Nicotinamide Adenine Dinucleotide Phosphate-Decanaldehyde Adduct as an Inhibitor of Beef Brain NADP-Linked Aldehyde Reductase

John F. Towell,[1] V. Gene Erwin,*

School of Pharmacy, University of Colorado, Boulder, Colorado 80309

and Richard A. Deitrich

Department of Pharmacology, University of Colorado Medical School, Denver, Colorado 80220. *Received December 15, 1978*

The synthesis and characterization of the addition product of NADP and decanaldehyde are described. This adduct is an inhibitor of beef brain NADP-linked aldehyde reductase (K_i = 2.3 × 10^{-6} M) along with the NADP adducts of butyraldehyde, phenylpropionaldehyde, and phenylacetaldehyde.

The catabolic processes of biogenic amines in neurons proceed via monoamine oxidase to the aldehyde intermediates,[2] which can be oxidized to the corresponding acid by aldehyde dehydrogenase (ALDH) or reduced to the alcohol by aldehyde reductase (ALRD). It has been shown that the metabolites of dopamine and serotonin are primarily acids, whereas norepinephrine is catabolized primarily to the alcohol.[3] However, following ethanol administration, a decrease in urinary acid metabolites and an increase in alcohol derivatives are observed for these biogenic amines.[4-8] This shift in metabolism is presumed to be the result of competition with biogenic aldehydes for the aldehyde dehydrogenase by acetaldehyde, the proximal metabolite of ethanol.

Various central nervous system (CNS) effects may be related to the result of an increase in endogeneous biogenic aldehyde levels,[9] due to inhibition of ALDH or ALRD by drugs or metabolites thereof. For example, Erwin and co-workers[10] have shown that biogenic aldehydes at micromolar concentrations are potent inhibitors of (Na^+,-K^+)ATPase of brain microsomes and have reported[11] that norepinephrine uptake in brain synaptosomes was similarly inhibited. Also, biogenic aldehydes condense with biogenic amines to form tetrahydroisoquinolines (TIQ)[12] which have been observed in vivo.[13] Cohen has demonstrated that TIQ's possess marked false and surrogate neurotransmitter activities, depending on the tissue investigated.[14] Recently, Melchior and Myers[15,16] reported that cerebral intraventricular infusion of small amounts of tetrahydropapaveroline (THP) into rats which normally demonstrated an aversion to ethanol resulted in animals that voluntarily drank large quantities of ethanol solution. This induction of preference for ethanol appeared to be long lasting.

In order to further assess the pharmacological consequences of inhibition of biogenic aldehyde metabolism, we have initiated a program aimed at the synthesis of specific inhibitors of brain NADP-dependent ALRD.

Everse et al.[17] have reported the synthesis and detailed structural characterization of the addition products of NAD with oxidized substrates of NAD-linked dehydrogenases. The mechanism of the reaction is a nucleophilic attack by the base-stabilized enol form of the carbonyl-containing substrate on the highly electrophilic 4 position of the oxidized nicotinamide ring. This is followed by a second nucleophilic attack by the carboxamide nitrogen on the substrate carbonyl to form a second ring system. A diagram of the basic adduct structure is shown in Table II. These adducts exhibited specific inhibition of only the corresponding enzyme; i.e., NAD-pyruvate only significantly inhibited lactate dehydrogenase and not malate, glutamate, and alcohol dehydrogenase. Everse et al. also reported the synthesis of NAD-acetaldehyde and NAD-butyraldehyde adducts but presented detailed structural studies only on the α-keto acid adduct NAD-pyruvate. However, because the spectral properties of the aldehyde adducts were very similar to those of the α-keto acids, it was assumed that the basic ring structures of the aldehyde and α-keto adducts were identical.

Table I. Absorption Maxima of NAD(P)-Aldehyde Adducts

	reduced adduct, nm	oxidized adduct, nm	
		pH 7	pH 12
NADP-decanal	346	373	422
NAD-acetaldehyde	345	358	405[a]
NAD-butyraldehyde	344	375	419[a]
NAD-pyruvate	340	360	415[a]
	ε (× 10^3 M^{-1} cm^{-1})		$A_{260}/A_{340(6)}$
NADPH	6.22 (340 nm)		2.38
NADPH-DEC	5.88 (346 nm)		2.32

[a] Reference 17.

