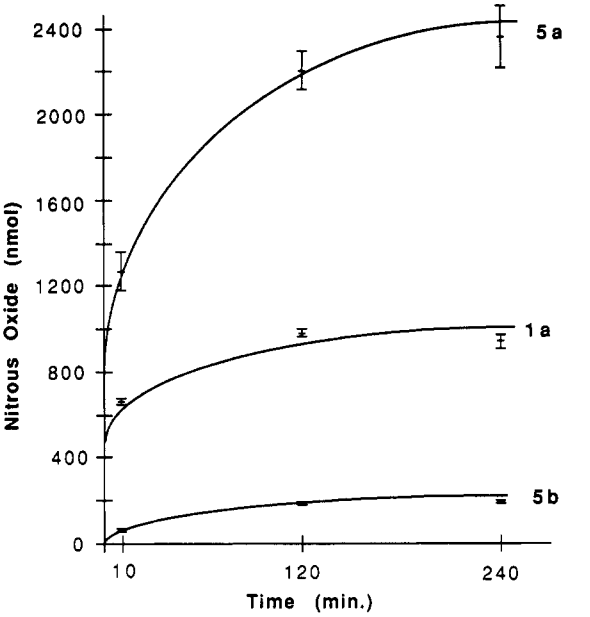


Figure 3. Generation of N_2O by hydrolysis of the N,O-diacylated *N*-hydroxyarylsulfonamides **1a**, **5a**, and **5b**, in dilute NaOH. See the Experimental Section for further details.

The relative activities of **1a**, **5a**, and **5b** in inhibiting yeast AlDH in vitro are compared in Figure 6. It is noteworthy that **1a** was the best inhibitor of this series, even though N_2O production by the action of yeast AlDH on **1a** was less than with porcine liver esterase which in turn was less than that from **5a** with the porcine esterase (Figure 2). Thus, the substrate specificity of yeast AlDH relative to its *esterase* activity must be different from that of the porcine enzyme and suggests that such differences might also be observed with the mammalian mitochondrial class II AlDH. Notwithstanding, these results lend support to our hypothesis that compounds represented by the general structure II are unstable and disproportionate to give 4-chlorobenzenesulfinic acid and acylnitroso intermediates that solvolyze to nitroxyl. Compounds that can release potential nitroxyl by metabolic action are, by definition, prodrugs of nitroxyl, and our observation that **1a**, **5a**, and **5b** inhibit yeast AlDH with intrinsic esterase activity suggests that these compounds are esterase-activated prodrugs of nitroxyl.

Whereas compounds of structure I were good inhibitors of the hepatic low Km AlDH when administered to rats in vivo, as reflected by the elevation of blood AcH levels following ethanol,[1] in preliminary experiments with a limited number of animals, the ester prodrugs **5a** and **5b** were found to be inactive in vivo (data not shown). This lack of in vivo activity of these ester prodrugs of nitroxyl is likely due to premature hydrolysis of the ester group, possibly by plasma esterases and lack of targeting to the liver. Nevertheless, the cumulative in vitro data presented here clearly implicate nitroxyl, generated by esterase action on these compounds, as the reactive species that inhibits AlDH. By deduction, the hepatic microsomal O-demethylase activated compounds that inhibit AlDH in rats and represented by structure I (see preceding paper[1]) must also give rise to nitroxyl in vivo. These latter compounds are therefore prodrugs of nitroxyl that are bioactivated by the microsomal O-demethylase of liver.

Experimental Section

The preceding paper[1] should be consulted for the general experimental parameters. Chemical ionization mass spectra were provided by the University of Minnesota Mass Spectrometry Service using a Finnegan 4000 mass spectrometer.

