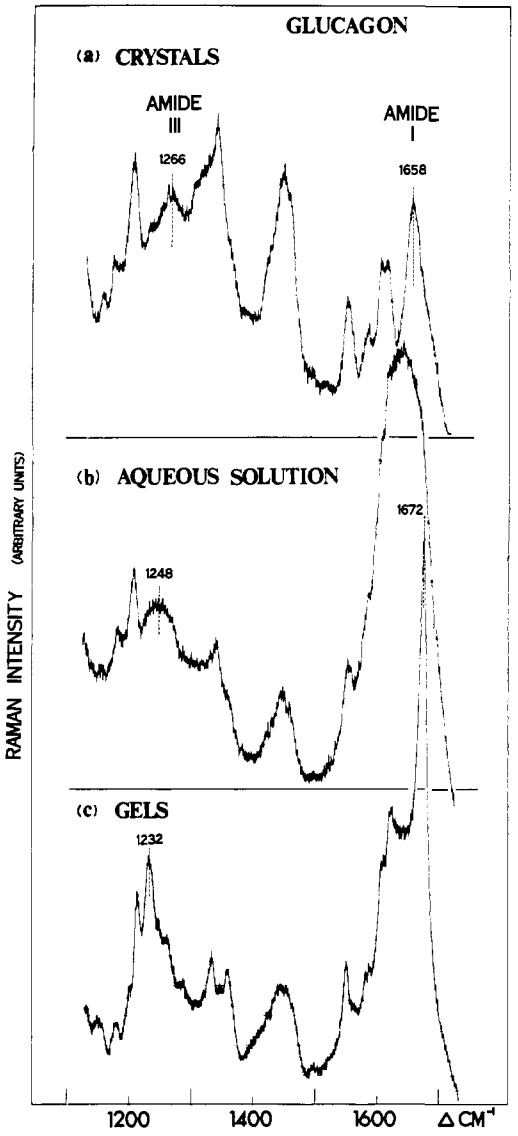

Figure 1. Raman spectra of glucagon in various states of aggregation. (a) Spectrum of crystalline glucagon (powder form): spectral slit width ($\Delta\sigma$), 4 cm^{-1}; sensitivity (s), 1000 cps full scale; rate of scan (γ), 10 cm^{-1}/min; standard deviation (sd), 1%; laser power (p) at the sample, 150 mW. (b) Spectrum of freshly prepared aqueous glucagon. The spectrum was obtained 1 hr after the solution was prepared: concentration (c), 20 mg/ml; pH 2.25; $\Delta\sigma$, 4 cm^{-1}; s, 2500 cps; γ, 10 cm^{-1}/min; sd, 1%; p, 250 mW. (c) Spectrum of gels formed from b on standing (~40 hr at 26°): $\Delta\sigma$, 5 cm^{-1}; s, 2500 cps; γ, 10 cm^{-1}/min; sd, 1%; p, 230 mW.

1b) has changed into a doublet: one at 1232 and the other at 1256 cm^{-1}. On further standing, we have observed that the intensity ratio of the 1232 cm^{-1} line to the 1256 cm^{-1} line increased and eventually approached the limit shown in Figure 1c. This indicates that an intermediate structure exists in the conversion of random-coiled to antiparallel β glucagon. A detailed discussion on the time-dependent Raman spectra of acidic glucagon solution will be given in a future publication.

It is certainly noteworthy to mention that infrared and Raman techniques do not detect the same amide I vibrations in both peptide homopolymers and proteins. In polyglycine I,[9] the infrared amide I bands appear at 1636 (strong) and 1685 cm^{-1} (medium), while the Raman amide I line shows up at 1674 cm^{-1}

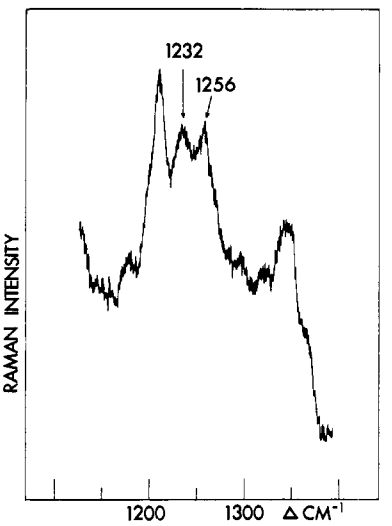

Figure 2. Raman spectra of incompletely formed gels of glucagon (from Figure 1b). This spectrum was obtained after 15 hr of standing at 26°. Conditions for the spectrum are the same as for Figure 1c.

