(11) Compound IV, mp 134.5–135.0°; purity in excess of 99% as judged by TLC and GLC; MS 440 (M; 100%), calcd for $C_{30}H_{48}O_2$: 440.3654, found: 440.3663; NMR 0.99 (d, J = 8 Hz, 4β-CH_3), 2.03 (s, 3 H, methyl of acetate), 4.78 (m, 1 H, C-3α-H).

(12) Prepared and developed as described previously (W.-H. Lee, R. Kammereck, B. N. Lutsky, J. A. McCloskey, and G. J. Schroepfer, Jr., *J. Biol. Chem.*, **244**, 2033 (1969).

(13) Compound V, mp 128.5–130°; purity in excess of 98% as judged by TLC and GLC; MS 442 (M; 100%), calcd for $C_{30}H_{50}O_2$: 442.3790, found: 442.3810; NMR 0.89 (d, 3 H, J = 7.2 Hz, 4β-CH_3), 2.07 (s, 3 H, methyl of acetate), 4.82 (m, 1 H, C-3α-H).

(14) Compound VI, mp 155–156.5°; purity in excess of 99% as judged by TLC and GLC; MS 400 (M; 100%), calcd for $C_{28}H_{48}O$: 400.3702, found: 400.3704; NMR, 0.92 (d, 3 H, J = 7–8 Hz, 4β-CH_3), 3.75 (m, 1 H, C-3α-H); $[\alpha]_{589}$ +41.6° ($CHCl_3$).

(15) The gift from Dr. A. A. Kandutsch of a sample of this sterol, isolated from a preputial gland tumor (A. A. Kandutsch and A. E. Russell, *J. Biol. Chem.*, **235**, 2253 (1960)), is gratefully acknowledged.

(16) This sterol was prepared by chemical synthesis (J. M. Midgley, A. F. A. Wallis, and W. B. Whalley, *Chem. Commun.*, 1297 (1967)) and was a gift from Dr. A. A. Kandutsch who received this sample from Dr. M. L. Black of the Parke, Davis, and Co., Ann Arbor, Mich. Midgley et al. reported that their synthetic sample was identical (melting point, mixture melting point, mass spectrum, and GLC) to the sterol isolated by Kandutsch and Russell.

(17) In a separate study (F. F. Knapp, Jr., and G. J. Schroepfer, Jr., manuscript submitted for publication) of a large number of synthetic 4α-methyl- and 4β-methyl-3β-hydroxysterols, it has been established that the 3α-proton resonance is consistently further downfield (0.57–0.61 ppm) and the C-4-methyl group coupling constant (J) is larger (1.2–1.8 Hz) for the 4β-methyl sterols.

(18) Prepared by the specific approach utilized for the preparation of [3α,4α-$^2H_2$]-4β-methyl-cholest-5-en-3β-ol (F. F. Knapp, Jr., and G. J. Schroepfer, Jr., *J. Org. Chem.*, **39**, 3247 (1974)).

(19) Over 90% of the incubated radioactivity was extracted into petroleum ether from saponified incubation mixtures, values comparable to those of boiled enzyme controls. The only labeled component recovered showed the chromatographic mobility of the incubated substrate. Under the incubation conditions employed, the label of [3α-^{3}H]-14α-methyl-5α-cholest-7-en-3β-ol was efficiently (40–60%) incorporated into cholesterol.

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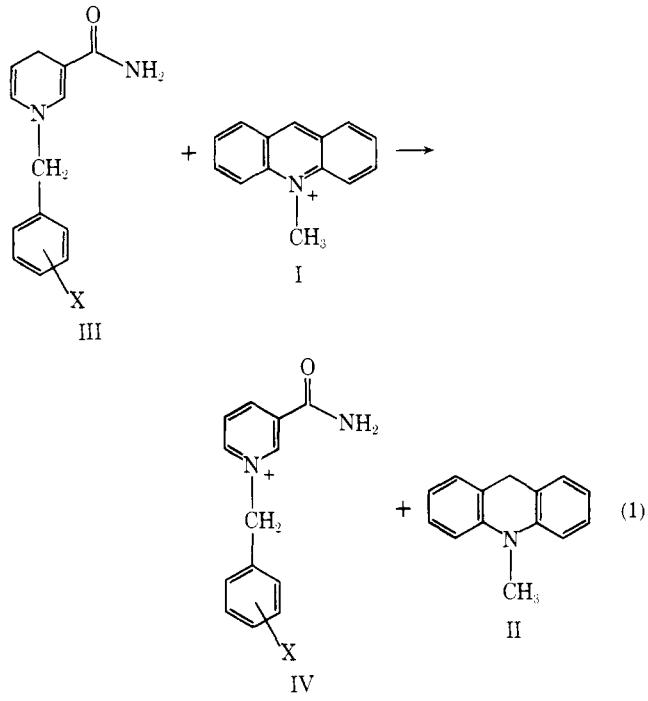
Model Dehydrogenase Reactions. Neighboring Group Effects in Dihydronicotinamide Reductions