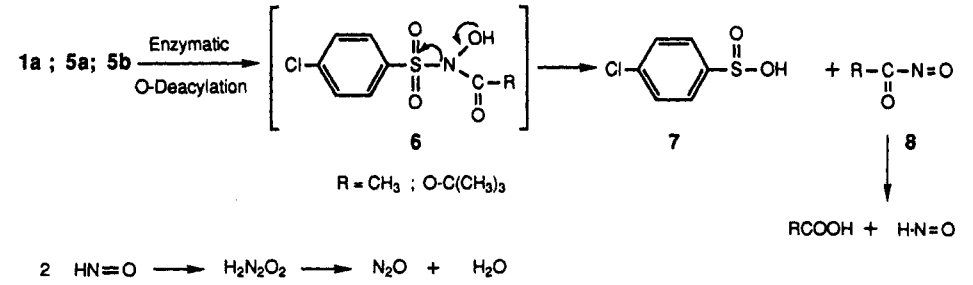
***N*-Acetyl-*N*-(acetyloxy)-4-chlorobenzenesulfonamide (1a).** To a cooled (ice bath) stirred solution of 4-chloro-*N*-hydroxybenzenesulfonamide[9] (1.04 g, 5 mmol) in 10 mL of THF (HPLC grade), acetic anhydride (0.95 g, 0.95 mL, 10 mmol) was added dropwise, followed by pyridine (1.02 g, 0.81 mL, 10 mmol). The reaction mixture was allowed to come to room temperature over 1 h. The THF was then evaporated, and the residue was treated with cold H_2O (20 mL). The solid which formed was collected and dissolved in EtOAc, and the solvent was dried over Na_2SO_4 and evaporated to dryness. The residue was recrystallized from Et_2O to give fluffy white crystals: 0.85 g (58% yield); mp 124–125 °C; IR (KBr, cm^{-1}) 1806 (O—C=O), 1736 (N—C=O), 1388 and 1175 (SO_2); ^1H NMR (200 MHz, δ) 2.18 (s, 3 H, $NCOCH_3$), 2.29 (s, 3 H, $OCOCH_3$), 7.49–7.99 (q, 4 H, 1,4-disubstituted benzene); ^{13}C NMR (δ) 17.90 (1 C, $OCOCH_3$), 22.51 (1 C, $NCOCH_3$), 129.5 and 130.6 (4 C, aromatic), 135.6 and 141.7 (2 C, aromatic substituted), 167.1 (ArCON), 167.6 (ArCOON). Anal. ($C_{10}H_{10}ClNO_5S$) C, H, N.

***N*-Benzoyl-*N*-(benzoyloxy)-4-chlorobenzenesulfonamide (1b).** To a cooled (ice bath) stirred solution of 4-chloro-*N*-hydroxybenzenesulfonamide (1.04 g, 5 mmol) in 10 mL of THF (HPLC grade), benzoyl chloride (1.41 g, 1.16 mL, 10 mmol) was added dropwise, followed by pyridine (0.79 g, 0.81 mL, 10 mmol). The reaction mixture was allowed to come to room temperature, and the precipitate of pyridine hydrochloride was removed by filtration. The filtrate was cooled until white crystals appeared; these were collected and recrystallized from THF, yielding 1.25 g (60% yield) of **1b**: mp 132–134 °C, IR (KBr, cm^{-1}) 1775 (C=O), 1698 (N—C=O), 1381 and 1176 (SO_2); ^1H NMR (300 MHz, δ) 7.44–8.18 (aromatic H); ^{13}C NMR (300 MHz, δ) 125.7–141.7 (18 C, aromatic), 163.5 (ArCOON), 168.3 (ArCO); CI-MS (CH_4) (positive ion) 105 (100) $[C_6H_5CO]^+$, 77 (8) $[C_6H_5]^+$; (negative ion) 294 (62) $[M - 121]^-$, 240 (69) $[C_6H_5COONCOC_6H_5]^-$, 121 (100) $[C_6H_5COO]^-$. Anal. ($C_{20}H_{14}ClNO_5S$) C, H, N.

(Acetyloxy)[(4-chlorophenyl)sulfonyl]carbamic Acid 1,1-Dimethylethyl Ester (5a). (Acetyloxy)carbamic acid 1,1-

(9) Przybylski, J.; Kupryszewski, G. O-[N'-Acylaminoacyl]-N-[arylsulfonyl]-hydroxylamines and their application in synthesis of peptide bonds. *Rocz. Chem.* **1975**, *49*, 529–537.