(strong) as mentioned previously. In glucagon fibrils,[16] the infrared amide I bands are at 1630 (strong) and 1685 (weak), while the Raman amide I line is found at 1672 cm^{-1} (strong). In an earlier communication,[1] we have pointed out that the denatured fibrous insulin has the infrared amide I band at 1637 cm^{-1} and the Raman amide I line at 1673 cm^{-1}.

In order to determine the effect of water molecules on the conformation of fibrous glucagon, we obtained the spectrum of glucagon fibrils in the solid state. The amide I and III frequencies are found to be the same as those of Figure 1c, indicating that the antiparallel β structure of glucagon gels remains in the solid state.

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Intrinsic Basicities of Ammonia, Methylamines, Anilines, and Pyridine from Gas-Phase Proton-Exchange Equilibria[1]

Sir:

Measurements of the proton affinities of a variety of organic compounds by several different methods have been reported.[2] However, many important organic bases have not been investigated. The present work was stimulated by a recent determination of gas-phase

(1) Presented in part at the 3rd Conference of Structure Energy Relationships, Tallahassee, Fla., Feb 17–19, 1972.
(2) For a review article, see J. L. Beauchamp, *Annu. Rev. Phys. Chem.*, 22, 527 (1971).

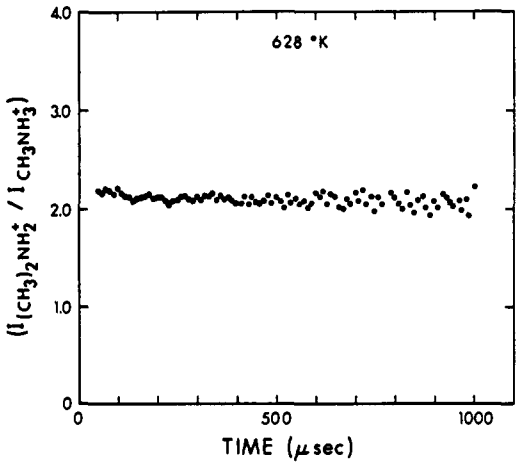

Figure 1. Ratio of ion signals for $(CH_3)_2NH_2^+$ and $CH_3NH_3^+$ observed after the 10-μsec electron pulse. Rapid establishment of the proton-transfer equilibrium leads to constant ion ratio over the entire observed time range: $p(CH_3NH_2)$ = 4 Torr, $p((CH_3)_2NH)$ = 10 mTorr, T = 628°K.

basicity orders of a large number of organic bases.[3] Since the technique used by Dzidic[3] did not give quantitative results, we proceeded to measure the equilibrium constants for the proton exchange reactions: $B_1H^+ + B_2 = B_1 + B_2H^+$. The organic bases used and the free-energy differences, $\Delta G° = -2.3RT \log K$, are given in Table I.

Table I. Proton-Transfer Reactions $M_1H^+ + M_2 = M_1 + M_2H^+$[a]

Direct equilibria measurements			Equilibria with $M_1 = NH_3$		
		$-\Delta G°$		$\Delta G°$	$\Delta G°$
M_1	M_2	(gas)	M_2	(gas)	(aqua)[b]
NH_3	CH_3NH_2	10.8	NH_3	0	0
CH_3NH_2	$(CH_3)_2NH$	7.5	$C_6H_5NH_2$	−8.9	+6.3
CH_3NH_2	$(CH_3)_3N$	12.5	CH_3NH_2	−10.8	−1.9
CH_3NH_2	$C_6H_5NHCH_3$	4.3	$C_6H_5NHCH_3$	−15.1	+6.1
$C_6H_5NHCH_3$	$(CH_3)_2NH$	3.5	$(CH_3)_2NH$	−18.3	−1.9
$C_6H_5NH_2$	CH_3NH_2	1.9	Pyridine	−18.6	+5.4
CH_3NH_2	Pyridine	7.8	$(CH_3)_3N$	−23.3	−0.8

[a] All values in kcal/mol. Gas-phase values at 600°K. Since temperature dependence is very small, $\Delta G° \approx \Delta H°$ and $\Delta G°_{600} \approx \Delta G°_{300}$. Numerical values in the second $\Delta G°$ column represent proton affinities relative to ammonia. The error in the $\Delta G°_{600}$ determinations is estimated at some 5%, *i.e.*, ~0.4 kcal/mol for the average determination. [b] Calculated from base constants K_{B_2}/K_{BNH_3} in aqueous solution (C. R. Noller, "Chemistry of Organic Compounds," 3rd ed, W. B. Saunders, Philadelphia and London, 1966, p 986), 300°K.

