saturated aqueous $NaHCO_3$ (50 mL), and saturated aqueous NaCl (50 mL). The solution was dried (Na_2SO_4), decanted, and evaporated. After drying overnight under vacuum, the weight of the crude product was 1.00 g. This material was used in subsequent steps without further purification. Flash chromatography of a portion (200 mg) of the crude product on silica gel eluting with 2% ethyl acetate in hexane produced an analytical sample (186 mg, 89% yield) of the methyl pyranoside **26**: $[\alpha]_D$ +79.8° (c = 1.0, $CHCl_3$); IR (CCl_4) 2960, 2920, 2870, 1800, 1375, 1345, 1175, 1135, 1080, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.26 (s, 1 H), 3.39 (s, 3 H), 3.33 (dq, 1 H, J = 9, 6 Hz), 1.90 (td, 1 H, J = 13, 4.5 Hz), 1.73 (broad dq, 1 H, J = 13, 3 Hz). 1.63 (dt, 1 H, J = 13, 3 Hz), 1.46-1.08 (m, 5 H), 1.37 (s, 3 H), 1.16 (d, 3 H, J = 6 Hz), 1.07-0.99 (m, 2 H), 0.95 (s, 9 H), 0.88 (t, 3 H, J = 7 Hz), 0.84 (d, 3 H, J = 7 Hz). Anal. Calcd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 67.20; H, 10.12.

(2*S*)-2-[(2*R*,5*R*,6*R*)-2-Methoxy-6-methyl-5-[(2*S*)-2-methylbutyl]tetrahydro-2*H*-pyran-2-yl]propanoic Acid Methyl Ester (5). Methanolic sodium methoxide was prepared by adding sodium (970 mg, 42 mg-atom) to dry methanol (20 mL, distilled from magnesium methoxide). The methyl pyranoside **26** was dried by repeatedly evaporating a toluene solution (3 × 12 mL) under vacuum. The residue was then dissolved in 10.0 mL of the methanolic sodium methoxide solution and stirred for 2 days. The reaction was quenched by addition of saturated aqueous NH_4Cl (50 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with saturated aqueous NaCl (25 mL), dried (Na_2SO_4), decanted, and evaporated. Flash column chromatography on silica gel (40 g), eluting with 12% ethyl acetate/hexane (800 mL), gave the methyl ester **5** as 658 mg (78% yield) of colorless oil: $[\alpha]_D$ +89.8° (c = 1.0, $CHCl_3$); IR (CCl_4) 3560, 2960, 2930, 1740, 1450, 1380, 1250, 1175, 1150, 1125, 1080, 1030, 980 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.78 (s, 3 H), 3.39 (s, 1 H), 3.38 (dq, 1 H, J = 9, 6.5 Hz), 3.37 (s, 3 H), 1.97-1.87 (m, 1 H), 1.79-1.60 (m, 2 H), 1.49-0.94 (m, 7 H), 1.43 (s, 3 H), 1.19 (d, 3 H, J = 6 Hz), 0.87 (t, 3 H, J = 7 Hz), 0.82 (d, 3 H, J = 6.5 Hz). Anal. Calcd for $C_{16}H_{30}O_5$: C, 63.55; H, 10.00. Found: C, 63.85; H, 9.94.

Acknowledgment. We are grateful to Drs. M. Hammond, W. Hagmann, and J. Boger for helpful discussions during the course of this work and in the preparation of the manuscript.

Registry No. **1**, 125228-51-5; **5**, 125228-52-6; **6**, 104194-02-7; **7**, 125228-53-7; **9**, 534-00-9; **10**, 29394-58-9; **11**, 3976-69-0; **12**, 125228-54-8; **12** (*O*-TES deriv), 125228-69-5; **13**, 125228-55-9; **14**, 125228-56-0; **15**, 125228-57-1; **16**, 125228-58-2; **17**, 125228-59-3; **18**, 125228-60-6; **19**, 125228-61-7; **20**, 125228-62-8; **21**, 125228-63-9; **22**, 125228-64-0; **23**, 125228-65-1; **24**, 125228-66-2; **25**, 125228-67-3; **26**, 125228-68-4; $Ph_3P{=}CHCOOMe$, 2605-67-6; 4-BrC_6H_4COOH, 586-76-5; (*R*)-2-methylbutaneboronic acid, 125228-70-8; (+)-potassium bis(pinanediol)borate, 125228-71-9; 2-(2-bromoethyl)-1,3-dioxane, 33884-43-4.