Sir:

Recent X-ray crystallographic studies of an abortive ternary complex of the NAD^+-dependent lactate dehydrogenase from dogfish have revealed that the carboxylate of a glutamyl residue is in close proximity to the nitrogen of the bound coenzyme's nicotinamide moiety.[1] We wish to report a nonenzymic reaction which suggests the catalytic function of this residue. Specifically, we have found that dihydronicotinamide derivatives in which a carboxylate is adjacent to the nicotinamide moiety reduce nonenzymic oxidants in anhydrous media much more rapidly than homologous dihydronicotinamides in which the carboxylate is absent. The acceleratory effect of the neighboring carboxylate group in acetonitrile is most likely due to the stabilization of the developing positive charge on the nicotinamide ring in the transition state. By analogy, the role of the active site glutamate may be to stabilize the partial positive charge which develops in the nicotinamide moiety of the coenzyme in the transition state during its reversible oxidation and reduction by pyruvate and lactate. These studies provide the first example of a noncovalent interaction capable of enhancing the reactivity of a dihydronicotinamide that is of potential importance in the mechanism of action of $NAD^+/NADP^+$-dependent dehydrogenases. Previous nonenzymic studies have focused on mechanisms of enhancing the reactivity of the hydride acceptors.[2-4]

Our most important observation, reported in Table I, is that the second-order rate constant for the reduction of N-methylacridinium ion by N-2′-carboxybenzyldihydronicotinamide (IIIh) in acetonitrile is two orders of magnitude greater than the corresponding rate constant for N-benzyldihydronicotinamide (IIIa) or any of its other 2′- or 4′-substituted derivatives (IIIa-g). The observed kinetic isotope effect for this reduction using monodeuterio-N-2′-carboxybenzyldihydronicotinamide (IIIh) is 1.11. The ratio of undeuterated N-methylacridan (m/e, 195) to monodeuterio-N-methylacridan (m/e, 196) formed, using the monodeutero form of IIIh as reductant, is 3.8. Similar isotope effects have been observed for the reduction of N-methylacridinium ion[5] and trifluoroacetophenone[6] by N-propyldihydronicotinamide in aqueous solution. They suggest the formation of a noncovalent complex between the "hydride donor" and "hydride acceptor" during the course of the reaction.[5,6]



Since the various neutral dihydronicotinamides react with I considerably faster in water than in acetonitrile, a significant amount of positive charge must develop on the nicotinamide ring in the transition state at the expense of the larger acridinium cation. As noted above, we propose that the ability of the negatively charged carboxylate of IIIh to stabilize this positive charge in acetonitrile is primarily responsible for the efficiency of IIIh as a reductant compared to the neutral dihydronicotinamides. Comparable rate accelerations are not observed in aqueous solution because water is probably better able to stabilize the incipient positive charge and the hydration of the carboxylate group in aqueous solution would restrict its access to the dihydronicotinamide ring. In acetonitrile, the carboxylate is less effectively solvated[7] and therefore able to approach the nicotinamide ring more readily. Moreover, electrostatic interactions would be greater in acetonitrile because of the lower dielectric constant. Consistent with the presence of a negative charge at the nitrogen of the dihydronicotinamide in acetonitrile, we have found a red shift in the characteristic dihydronicotinamide absorption of IIIh in acetonitrile.[8]

Inductive effects are not responsible for the enhanced reactivity of IIIh because the rate constants for the reduction by other N-benzyldihydronicotinamides exhibit little sensitivity to substituents on the phenyl ring. The possibility that the carboxylate group permits the formation of a reactive