We have undertaken syntheses of the addition products of NADP and aldehyde substrates of ALRD in order to develop a specific inhibitor of ALRD. However, the techniques for synthesis and purification of NAD adducts described by Everse et al. were not suitable for our purposes. Because the aliphatic and aryl aldehyde substrates of ALRD are not water soluble, it was necessary to modify the reaction conditions. Also, the technique for adduct purification described by Everse et al. introduced $BaCO_3$ as an impurity, since the adduct was obtained as the barium salt. Introduction of barium, even in trace amounts, was unsuitable for in vivo studies. We report here the synthesis and characterization of an NADP-decanaldehyde (NADP-DEC) adduct employing modified reaction and purification techniques. Steady-state inhibition studies of beef brain ALRD using NADP-DEC and NADP adducts of butyraldehyde, phenylacetaldehyde, and phenylpropionaldehyde are described.

Results

The absorption spectrum of reduced NADP-DEC is very similar to that of the reduced adducts reported by Everse et al.[17] and is therefore not included. However, Table I shows a comparison of the absorption maxima of NADP-DEC with NADPH and the adducts reported by Everse et al. The extinction coefficient for NADP-DEC was calculated by correlation of phosphate determination with 346-nm absorbance. The spectral measurements were carried out in 0.1 M sodium phosphate buffer, pH 7.0. The NMR spectra in Figure 1 demonstrate the dihydronicotinamide configuration of reduced NADP-DEC and are very similar to the NMR spectra reported by Everse

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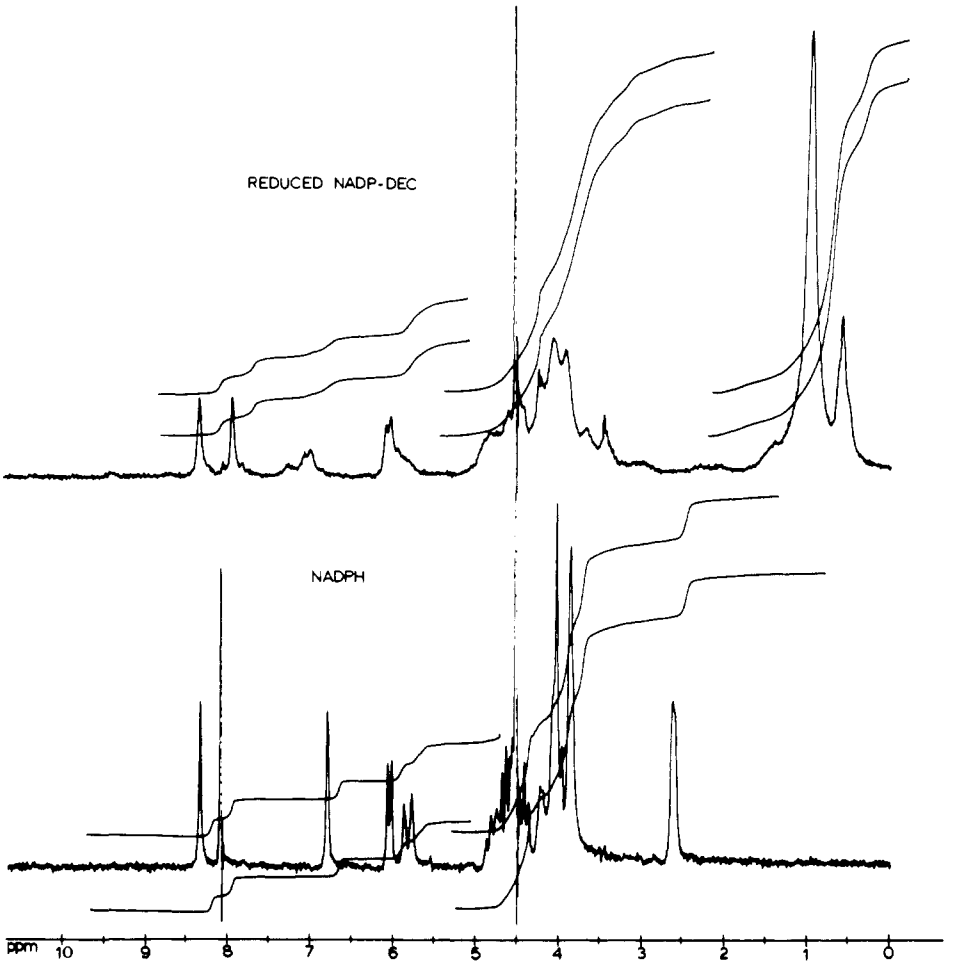
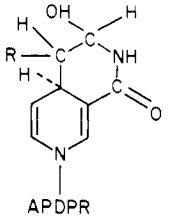


Figure 1. NMR spectra of reduced NADP–DEC and NADPH measured in D_2O at ambient temperature. Residual H_2O signal was used as the internal standard at 4.5 ppm. Peaks: 8.1 and 8.4 ppm, adenine C_2 and C_8 H, respectively; 6.7 ppm, dihydropyridine C_2 H; 6.1 ppm, adenine–ribose C_1 H and dihydropyridine C_1 H; NADP–DEC, 3.5 ppm, dihydropyridine C_4 H; NADPH, 2.6 ppm, dihydropyridine C_4 H; NADPH–DEC, 0.5–1.0 ppm, methyl and methylene protons of the decanal chain.