The equilibrium constants, $K = [B_1][B_2H^+]/[B_1H^+][B_2]$, were obtained with a high-pressure mass spectrometer.[4] Generally the gas with the lower basicity was used at a known pressure of 1–5 Torr while the stronger base was in the 5–100-mTorr range. Ionization was achieved by electron beam pulses of 10-μsec duration fired at intervals of several milliseconds. The ions escaping through a narrow slit of the reaction chamber into a vacuum chamber were mass analyzed and detected. The time dependence of the ion count ratio for $CH_3NH_3^+$ and $(CH_3)_2NH_2^+$ is shown in Figure 1. The ratio is constant over the total observational period.

(3) I. Dzidic, unpublished results.
(4) R. Yamdagni and P. Kebarle, *J. Amer. Chem. Soc.*, **93**, 7139 (1971).

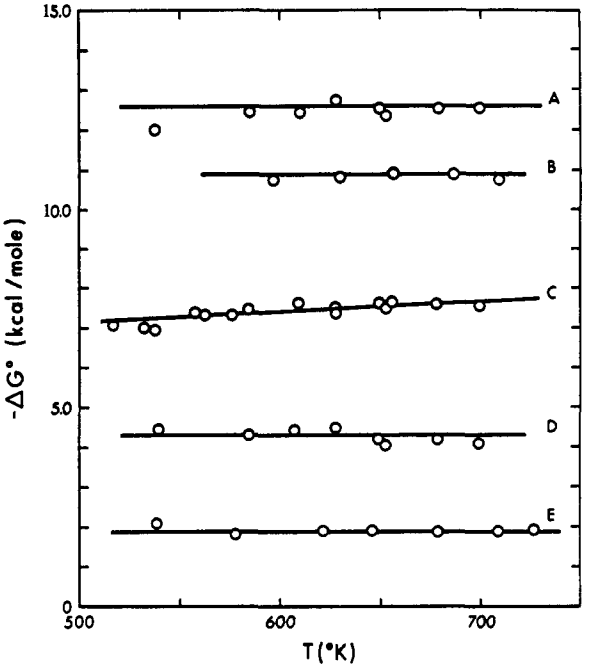

Figure 2. Temperature dependence of $\Delta G° = -2.3\ RT \log K$ for proton-transfer equilibria: $M_1H^+ + M_2 = M_1 + M_2H^+$. M_1, M_2 are: A, CH_3NH_2, $(CH_3)_3N$; B, NH_3, CH_3NH_2; C, CH_3NH_2, $(CH_3)_2NH$; D, CH_3NH_2, $C_6H_5 \cdot NH \cdot CH_3$; E, $C_6H_5NH_2$, CH_3NH_2.

Since the proton-transfer reactions require only tens of microseconds for completion,[2] the observed constant ratio should correspond to the equilibrium ratio. The equilibrium constants were found to remain unchanged for a variation of the neutral reactant ratio by a factor from 3 to 10.

Since neutrals ratios as large as 1000 could be used and the multiscaling detection allowed ion ratios as large as 10^3 to be measured, equilibrium constants as large as 10^5 could be determined directly. Simultaneous equilibria caused by the presence of small impurities (*i.e.*, dimethylamine in methylamine) were observed but did not interfere with the measurements.