Table I. Second-Order Rate Constants for the Reduction of *N*-Methylacridinium Ion by a Series of *N*-Benzyldihydronicotinamides[a]

Abbreviation	Substitution on benzyl group	Aq buffer 0.05 *M* tris pH 8[d]	k_2, l. mol^{-1} sec^{-1} [e]	
			Methanol[b]	Acetonitrile[b,c]
IIIa	H	5.5×10^2	1.8×10^2	65
IIIb	4'-CN	1.8×10^2		26
IIIc	4'-$COOCH_3$	2.7×10^2		42
IIId	4'-CH_3	5.2×10^2		80
IIIe	4'-CH_3O	5.3×10^2		92
IIIf	2'-CH_3	5.4×10^2		70
IIIg	2'-$COOCH_3$	4.6×10^2	2.2×10^2	70
IIIh	2'-COO^-Na^+	1.45×10^3	4.0×10^3	1.0×10^4
	2'-COO^-K^+ [g,h]			1.3×10^4
	2'-COO^-K^+ + crown[i]			1.5×10^4
IIIi	4'-COO^-Na^+	6.1×10^2	No reaction[f]	
	N-propyldihydronicotinamide	2.0×10^3	6.0×10^2	2.4×10^2

[a] All dihydronicotinamides were chromatographed on a Sephadex LH-20 column prior to the kinetic measurements. The anhydrous methanolic solutions were diluted into the solvent chosen for the reaction. [b] Distilled from *N*-methylacridinium iodide. [c] Contained 1% methanol. [d] Methanol up to 10% had no effect on the rates. [e] All kinetic runs were carried out under pseudo-first-order conditions using 2×10^{-5} *M* *N*-methylacridinium chloride or tetrafluoroborate interchangeably. Neither anion showed any effect on the rate. The concentration of the dihydronicotinamides was increased up to 6×10^{-3} *M*. Within this concentration range all the logarithmic pseudo-first-order plots were strictly linear. The absolute value of the second-order rate constants are accurate to within ±8%. [f] The absorption of the dihydronicotinamide did not decrease on adding the oxidant. [g] The formation of the *N*-methylacridan was confirmed and the products isolated by chromatography. [h] 10^{-3} *M* $Me_4N^+Br^-$ had no effect on the rate. [i] 18-Crown-6 purchased from PCR Inc., Gainesville, Fla.

Table II. Rates of Reaction between Tetrachlorobenzoquinone (chloranil)[a] and a Series of Dihydronicotinamides

Dihydronicotinamide	$k_2^{25°}$, l. mol^{-1} sec^{-1} [b,c]	
	Methanol	Acetonitrile
IIIa	1.1×10^3	1.9×10^3
IIIh (K^+ salt)	3.0×10^3	2.25×10^4
IIIh (K^+ salt) + crown[d]	3.0×10^3	2.25×10^4
IIIi (K^+ salt) + crown[d]	2.8×10^3	3.5×10^3

[a] We have found chloranil to be unstable in aqueous buffer. On dilution into 0.05 *M* Tris, pH 8.0, the characteristic uv absorption associated with the tetrachlorobenzoquinone disappears. The product solution is still capable of oxidizing dihydronicotinamides at considerably lower rates; these systems were not investigated further. [b] The rates in methanol and acetonitrile were determined by monitoring the absorption of the anion formed in the reduction λ_{max}^{MeCN} 448 nm; λ_{max}^{MeOH} 453 nm. [c] The kinetic runs were carried out with 2×10^{-5} *M* chloranil under pseudo-first-order conditions. The reaction was strictly first order with respect to the reductant, up to 3×10^{-3} *M*. [d] 18-Crown-6 purchased from PCR Inc., Gainesville, Fla.

intermediate and thus provides an entirely different reaction pathway for IIIh seems excluded by the observation that *N*-4'-carboxybenzyldihydronicotinamide (IIIi) does not reduce I in acetonitrile or methanol. In these nonaqueous solvents, the carboxylate group of IIIi adds to the electrophilic 9 position of I to form a stable nonreducible covalent adduct which readily dissociates upon addition of water. The corresponding adduct between IIIh and I has not been detected in either methanol or acetonitrile.