Table II. Inhibition of Beef Brain NADP-Dependent Aldehyde Reductase by NADP-Aldehyde Adducts[a]



aldehyde	R	var substr	fixed substr	type	K_i, M
butyraldehyde	CH_3CH_2-	NADPH	*p*-nitrobenzaldehyde	comp	1.3×10^{-5}
decanaldehyde	$CH_3(CH_2)_7$-	NADPH	*p*-nitrobenzaldehyde	comp	2.3×10^{-6}
phenylpropionaldehyde	$PhCH_2$-	NADPH	*p*-nitrobenzaldehyde	comp	7.0×10^{-6}
phenylacetaldehyde	Ph-	NADPH	*p*-nitrobenzaldehyde	comp	5.1×10^{-6}

[a] $T = 37$ °C.

et al. The broad bands in the NADP–DEC spectrum are due to the high viscosity of the solution. The integrations indicate that the methyl and methylene protons of the NADP–DEC aliphatic chain (between 0 and 2 ppm) are in proper proportion to the total number of protons present (calcd, 43.2; found, 44.9).

Figure 2 shows a pH titration of oxidized NADP–DEC which was prepared from reduced NADP–DEC according to Everse et al. The stable isosbestic point at 395 nm suggests the interconversion of the acid and base forms of the same molecule. The pH dependence of the relative absorbances at 422 (basic form) and 373 nm (acid form) was used to calculate a pK_a of 9.3. The pK_a values of the oxidized forms of NAD–pyruvate and NAD–acetone are 9.6 and 9.5, respectively.[17,18]

ALRD Inhibition. Beef brain NADP-linked aldehyde reductase was prepared and assayed according to Tabakoff and Erwin.[19] Steady-state enzyme inhibition studies were performed in sodium phosphate buffer, pH 7.0, $T = 37$ °C. The reactions were initiated by adding microliter amounts of the enzyme to a cuvette containing appropriate concentrations of *p*-nitrobenzaldehyde, NADPH, and reduced NADP–DEC. Figure 3 shows that NADP–DEC is competitive with NADPH as an inhibitor of ALRD, $K_i = 2.3 \times 10^{-6}$ M. Table II shows the results of aldehyde reductase inhibition by reduced NADP adducts of other aldehydes

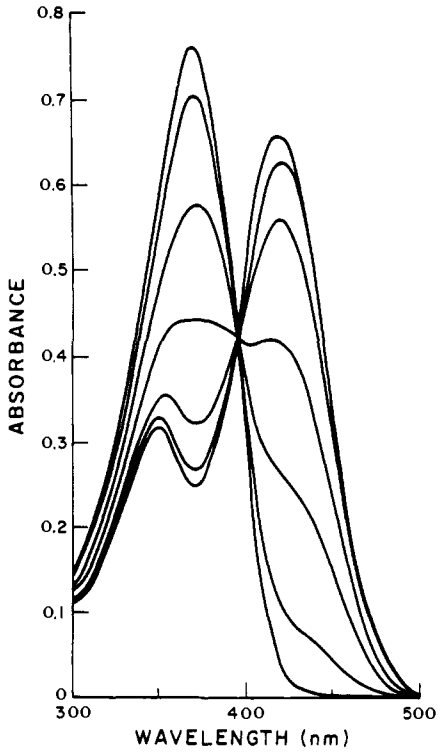


Figure 2. Titration of oxidized NADP–DEC. The decrease in absorbance at 422 nm and concurrent increase at 373 nm corresponds to pH values of 11.50, 10.48, 9.94, 9.43, 8.92, 8.12, and 7.14, respectively.

prepared as described here for NADP–DEC. The concentrations of these reduced adducts were determined by using $\epsilon_{346} = 5.88 \times 10^3\ M^{-1}\ cm^{-1}$.