Reactivity of Biologically Important Reduced Pyridines. 7.† Energetics and Effect of Substitution on Hydride versus Electron Transfer in Dihydropyridines, Dihydroquinolines, and Dihydroisoquinolines

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Received October 17, 1989

The hydridic and electronic oxidation of a variety of 1-methyl-3-substituted-1,4-dihydropyridines, 1-methyl-3-substituted-1,4-dihydroquinolines, 1-methyl-3-substituted-1,2-dihydroquinolines, 2-methyl-4-substituted-1,2-dihydroisoquinolines, and 1-(4-substituted-phenyl)-1,4-dihydronicotinamides were examined using a semiempirical molecular orbital (AM1) method. The data obtained indicate that a significant correlation (r > 0.97) is generated when the energies associated with either electron loss or hydride transfer are compared. The slope of such relationships approaches unity. In addition, the relative stabilities of various derivatives determined theoretically are consistent with experimentally derived kinetic stabilities. A group of five substituents ($(CH_3)_2N$, CH_3O, CH_3S, Cl, F) invariably deviate from the relationship defined by the remaining compounds. This deviation may be due to the differential effect of electron donation on the ground state which controls electronic oxidation and on the hydride-transferring transition state which determines hydridic oxidative reactivity.

Introduction

Dihydropyridines constitute the basic operational subunit of a number of biologically important coenzymes.[1] These compounds can accept a pair of electrons from the respiratory chain resulting in pyridinium salt formation. The nature of this oxidation, which is fundamental to the understanding of biochemical respiration, has been extensively studied through the use of various model systems.[2] In these investigations, the mechanism of this oxidation has been narrowed to two main possibilities, namely, concerted hydride transfer or sequential elec-

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† Part VI of this series: Lack of Through Resonance Stabilization in the Ferricyanide-Mediated Oxidation of Substituted 1-Phenyl-1,4-dihydronicotinamides. *Tetrahedron*, in press.

‡ University of Florida.

§ Pharmatec, Inc.

⊥ Schering-Plough Research.

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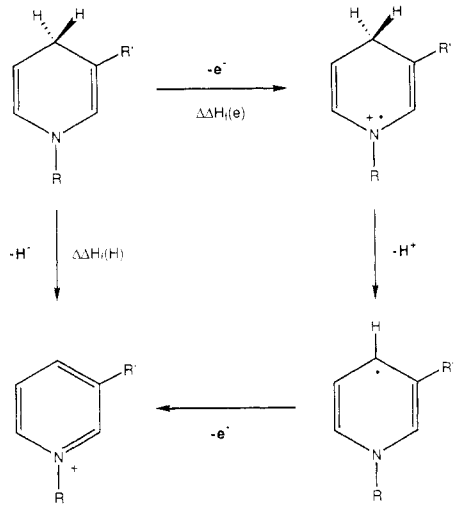
0022-3263/90/1955-2361$02.50/0

Scheme I



tron–proton–electron transfer.[3] In the latter mechanism, an initial electron loss gives rise to a radical cation which undergoes proton abstraction to yield a free radical. This species can participate in a second electronic loss to produce the pyridinium salt. In hydridic oxidation, the proton and two attendant electrons are simultaneously transferred from the dihydropyridine to an acceptor. Hydride acceptors can be any one of a number of compounds including the corresponding pyridinium salt. These processes are presented in Scheme I. Since both of these mechanisms produce the pyridinium salt as the ultimate product, the energy associated with oxidation via hydride loss or electron–proton–electron transfer is the same (i.e., the Law of Hess). In attempting to distinguish between the two possible mechanisms, the nature of the hydride transfer and the nature of the initial electron ionization have been experimentally considered.[4] Unfortunately, since both processes involved the formation of a formal positive charge, it is difficult to differentiate between the mechanisms based on electronic influences.

The purpose of this report is to examine relative energetics of hydridic and electronic oxidation for five groups of compounds namely 1-(4-substituted-phenyl)-1,4-dihydronicotinamides, 1-methyl-3-substituted-1,4-dihydropyridines, 1-methyl-3-substituted-1,4-dihydroquinolines, 1-methyl-3-substituted-1,2-dihydroquinolines, and 2-methyl-4-substituted-1,2-dihydroisoquinolines. The tool used to approach this problem was the AM1 method,[5] a semiempirical all valence electron molecular orbital technique. This technique has been shown to be a reliable method for examining substituent effects in a variety of reactions, including ionization of benzoic acid derivatives.[6] In addition, this formalism allows for an extremely flexible investigative paradigm in that highly unstable compounds can be considered. The accuracy of those calculations can then be estimated by comparing the results obtained theoretically to experimentally derived values.