Because the pattern of isotope effects exhibited in the reduction of I indicates the obligatory formation of some type of noncovalent complex during the reaction,[5] the observed rate enhancement could be due to an increased concentration in acetonitrile of the kinetically important intermediate as a result of ion pairing between the negatively charged IIIh and the positively charged *N*-methylacridinium ion. Although this possibility cannot be rigidly excluded, the reduction of the neutral oxidant chloranil[9] by IIIh with a rate constant tenfold greater than that observed with IIIa in acetonitrile (Table II) strongly supports the previously stated view that the primary kinetic effect of the carboxylate in reductions involving IIIh is to stabilize the positive charge developed in the nicotinamide ring in the transition state. The more pronounced acceleratory effect of the carboxylate group in the reduction of *N*-methylacridinium than in the reduction of chloranil most probably results from the greater positive charge on the nicotinamide in the transition state in the *N*-methylacridinium ion reaction since chloranil is a stronger oxidant. As a result, a more substantial stabilization of the transition state by an electrostatic interaction with the carboxylate group can be expected for the reduction of *N*-methylacridinium ion than for chloranil.[10]

Additional studies using dihydronicotinamides of different structures in the reductions of other oxidants will be necessary to evaluate the scope of neighboring group effects in nonenzymic dihydronicotinamide reactions. Obviously further support for the suggested function of the glutamyl residue in lactate dehydrogenase will be the demonstration that other $NAD^+/NADP^+$ dehydrogenases, besides the closely homologous malate dehydrogenase,[11] contain a carboxylate or another nucleophilic amino acid side chain in a comparable position in their active sites. Nevertheless, the present studies clearly demonstrate that neighboring carboxylate groups can greatly accelerate the rate of dihydronicotinamide reductions under appropriate conditions.

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The Chlorine Hexafluoride Radical. Preparation, Electron Spin Resonance Spectrum, and Structure[1]

Sir:

Chlorine hexafluoride is the only unreported molecule of the series ClF_n (n = 1-6) to date. This hypervalent radical possesses one electron more than SF_6 and one valence electron less than XeF_6 so that the unpaired electron is expected to occupy the a^*_{1g} orbital in O_h symmetry. Since the a^*_{1g} orbital is thought to play a pivotal role in the unusual structural dynamics of XeF_6,[2-4] it is of interest to ascertain the composition of this antibonding orbital in related radicals by ESR studies. We now wish to report the preparation and ESR identification of the ClF_6 radical.

The title radical was generated by γ radiolysis of SF_6 containing 5 mol % of ClF_5 at −196°. Fessenden and Schuler[5] have described the use of solid SF_6 as a suitable matrix for the observation of isotropic ESR spectra during in situ irradiation.[6] Similarly, we find that isotropic spectra are observed for radicals which are sufficiently long-lived to be detected in a γ-irradiated SF_6 matrix at −165°.

In the spectrum shown in Figure 1, the outer lines consist predominantly of two separate 1:6:15:20:15:6:1 septets at low field together with a corresponding septet at high field. Allowing for the effect of radical decay during the sweep from low to high field, these three septets are of comparable intensity. Accordingly, the pattern is interpreted as a 1:1:1:1 quartet of binomial septets, the missing septet being masked by the more intense lines from SF_5[7] and ClF_4[8] in the center of the spectrum. The septet substructure is attributed to hyperfine interaction with six equivalent fluorine (I = ½) nuclei while the quartet splitting originates from coupling to one ^{35}Cl (I = ³/₂) nucleus.[9] This interpretation is verified by the observation of the outer components from the weaker spectrum of the ^{37}Cl (I = ³/₂) radical, as indicated in Figure 1.

Because of the large hyperfine interactions, the ESR parameters were calculated by including terms up to fourth order in the solution of the isotropic ESR spin Hamiltonian.[10] These values were then refined until forward calculations by means of an accurate expression[11] derived from the Breit-Rabi equation reproduced the experimental field positions of the outer ($M_I(Cl) = \pm 3/2$) components, the following results being obtained: $a(^{35}Cl)$ = 771 G, $a(^{37}Cl)$ = 642 G, $a(^{19}F)$ = 89 G, and g = 2.015 ± 0.001. It is estimated that the hyperfine coupling constants are accurate to ±1 G. The ratio of $a(^{35}Cl)/a(^{37}Cl)$ is 1.201 ± 0.002, in satisfactory agreement with the value of 1.2015 for the $^{35}Cl/^{37}Cl$ ratio of nuclear g factors. An additional check on the parameters was made by calculating the field positions for the $M_I(^{35}Cl) = +1/2$ set of components, these values agreeing with the observed positions to better than 1 G.