Conclusions and Discussion

Erwin and Deitrich[20] and others[21,22] have observed that drugs, such as barbiturates, benzodiazepines, and anticonvulsants, which display a cross-tolerance with alcohol and are used clinically to control alcohol withdrawal seizures and other seizures are inhibitors of brain ALRD. In search for more potent and specific inhibitors of brain ALRD, which would allow further examination of the role of biogenic aldehydes in the brain, we have synthesized, using modifications of the procedures of Everse et al.,[17] the addition products of NADP with long- and short-chain aliphatic and aryl aldehydes and have developed alternative purification methods which provide a compound which is free of Ba and pure enough to be introduced into a biological system. Enzyme-inhibition studies indicate that the NADP–aldehyde adducts are competitive inhibitors of beef brain ALRD with K_i values between 10^{-6} and 10^{-5} M.

Preliminary studies have indicated that reduced NADP–DEC also inhibits other NADP-linked enzymes, e.g., glucose-6-phosphate and isocitrate dehydrogenases, and malic enzyme but not glutamate dehydrogenase. Greater specificity and potency may be achieved by forming the NADP adducts of more substrate-like aldehydes, i.e., 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycoaldehyde. These studies are currently underway.

Experimental Section

All solvents employed in the syntheses described were previously gassed with N_2.

Absorption and NMR spectra were recorded on a Beckman 25 spectrophotometer and Varian EM-390 (90 MHz) spectrometer,

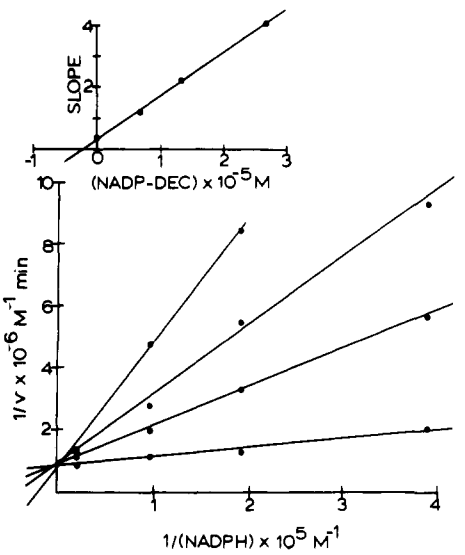


Figure 3. Lineweaver–Burke plot of the inhibition of beef brain NADP-linked aldehyde reductase by reduced NADP–DEC, T = 37 °C. *p*-Nitrobenzaldehyde was the fixed substrate at a concentration saturating the enzyme (0.25 mM). NADPH was the variable substrate. Reactions were initiated with microliter amounts of enzyme with a specific activity generally within 0.2 and 0.4 μmol of NADPH oxidized min^{-1} (mg of protein)$^{-1}$. Insert: Replot of slope vs. NADP–DEC concentration. K_i determined from x intercept of replot.

respectively. The elemental analysis was performed by Galbraith Laboratories, Knoxville, Tenn. Phosphate determinations were conducted using the King Method.[23]

Reduced NADP–Decanal. NADP (330 mg, Sigma no. N-0505) dissolved in 5 mL of H_2O and decanaldehyde (0.36 mL, Sigma no. D-7384) dissolved in 5 mL of EtOH were mixed in a two-necked round-bottom flask and degassed with N_2. The flask was sealed with a pH electrode and dropping funnel which contained 1 N NaOH. The mixture was adjusted to pH 11 with NaOH with stirring (magnetic) and maintained at pH 11 and ambient temperature for 1 h. During this time, the cloudy reaction mixture turned yellow. After 1 h, the volume of the reaction mixture was adjusted to 25 mL with H_2O, and a small aliquot was taken for absorbance measurement at 346 nm to estimate initial yield (using $\epsilon_{346} = 5.88 \times 10^3\ M^{-1}\ cm^{-1}$), which was generally 60%. Then the reaction mixture was adjusted to pH 8.5 with 1 M Tris-Cl buffer (pH 8) and then extracted three times with benzene. The aqueous solution was then washed onto a DEAE-cellulose column (100 × 1.5 cm) which had been equilibrated with 0.01 M NaCl, pH 7. A linear NaCl gradient was applied (0.1–0.3 M NaCl, total volume = 1 L), and an elution profile similar to that reported by Everse et al.[17] was typically observed. The oxidized adduct can be observed on the DEAE-cellulose column with a UV lamp as a bright yellow band, whereas the reduced adduct appears white. Fractions were collected and the absorbance was measured at 346 and 260 nm. Only those fractions with an A_{260}/A_{346} value of less than 2.40 were pooled, and the solvent was removed by rotary evaporation. The bath temperature was maintained below 40 °C during rotary evaporation, coolant was maintained at 3 °C, and the receiving vessel was kept on ice. The dried adduct–salt mixture was dissolved in a minimal volume of H_2O and desalted on a Sephadex G-10 column (5 × 100 cm) equilibrated with H_2O. Chloride in the eluant was tested with concentrated $AgNO_3$, and those adduct fractions without chloride were pooled, concentrated by rotary evaporation, and stored over P_2O_5 at −20 °C in a vacuum dessicator, yield 100 mg (28%). Anal. ($C_{31}H_{44}N_7O_{18}P_3Na_4$) H, N, Na; C: calcd, 37.7; found, 33.9.