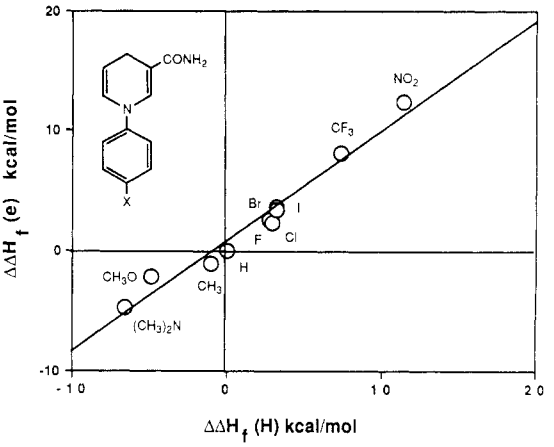


Figure 1. A comparison of the energy required to removed a hydride species ($\Delta\Delta H_f$(H)) and that required to cause the loss of an electron ($\Delta\Delta H_f$(e)) for a series of 1-(4-substituted-phenyl)-1,4-dihydronicotinamide. The data is shown relative to 1-phenyl-1,4-dihydronicotinamide (0,0). The correlation produced was significant (r = 0.982) and the slope of the line was 0.918. This graph uses data generated from the vertical ionization potentials although a plot of hydride transfer versus adiabatic ionization potential was similar (r = 0.970, slope = 0.900).

Experimental Section

Theoretical studies were performed using the AM1 molecular orbital method.[5] Calculations were obtained from either an IBM 3084 Model K dual processor computer or a MicroVax II (Digital Equipment Corp.) system. The AM1 program was obtained through the Quantum Chemistry Program Exchange (QCPE) and adapted to run on the IBM computer. Structural inputs were generated using an AMPAC/SYBYL interface, and all starting geometries represent the MM2 minimized structures. Final geometries and energetics were estimated by minimizing the total molecular energy with respect to all structural variables using the standard Davidon–Fletcher–Powell optimization procedure. Vertical ionization potentials were obtained using Koopman's theorem from the generated table of eigenvalues. Radical cations were examined using the half-electron (HE) method with restricted Hartree–Fock (RHF) formalism.[7]

In calculating the energy of hydride transfer ($\Delta\Delta H_f$(H)), the heat of formation of the pyridinium salts were subtracted from the corresponding values for the dihydropyridines. A similar procedure using the ΔH_f on the radical cations generated the energy associated with electron loss. In plotting data, it was convenient to relate hydridic and electronic oxidative energies to a reference compound. The compound selected for this marker was the 3-substituted carboxamide in the case of the dihydropyridines and dihydroquinolines, the 4-substituted carboxamide in the case of the dihydroisoquinolines, and 1-phenyl-1,4-dihydronicotinamide in the case of the 1-(4-substituted-phenyl)-1,4-dihydronicotinamides.

Statistical evaluation of slopes was performed using a Student t test for parallelism.[8] In all cases the level of significance was set at $p < 0.01$.

Results and Discussion

In order to analyze electronic versus hydridic oxidation for a series of substituted dihydropyridines, semiempirical heats of formation (ΔH_f) were estimated for the parent compound as well as for the corresponding radical cations and pyridinium salts. The differences between the ΔH_f's of the radical cations and the dihydropyridines gave an

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Table I. Semiempirical Heats of Formation (AM1) (ΔH_f, kcal/mol) and Vertical Ionization Potentials (vert IP) for a Series of 1-(4-Substituted-phenyl)-1,4-dihydronicotinamides and ΔH_f Values for the Corresponding Radical Cations and Pyridinium Salts

substituents	X	vert IP, eV	X	X
$N(CH_3)_2$	34.37	7.91	199.08	186.93
OCH_3	-12.19	8.02	160.06	144.85
CH_3	17.63	8.07	190.71	175.60
H	25.48	8.12	199.22	184.46
F	-19.32	8.22	157.26	142.63
Cl	18.55	8.22	195.01	180.25
Br	30.38	8.26	207.29	192.56
I	41.66	8.27	218.36	203.82
CF_3	-130.55	8.47	49.79	35.79
NO_2	29.17	8.65	213.40	199.52