Scheme III



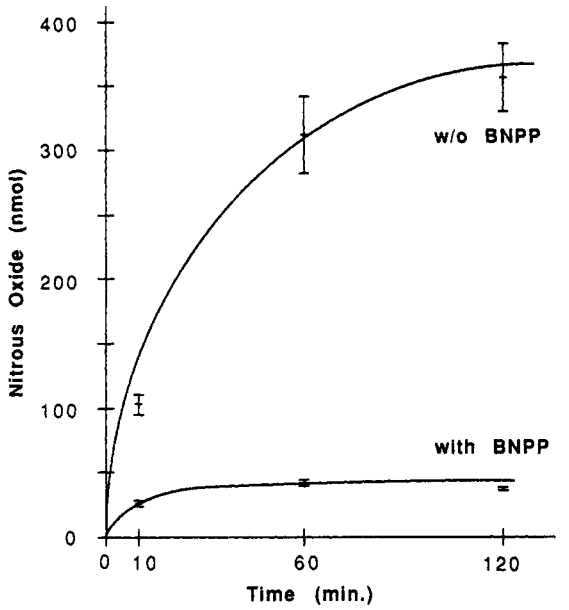
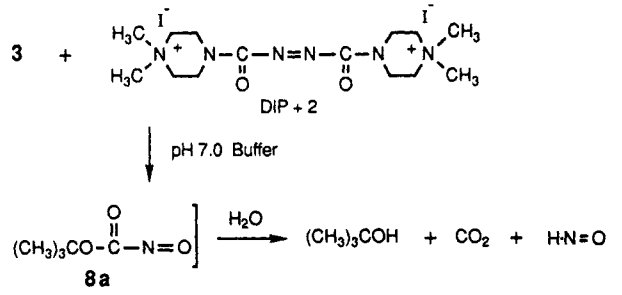


Figure 4. Inhibition of the action of porcine liver esterase on **5a** by BNPP. Details of this experiment are described in the Experimental Section.

Scheme IV



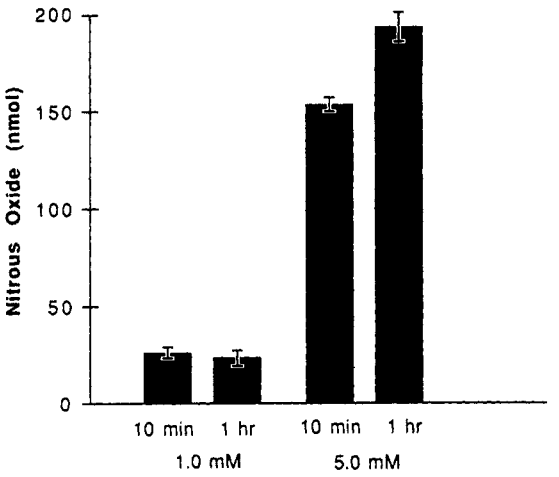


Figure 5. Generation of N_2O by oxidation of **3** with DIP+2. The concentrations of **3** are as indicated. See the Experimental Section for further details.

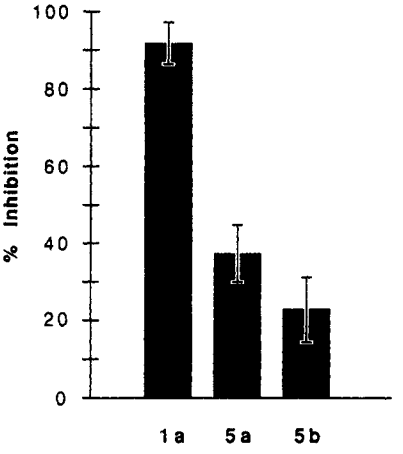


Figure 6. Inhibition of yeast AlDH by the N,O-diacylated *N*-hydroxyarylsulfonamides, **1a**, **5a**, and **5b**. Details of this experiment are described in the Experimental Section.