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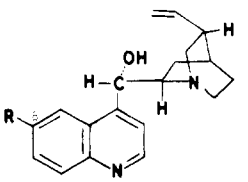
Partial Synthesis of 6′-Hydroxycinchonine and Its Antiarrhythmic Activity in Mice

LaVerne D. Small,* Harry Rosenberg, Peter U. Nwangwu, Terry L. Holcslaw, and Sidney J. Stohs

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 68105. *Received March 15, 1979*

The synthesis of 6′-hydroxycinchonine [(8*R*,9*S*)-cinchonan-6′,9-diol] was achieved by demethylating quinidine with boron tribromide in dichloromethane at −75 °C. The antiarrhythmic activities of 6′-hydroxycinchonine and quinidine were compared following the infusion of aconitine into the tail veins of mice to induce arrhythmias. Comparative ED_{50} and LD_{50} studies for quinidine and 6′-hydroxycinchonine revealed equivalent antiarrhythmic potencies for the two drugs but a smaller acute toxicity for 6′-hydroxycinchonine.

Since its initial use, quinidine has remained one of the



quinidine (6′-methoxycinchonine), R = CH_3O-
cupreidine (6′-hydroxycinchonine), R = HO-

most important and effective drugs for maintaining normal heart rhythm. Patient response to treatment with the drug, however, is often observed to be quite erratic. Side effects frequently require cessation of therapy. Quinidine has a narrow therapeutic range of about 2–5 μg/mL in plasma and exhibits a moderate duration of action, lasting approximately 4–6 h after a single dose.[1] These are undesirable attributes, since the drug is usually administered on a chronic basis for its prophylactic property.

The other major cinchona alkaloids, quinine, cinchonidine and cinchonine, also possess antiarrhythmic activity, although they are somewhat less potent than quinidine. Neither cinchonidine nor cinchonine possesses a 6′-methoxy group. It therefore appears that the 6′-methoxy group of quinidine is not essential for antiarrhythmic activity. Cleavage of the methyl group of the 6′-methoxy grouping of quinidine would form 6′-hydroxycinchonine [(8*R*,9*S*)-cinchonan-6′,9-diol, also called cupreidine].[2] Such cleavage will provide a phenolic group through which a large variety of chemical structures could be attached to yield quinidine analogues having different distribution patterns, tissue affinities, rates of elimination and duration of action. Previous attempts to prepare cupreidine from quinidine failed because the traditional methods for cleaving ethers with hot, concentrated mineral acids produced rearrangements of the quinuclidine and C-3 vinyl groups.[3] 10,11-Dihydroquinidine, however, is stable to hot mineral acids and various ethers of the dihydrophenolic derivative have been synthesized.[2]

Cleavage of the methyl group from the 6′ position of quinidine has been achieved for the first time, by reacting quinidine with boron tribromide in dichloromethane at −75 °C, using a procedure similar to that reported by McOmie, Watts, and West for the demethylation of aryl methyl ethers.[4]

Pharmacological Results and Discussion. The antiarrhythmic activity of 6′-hydroxycinchonine dihydrochloride was compared with that of quinidine sulfate and a control group pretreated with the saline vehicle. Groups of ten mice were pretreated by intravenous injection of either of the test drugs or the saline vehicle. After a period of 3 min, aconitine (5 μg/mL) was infused into the tail vein at a constant rate (0.25 mL/min) to induce arrhythmias, and the electrocardiogram (lead II) pattern was monitored as previously described.[5] The times to onset of aconitine-induced initial arrhythmia and onset of ventricular tachycardia were determined and compared in mice pretreated with quinidine sulfate, 6′-hydroxycinchonine hydrochloride, or the saline vehicle. The time to onset of initial arrhythmia is the time following the initiation of the aconitine infusion to the first discernible sign of persistant (>5 s) deviation from normal sinus rhythm as observed on the ECG recording. This induced

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