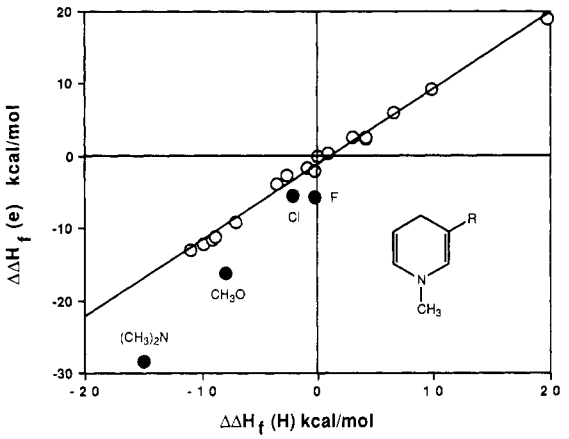

Figure 2. The relationship between electronic and hydridic oxidation for a series of 1-methyl-3-substituted-1,4-dihydropyridines. The data ($\Delta\Delta H_f$) are shown relative to 1-methyl-1,4-dihydronicotinamide (0,0). The correlation produced was significant (r = 0.996) and the slope of the line was 1.056. The correlated data (open circles) did not include four compounds (closed circles) containing electron-donating substituents. These derivatives were the 3-fluoro, 3-chloro, 3-methoxy, and 3-(dimethylamino) compounds. A line fitted to these values produced a slope which was significantly ($p < 0.01$) different from the well behaved values.

estimate of the energy associated with electron removal (adiabatic ionization potential) or the $\Delta\Delta H_f$(e). The corresponding hydridic process is described by $\Delta\Delta H_f$(H), which is the difference between the ΔH_f of the pyridinium salt and the dihydropyridine.

The first set of compounds examined was a series of ten 1-(4-substituted-phenyl)-1,4-dihydronicotinamides. Heat of formation information for the various components of the oxidative cycle is given in Table I. In addition, vertical ionization potentials are presented for the dihydronicotinamides. Correlation of the hydridic and electronic oxidation energies relative to the oxidation of the unsubstituted compound generates a straight line (r = 0.982) with a slope close to unity (0.92) (Figure 1). These data suggest that the effect of substitution on the energy differences associated with either electron removal or hydride transfer are similar. This is corroborated experimentally in that when the log rate of electron loss from dihydropyridines (as measure by ferricyanide-mediated oxidation) is compared to the log rate of hydride transfer (as measured by *N*-methylacridinium oxidation), a linear relationship is obtained with a slope of approximately one.[4]

Table II. Semiempirical Heats of Formation (AM1) (ΔH_f, kcal/mol) for a Series of 1-Methyl-3-substituted-1,4-dihydropyridines and Their Corresponding Radical Cations and Pyridinium Salts

substituent	R, CH_3	R, CH_3	R, CH_3
R			
$CONH_2$	-7.3	166.7	152.0
$COOCH_3$	-54.2	122.6	108.3
$COCH_3$	-6.8	167.9	153.9
(*E*)-C(=NOH)CH_3	27.5	194.8	182.0
COC_6H_5	27.9	200.5	186.0
F	-13.3	154.4	148.0
Cl	24.3	195.2	183.7
CF_3	-127.0	55.5	41.2
CF_3,5-CF_3	-283.8	-88.5	-102.5
$COCF_3$	-154.3	31.4	17.1
(*E*)-C(=NOH)CF_3	-119.1	55.3	42.3
CH_3O	-5.1	155.1	148.6
CH_3S	33.7	*a*	189.0
CH_3SO	45.3	244.1	211.0
CN	61.1	240.0	226.8
$(CH_3)_2N$	41.4	189.4	188.0
$(CH_3)_2NO$	66.5	240.2	225.5
CH_3	23.9	189.2	176.7
CH_3CH_2	18.2	183.1	170.7
$(CH_3)_2CH$	14.5	178.8	166.3
$(CH_3)_3C$	13.4	176.9	164.0

[a] Unable to achieve self-consistency.

In addition, the theoretical studies accurately predict the kinetic stability of the derivatives in that the compounds, as ranked from most stable (highest $\Delta\Delta H_f$'s) to least stable (lowest $\Delta\Delta H_f$'s), perfectly mirror the rank according to rate constants for oxidation.[9]

The next group of compounds was a set of 21 1-methyl-3-substituted-1,4-dihydropyridines. The heats of formation of the derivatives studied are given in Table II. The plot of $\Delta\Delta H_f$(H) versus $\Delta\Delta H_f$(e) for these systems is shown in Figure 2. The majority of the compounds are well described by a linear relationship as in the previous example. The slope of this line is 1.06 and the correlation is r = 0.996. Again, experimental data is in close agreement with the theoretical predictions as the data indicate that electron-withdrawing moieties stabilize the dihydro-