The spectrum of interest is assigned to the ClF_6 radical formed by fluorine atom addition or transfer to ClF_5. The most remarkable feature is the large ^{35}Cl coupling of 771 G which is more than twice the value (288 G) for ClF_4.[8] Since an isotropic coupling of only 82 G has been calculated for complete occupancy of the chlorine 4s orbital,[8a] it is evident that the unpaired electron in ClF_6 must populate the chlorine 3s orbital. In this case the coupling corresponds to a spin density of 0.46. This finding is consistent with the occupation of the totally symmetric a^*_{1g} orbital for a regular octahedral geometry. Also, the interaction with six equivalent fluorines accords with this description.

Although the results conform to O_h symmetry, they do not eliminate the possibility that ClF_6 undergoes deformations similar to those deduced for XeF_6.[2a] First, such motions would lead to such a rapid modulation of the spin distribution that the fluorines would be equivalent on the ESR time scale. Secondly, the results of Hückel MO calculations indicate that the HOMO of XeF_6 is stabilized by deformations from O_h to C_{3v} symmetry when there is a large contribution from the xenon 5s orbital to this antibonding orbital.[2b] Therefore the large spin density in the chlorine 3s orbital of ClF_6 is quite compatible with a fluctuating structure involving deformations to nonoctahedral configurations.

Finally, it is noteworthy that the lines of the ClF_6 spec-

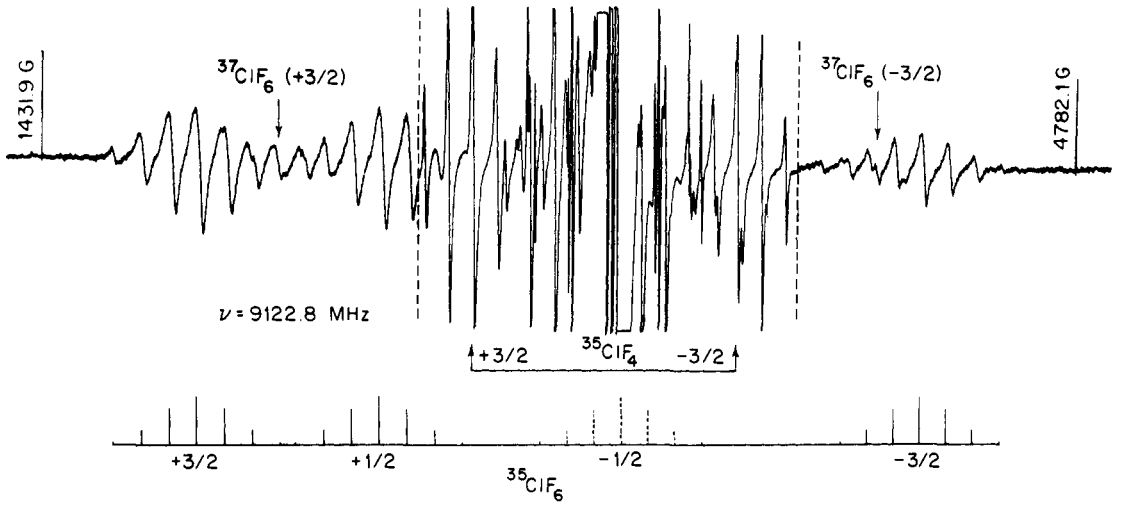

Figure 1. First-derivative ESR spectrum of γ-irradiated SF_6 containing ClF_5 at −165°. The center portion of the spectrum was recorded at approximately half the gain in order to show the outer quintets of the ClF_4 spectrum; strong lines from SF_5 are also present in the center region. The line positions for the $^{35}ClF_6$ spectrum are indicated by the stick diagram and the arrows above the spectrum mark the center lines of the outer septets of $^{37}ClF_6$.