dimethylethyl ester[10] (0.81 g, 4.6 mmol) was dissolved in 5 mL of dimethylacetamide, and NaH (0.22 g of a 50% dispersion, 4.6 mmol) was added. This mixture was stirred for 30 min after which time 4-chlorobenzenesulfonyl chloride (0.97 g, 4.6 mmol) was added and the reaction allowed to proceed at room temperature for 2 h. The mixture was then added to 25 mL of H_2O and extracted with ether (4 × 15 mL). The ether layer was separated and evaporated on the steam bath, and replaced with CH_2Cl_2 (30 mL). After drying, the mixture was concentrated to a clear oil, which was applied to a silica gel column and eluted with THF–hexane, 1:5. Fractions 9–13 were collected and the solvent evaporated to give **7a** as a white, fluffy solid: 0.44 g (27% yield); IR (KBr, cm^{-1}) 1814 (acetyl carbonyl), 1762 (BOC carbonyl), 1389 and 1191 (SO_2); ^1H NMR (200 MHz, δ) 1.40 (s, 9 H, $(CH_3)_3CO$), 2.28 (s, 1 H, $COCH_3$), 7.51–7.99 (q, 4 H, 1,4-disubstituted benzene); ^{13}C NMR (δ) 17.7 (1 C, $COCH_3$), 27.78 (3 C, $(CH_3)_3CO$), 86.75 ($(CH_3)_3CO$), 129.2 and 130.4 (4 C, aromatic), 136.2 and 141.1 (aromatic, substituted), 148.4 (1 C, carbamate carbonyl), 167.2 (CH_3CO). Anal. ($C_{13}H_{16}ClN_2O_6S$) C, H, N.

(Benzoyloxy)[(4-chlorophenyl)sulfonyl]carbamic Acid 1,1-Dimethylethyl Ester (5b). (Benzoyloxy)carbamic acid 1,1-dimethylethyl ester[10] (0.47 g, 2.0 mmol) was stirred in 10 mL of *N,N*-dimethylacetamide, and NaH (0.1 g of a 50% dispersion, 2 mmol) was added. This mixture was stirred for 30 min and 4-chlorobenzenesulfonyl chloride (0.42 g, 2 mmol) was added. The reaction was allowed to proceed at room temperature for 18 h, after which time the mixture was added to 25 mL of H_2O and extracted with ether (4 × 15 mL). The ether was evaporated on the steam bath and replaced with CH_2Cl_2 (30 mL). The solvent was dried and concentrated to a clear oil which solidified when placed in the freezer compartment of a refrigerator. The solid was collected, washed with H_2O, and redissolved in EtOAc. Recrystallization from EtOAc–hexane gave 0.44 g of **7b** (54%

(10) Carpino, L.; Giza, C.; Carpino, B. O-Acylhydroxylamines. I. Synthesis of O-benzoylhydroxylamine. *J. Am. Chem. Soc.* **1959**, *81*, 955–957.

yield): mp 133–138 °C; IR (KBr, cm^{-1}) 1779 (PhC=O), 1757 (BOC C=O), 1387 and 1149 (SO_2); ^1H NMR (200 MHz, δ) 1.41 (s, 9 H, $(CH_3)_3CO$), 7.26–8.13 (m, 9 H, aromatic H's); ^{13}C NMR (δ) 27.62 (3 C, $(CH_3)_3CO$), 86.79 ($(CH_3)_3CO$), 126.1 (1 C, aromatic), 129.8, 129.2, 130.3, and 130.6 (8 C, aromatic), 134.5, 136.3, and 141.2 (3 C, aromatic, substituted), 148.5 (1 C, $(CH_3)_3COCON$), 163.6 (PhCO). Anal. ($C_{18}H_{18}ClNO_6S$) C, H, N.

Headspace Gas Chromatographic Analysis of N_2O. (a) By Action of Yeast AlDH on 1a. Gas chromatography bottles (25 mL) containing 0.5 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.5 mL of 0.15 mM KCl, and 0.70 mL of H_2O were prepared in triplicate. Compound **1a** was dissolved in DMSO at a concentration of 200 mM, and a 100-μL aliquot was added to the GC bottle, followed by addition of 200 μL of yeast AlDH (Sigma Lot 12H820; 11 mg of protein/mL; 230 units/mg of protein) to initiate the reaction. The bottles were immediately stoppered and placed in a 37 °C shaking water bath and sampled periodically for N_2O determination by gas chromatography as previously described.[2b] Controls contained 200 μL of H_2O in place of yeast AlDH.