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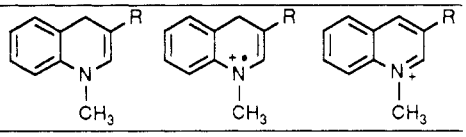
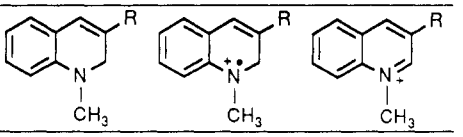
Table III. Semiempirical Heats of Formation (AM1) (ΔH_f, kal/mol) for a Series of 1-Methyl-3-substituted-1,4-dihydroquinolines and Their Corresponding Radical Cations and Pyridinium Salts



substituent			
R			
$CONH_2$	3.1	179.7	167.4
$COOCH_3$	-41.8	135.8	123.2
$COCH_3$	3.8	180.5	167.8
$COCF_3$	-142.0	43.6	31.0
COC_6H_5	40.2	214.1	201.7
(*E*)-C(=NOH)-CH_3	39.9	208.0	198.4
(*E*)-C(=NOH)-CF_3	-106.4	68.5	57.9
CF_3	-114.3	67.9	55.8
F	-0.5	170.4	164.5
Cl	37.0	208.4	199.9
CH_3	8.1	168.2	165.7
CH_3S	46.5	210.3	205.3
CH_3SO	58.0	237.2	223.3
CN	73.8	252.8	242.0
$(CH_3)_2N$	54.4	203.0	208.3
$(CH_3)_2NO$	79.4	253.4	241.3

Table IV. Semiempirical Heats of Formation (AM1) (ΔH_f, kcal/mol) for a Series of 1-Methyl-3-substituted-1,2-dihydroquinolines and Their Corresponding Radical Cations and Pyridinium Salts



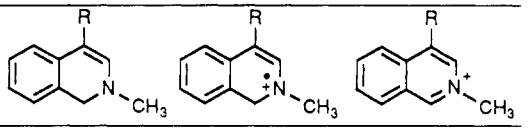
substituent			
R			
$CONH_2$	9.7	183.6	167.4
$COOCH_3$	-35.6	140.2	123.2
$COCH_3$	10.5	184.8	167.8
$COCF_3$	-134.6	47.7	31.0
COC_6H_5	46.0	218.3	201.7
(*E*)-C(=NOH)-CH_3	45.2	214.1	198.4
(*E*)-C(=NOH)-CF_3	-100.7	73.7	57.9
CF_3	-108.6	71.6	55.8
F	3.0	176.7	164.5
Cl	41.2	214.3	199.9
CH_3O	9.5	175.2	165.7
CH_3S	50.5	217.8	205.3
CH_3SO	66.2	241.2	223.3
CN	79.3	257.7	242.0
$(CH_3)_2N$	56.9	212.6	208.3
$(CH_3)_2NO$	85.1	257.2	241.3

pyridines toward oxidation while electron-donating groups destablize the compounds. In addition, in cases where both kinetic and theoretical data are available, the rank order of stability compares reasonably well.[9] Both experimental and theoretical measures indicate that the 3-cyano derivative is the most stable toward oxidation and that the 3-methyl ester and 3-carboxamide are less stable in that order. In contrast, the stability of the 3-acetyl compound is inappropriately low based on theoretical calculations in that it ranks above the carboxylic acid ester in reactivity.

There are four derivatives which clearly deviate from the linear relationship given by the other 17 compounds. These points could be fitted to a unique line which had a slope (1.692) significantly different from the remaining values ($p < 0.01$). These dihydropyridines contain substituents which are characterized as having lone pairs of electrons which are available for donation to the dienamine system. The direction of the deviation indicates that these species undergo an unusually endergonic hydride transfer relative to the ease of electron loss. One reason for this is that the electronic oxidation is dependent mainly on the energy of the highest occupied molecular orbital (HOMO). The effects of substitution on electron loss are, therefore, mediated through the ground state and associated with the donor or acceptor nature of the substituent. In the case of hydride transfer, the substituent exerts an effect on an intermediate which is undergoing a change in symmetry (odd alternate to even configuration). In this scenario, conjugative, destabilizing electron donation is attenuated. The result of this differential effect is that in the ground state, groups like dimethylamino or methoxy raise the energy of the HOMO and increase the reactivity of the dihydropyridine to electronic oxidation. In contrast, these groups are prevented from exerting a similarly potent effect in the hydride-transferring intermediate, resulting in a more stable compound than would be predicted. Halogens, like chlorine and fluorine, inductively withdraw electrons and conjugatively donate them. As this latter effect is not as quantitatively significant as it is in the case of alkoxy or dialkylamino groups, the deviation of the halogen-containing dihydropyridines from linearity is modest.

This observation is precedented in the effect of substitution on allyl anions. In these systems, which represent an active fragment of the dihydropyridines undergoing hydridic oxidation, electron-withdrawing substituents such as trifluoromethyl, nitro, or carboxaldehydes stabilize the incipient anion as expected. On the other hand, electron-donating species such as hydroxy or amino groups, which would be expected to destablize the anion, exert a paradoxically stabilizing influence.[10]

Table V. Semiempirical Heats of Formation (AM1) (ΔH_f, Kcal/mol) for a Series of 2-Methyl-4-substituted-1,2-dihydroisoquinolines and Their Corresponding Radical Cations and Pyridinium Salts



substituents			
R			
$CONH_2$	8.6	179.0	168.5
$COOCH_3$	-37.5	135.4	125.2
$COCH_3$	8.7	179.6	170.0
$COCF_3$	-138.6	42.1	32.1
COC_6H_5	44.9	212.8	202.7
(*E*)-C(=NOH)-CH_3	43.2	214.4	205.7
(*E*)-C(=NOH)-CF_3	-104.5	73.6	63.9
CH_3	-111.9	66.7	55.8
F	2.7	168.8	161.9
Cl	40.1	206.6	198.3
CH_3O	11.0	174.2	168.3
CH_3S	48.7	212.0	207.2
CH_3SO	59.8	234.2	224.5
CN	75.3	250.8	240.9
$(CH_3)_2N$	58.7	208.7	207.4
$(CH_3)_2NO$	85.2	253.8	245.6

Tables III, IV, and V give heat of formation information for a series of 1-methyl-3-substituted-1,4-dihydroquinolines, 1-methyl-3-substituted-1,2-dihydroquinolines,

(10) (a) Khalil, S.; Jarjis, H. *Z. Naturforsch.* **1987**, *42a*, 761–766. (b) Jarjis, H.; Khalil, S. *Z. Naturforsch.* **1987**, *42a*, 297–304. (c) Dewar, M. J. S.; Nelson, D. *J. Org. Chem.* **1982**, *47*, 2614–2618. (d) Inagaki, S.; Iwase, K.; Goto, N. *J. Chem. Soc. Perkin Trans. 2* **1984**, 2019–2022.

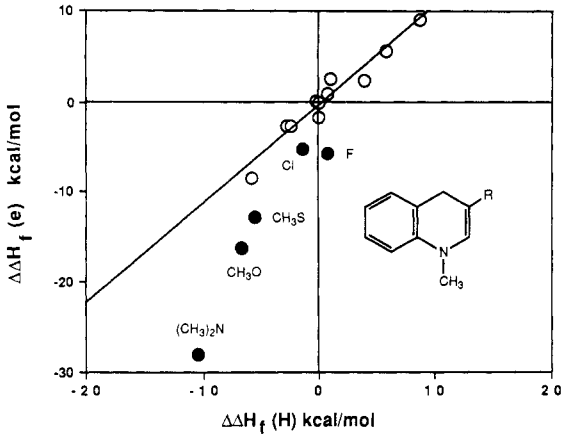


Figure 3. The energetic relationship between electronic and hydridic oxidation for a series of 1-methyl-3-substituted-1,4-dihydroquinolines. The data ($\Delta\Delta H_f$) are shown relative to 1-methyl-3-carbamoyl-1,4-dihydroquinoline (0,0). The correlation produced was significant (r = 0.97) and the generated line had a slope of 1.096. The correlated data (open circles) did not include compounds containing dimethylamino, methoxy, methylthio, chloro, or fluoro groups (closed circles). Correlation of the latter data generated a significantly different slope ($p < 0.01$) than that of the open circle values.

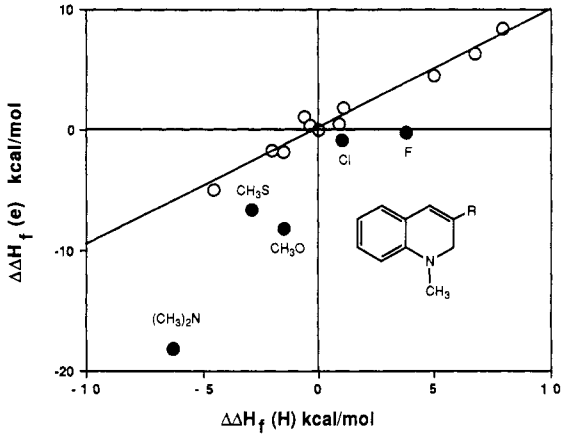


Figure 4. The relationship between electronic and hydridic oxidation for a series of 1-methyl-3-substituted-1,2-dihydroquinolines. The data ($\Delta\Delta H_f$) are shown relative to 1-methyl-3-carbamoyl-1,2-dihydroquinoline (0,0). The correlation produced was significant (r = 0.98) and the slope of the line was 0.983. The correlated data (open circles) did not include compounds containing dimethylamino, methoxy, methylthio, chloro, or fluoro groups (closed circles). Lines drawn through each data set had significantly different slopes ($p < 0.01$).