(b) By Base Hydrolysis of 1a, 5a, and 5b. Gas chromatography bottles (25 mL) containing 1.25 mL of 80 mM potassium phosphate buffer (pH 7.0) and 0.35 mL of H_2O were prepared in triplicate for each sample. The compounds to be tested were dissolved in THF at a concentration of 100 mM, and an aliquot (200 μL) was added to the GC bottle, followed by 200 μL of 1 N NaOH. Depending on the compound tested, the final concentrations of the sample in the reaction mixture were 1, 5, or 10 mM. The bottles were incubated and the N_2O was determined as above.

(c) By Action of Porcine Liver Esterase on 1a, 5a, and 5b. Gas chromatography bottles (25 mL) containing 1.25 mL of 80 mM potassium phosphate buffer (pH 8.0) and 0.525 mL of H_2O were prepared in triplicate for each sample. The compounds to be tested were dissolved in THF at a concentration of 100 mM, and an aliquot (200 μL) was added to the GC bottle, followed by addition of 25 μL of porcine liver esterase (Sigma Lot 107F-8235; 19 mg of protein/mL; 335 units/mg of protein) to initiate the reaction. The bottles were immediately stoppered and placed in a 37 °C shaking water bath for 10 min before sampling for N_2O determination as above. Controls contained 25 μL of H_2O in place of porcine liver esterase.

Inhibition of Porcine Liver Esterase on 5a by Bis(*p*-nitrophenyl)phosphate (BNPP). Gas chromatography bottles (25 mL) containing 1.00 mL of 100 mM potassium phosphate buffer (pH 8.0) and 0.8 mL of 37.5 mM BNPP were prepared in quadruplicate for each run. A solution of (100 μL) porcine liver esterase (Sigma Lot 29F-8055; 11 mg of protein/mL; 230 units/mg of protein) was diluted with H_2O (1.53 mL) to give an activity of 100 μmol/mL per min. A solution of **1a** in THF was made at a concentration of 100 mM, and an aliquot (100 μL) was added to the GC bottle, followed by addition of 100 μL of esterase solution to initiate the reaction. The ratio of BNPP concentration to substrate concentration was 3:1. The bottles were immediately stoppered and placed in a 37 °C shaking water bath. Samples for N_2O determination as described above were taken at 10, 60, and 120 min. The uninhibited reaction contained 0.8 mL H_2O in place of BNPP. Controls contained 100 μL of H_2O in place of porcine liver esterase.

N_2O Formation by the Oxidation of (Acetyloxy)carbamic Acid 1,1-Dimethylethyl Ester (3). Two reaction mixtures of **3** where the final concentrations were 1.0 and 5.0 mM in a total of 5.0 mL were prepared in phosphate buffer (pH 7.0), and 200 μL of a 100 mM aqueous solution of DIP+2 was added to each sample. The N_2O formed at 10 min and after 1 h of incubation at 55 °C was analyzed in triplicate by gas chromatography as described above.

Inhibition of Yeast AlDH. A primary mix containing 50 mM potassium phosphate buffer (pH 7.5), yeast AlDH (20–30 units/mg, 0.07 IU), 1.0 mM NAD^+, and 5 μL of the test compound dissolved in DMSO, in a final volume of 0.1 mL, was incubated at 37 °C for 10 min. A 20-μL aliquot was then removed for assay of the remaining AlDH activity as described previously.[11]

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Registry No. **1a**, 142867-52-5; **1b**, 142867-53-6; **2**, 142867-54-7; **3**, 36016-38-3; **4a**, 99768-83-9; **4b**, 105340-85-0; **5a**, 142867-55-8; **5b**, 142867-56-9; *p*-$ClC_6H_4SO_2Cl$, 98-60-2; *p*-$ClC_6H_5O_2NHOH$, 50695-53-9; AcCl, 75-36-5; PhCOCl, 98-88-4; N_2O, 10024-97-2; HNO, 14332-28-6; aldehyde dehydrogenase, 9028-86-8; esterase, 9013-79-0.

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