and 2-methyl-4-substituted-1,2-dihydroisoquinolines, respectively, as well as the corresponding radical cations and quaternary salts. Plots of relative energies associated with hydridic or electronic oxidation are provided in Figures 3, 4, and 5 for the 1,4-dihydroquinolines, the 1,2-dihydroquinolines, and the 1,2-dihydroisoquinones, respectively. In all three cases, a linear relationship was found to exist between $\Delta\Delta H_f$(H) and $\Delta\Delta H_f$(e)'s with corresponding slope values close to unity. As in the case of the 1,4-dihydropyridines, compounds bearing substituents capable of conjugative donation of electrons to the dienamine system ($(CH_3)_2N$, CH_3O, CH_3S, Cl, and F) significantly deviated from the relationships defined by the remaining compounds, and the degree of deviation appeared to be related to the ease of electron donation associated with the substituent. As in previous examples, lines fitted to the deviating data had significantly different slopes ($p < 0.01$) than did the well-behaved examples. Interestingly, when the nitrogen of the dimethylamine group is oxidized

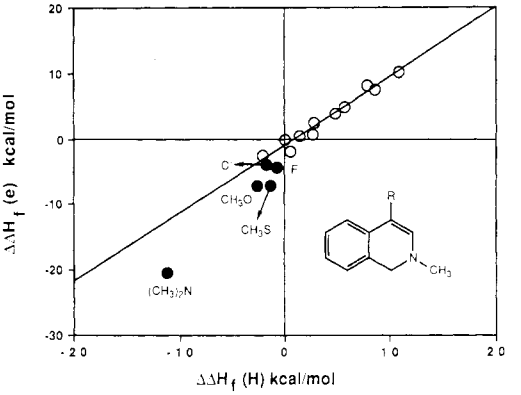


Figure 5. A correlation between the energy associated with hydride loss and with electron loss for a series of 2-methyl-4-substituted-1,2-dihydroisoquinolines. The data ($\Delta\Delta H_f$) are shown relative to 2-methyl-4-carbamoyl-1,2-dihydroisoquinoline (0,0). The correlation produced was significant (r = 0.98), and the slope of the generated relationship was 1.05. The correlated data (open circles) did not include compounds containing dimethylamino, methoxy, methylthio, chloro, or fluoro groups (closed circles). Lines drawn through each of these data sets had significantly different slopes ($p < 0.01$).

forming the N-oxide or when the sulfur of the methyl sulfide group is oxidized forming the sulfoxide, the resulting compounds behave normally. Thus, when the electron-donating potential of the substituents is oblated, the derivatives can be described by a linear energetic relationship.

Collectively, these data indicate that the electronic and hydridic oxidation of dihydropyridines and related heterocycles are responsive to similar substituent effects resulting in similar changes in free energies of reaction. In cases where experimental data is available, excellent agreement with theoretical predictions is obtained for oxidative stabilities and chemical proclivities. The results collected here indicate that the mechanistic route taken by a particular dihydropyridine may be distinguishable. These studies have shown that substitution of dihydropyridines and related heterocycles with a 3-methoxy or 3-(dimethylamino) group causes a clear deviation from the characteristic unitary, linear slope produced by comparing electronic and hydridic oxidation. This deviation is apparently related to the inability of the potentially destabilizing moieties to exert their full influence in the hydride-transferring intermediate. In contrast, electron-withdrawing groups exert stabilizing influences on the ground state in both electronic and hydridic oxidation. Thus, one might expect deviation from linearity in reactions involving hydride transfers when measured by a variety of electronic parameters. Unfortunately, this effect may not be an experimentally verifiable prediction in that the compounds which were shown to deviate significantly in this study would be highly unstable products. In general, derivatization of the 3-position with electron-withdrawing groups capable of conjugative interaction are required to produce chemically stable dihydropyridines.[11] The unsubstituted 1-methyl-1,4-dihydropyridine has been prepared, but is highly unstable. It is doubtful that this material would be of appropriate stability to endure experiment manipulation associated with the necessary kinetic determinations.[12] The study of even more unstable derivatives is therefore problematic. In this sense, these

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theoretical investigations allow the knowledge base of chemically and biologically important dihydropyridine to expand, unencumbered by the practical confines imposed by experimental methodologies.

Acknowledgment. We are indebted to M. J. S. Dewar for his many useful discussions on this project. In addition, the expert editorial skills of Joan Martignago are thankfully appreciated.

Synthesis of Ester Derivatives of Chloramphenicol by Lipase-Catalyzed Transesterification in Organic Solvents

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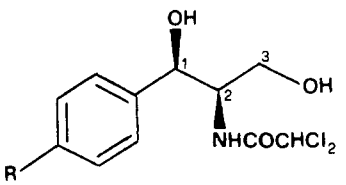
Received September 11, 1989

Regioselective esterification of chloramphenicol (1) and its synthetic analogue thiamphenicol (2) has been achieved by the action of lipase in acetone and several methyl carboxylates. Aliphatic and aromatic esters of different sizes and natures have been introduced selectively on the primary hydroxyl group of these molecules by modification of the reaction conditions (e.g., temperature, solvent, and lipase source).

Introduction

Chloramphenicol (1) is a natural antibiotic with a fairly wide spectrum of antimicrobial activity.[1] It inhibits protein synthesis in bacteria and, to a lesser extent, in eukaryotic cells by binding to the 50S ribosomal subunit, thus preventing the access of aminoacyl tRNA to the ribosome. Chloramphenicol is widely employed in veterinary medicine against infections of the urinary tract and other bacterial diseases. On the other hand, human therapy with 1 is limited to those infections (typhoid fever, bacterial meningitis) in which the benefits of the drug outweigh the risks of potential toxicity (1 can cause serious and fatal blood dyscrasias). Chloramphenicol may be administered



1 : R = NO_2

2 : R = CH_3SO_2

orally, usually as the water-insoluble 3-*O*-palmitate, or intravenously as the inactive 3-*O*-succinate ester. Both these derivatives are rapidly hydrolyzed in vivo to the biologically active drug.[1,2] Since the preparation of several other esters of 1 has been reported in the patent literature,[3] we focused on exploiting enzymatic acylation of chloramphenicol as a model for the production of useful simple drug derivatives. Here we report our results for the synthesis of various 3-*O*-esters of 1 by the action of lipase in organic solvents.

Results and Discussion

The standard production method for fatty acid 3-*O*-esters of chloramphenicol (*threo*-(1*R*,2*R*)-1-(4-nitrophenyl)-2-(dichloroacetamido)-1,3-propanediol (1)) involves the reaction of 1 with a suitable acyl anhydride or acyl chloride in the presence of a tertiary amine.[3] More recently, chloramphenicol esters have been obtained through biological catalysis. An initial paper[4] reported the isolation of a mixture of acyl derivatives of 1 upon incubation with spores, washed mycelium, or whole cultures of *Streptomyces griseus*. A second approach[5] took advantage of the catalytic action of a specific enzyme (chloramphenicol acetyltransferase from *Streptococcus faecalis*) to achieve the synthesis of 3-*O*-acetyl-1. A serious drawback of this last method is the requirement for a stoichiometric amount of the expensive cofactor acetyl coenzyme A.

Looking for a different and more effective enzymatic approach, we noticed that the inert 3-*O*-palmitate ester of 1, used in the pharmaceutical preparation to circumvent the bitter taste of 1, is hydrolyzed to the free, biologically active chloramphenicol by intestinal and pancreatic lipase.[1] We decided to take advantage of this class of enzymes by reversing their hydrolytic activity. It is well-known that lipases, when suspended in a suitable organic solvent, can catalyze esterification or transesterification reactions,[6]

Table I. Percentage Acylation (%) in the Transesterification Reactions between Chloramphenicol and Various Methyl Carboxylates[a]

	methyl carboxylate				
lipase	acetate	propionate	butanoate	hexanoate	octanoate
pancreatic[b]	45.0	57.5	72.2	47.4	50.7
P[c]	76.9	76.6	85.1	74.0	73.2
CE-5[d]	53.4	70.6	71.1	72.8	65.8
Ch.v.[e]	83.0	83.6	85.6	81.8	78.5
G[f]	82.6	83.0	83.6	81.1	81.6

[a] Conditions: 50 mM chloramphenicol and 100 mg of lipase "straight from the bottle" in 1 mL of anhydrous methyl carboxylate were shaken at 250 rpm for 96 h at 45 °C. Conversion estimated by HPLC: Partisil 10 column (Whatman) eluted with hexane–propanol, 9:1; flow rate, 1 mL min^{-1}; readings were made at 300 nm. [b] Sigma Chemical Co. [c] Lipase from *Pseudomonas fluorescens* (Amano Pharm. Ltd.). [d] Lipase from *Humicola langinosa* (Amano Pharm. Ltd.). [e] Lipase from *C.v.* (Finnsugar Biochem. Inc.). [f] Lipase from *P. cyclopium* (Amano Pharm. Ltd.).

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