B_1H^+ and B_2H^+ were the two major ions observed between 700 and 600°K. The concentrations of the dimers $(B_1)_2H^+$, $(B_1B_2)H^+$, and $(B_2)_2H^+$ increased progressively as the temperature was lowered. At 500°K they became the dominant ions and the BH^+ intensities became difficult to measure. The equilibria involving the dimers, trimers, etc., contain interesting information which will be reported in a separate publication.[5]

The temperature dependence of the monomer equilibria is shown in Figure 2. Evidently $\Delta G° = \Delta H° - T\Delta S°$ changes very little with temperature, *i.e.*, $\Delta S°$ is very small. A small $\Delta S°$ is expected for the proton-exchange reactions. The results are not accurate enough to permit an evaluation of the small entropy change. Least-squares treatment of the line with the largest slope (Figure 2C) gives $\Delta S°$ = 3.7 eu. However, if the three less accurate, low-temperature points are omitted one obtains $\Delta S°$ = 1.9 eu only. Obviously $\Delta G° = \Delta H°$ to a good approximation.

The second last column in Table I gives the $\Delta G°$ differences relative to ammonia. These differences should be close to the proton-affinities differences. The

(5) R. Yamdagni and P. Kebarle, unpublished results.

gas-phase basicities of the amines increase in the order ammonia, methylamine, dimethylamine, and trimethylamine, *i.e.*, with increasing methyl substitution. This is the order that can be expected on the basis of the electron-donating effect of the methyl groups. This simple order is not observed in aqueous solution (see last column of Table I).

Aniline and pyridine are stronger gas-phase bases than ammonia, while aniline and pyridine are much weaker bases in aqueous solution. Most organic chemistry textbooks explain the relative weak aqueous basicity of aniline by the resonance[6] and the electron-withdrawing inductive effect of the phenyl group. Both of these are intrinsic molecular properties which should show up in the gas phase. Since this is not the case, the low basicity of aniline (relative to ammonia) is largely due to solvent effects. Perhaps it is more appropriate to compare aniline to an amine of similar size, *i.e.*, cyclohexylamine. A preliminary determination shows that $\Delta G^\circ \simeq 11$ kcal/mol for proton transfer from cyclohexylamine to aniline. The much greater base strength of cyclohexylamine thus is in qualitative agreement with a relative electron withdrawal from the N atom in aniline.

Arnett[7] has recently proposed a simple thermodynamic cycle treatment based on gas-phase basicities which promises to lead to a meaningful separation of solvent and intrinsic molecular effects.

The above measurements were completed before we learned that similar organic bases have recently been investigated by the ion-cyclotron resonance technique.[8] The results from these measurements which were done at room temperature and pressures of around 10^{-5} Torr are in good agreement with the present findings.

(6) G. W. Wheland, "Resonance in Organic Chemistry," Wiley, New York, N. Y., 1965, pp 355–357.

(7) E. M. Arnett, "Thermodynamic Properties for Ionization and Solvation of Amines and Their Conjugate Ions," paper presented at 3rd Conference of Structure Energy Relationships, Tallahassee, Fla., Feb 17–19, 1972. A very similar cycle was used earlier for the related proton transfer $HA + B^- = A^- + BH$ [J. D. Payzant, R. Yamdagni, and P. Kebarle, *Can. J. Chem.*, **49**, 3308 (1971)].

(8) (a) M. Taagepera, W. H. Henderson, R. T. C. Brownlee, J. L. Beauchamp, D. Holtz, and R. W. Taft, results quoted at the 3rd Conference of Structure Energy Relationships, Tallahassee, Fla., Feb 17–19, 1972. (b) R. T. McIver, and R. W. Taft, ref 8a.

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Analysis of Steroid Nuclear Magnetic Resonance Spectra Using Paramagnetic Shift Reagents

Sir:

Recent publications have indicated the potential utility of the so-called shift reagents in interpreting nmr spectra of steroids.[1-3] In such studies, assignment of resonances is generally made by following changes in the chemical shifts for protons of the steroid as the concentration of the shift reagent is increased in the solution under observation. These changes in chemical shifts, $\Delta\delta$, are assumed to be linear and usually to lower field with increasing concentration of shift reagent. In most cases $\Delta\delta$ has been interpreted as arising from a contact interaction, pseudocontact interaction, or combination of the two, between the shift reagent and the substrate. In the case of pseudocontact interaction, the magnitude of $\Delta\delta$ has been assumed to be inversely proportional to the cube of the distance between the metal ion of the shift reagent and the proton giving rise to the resonance in question.[3]

Recently, our laboratory had occasion to apply this technique to an attempt to analyze the stereochemistry of several cholesterol derivatives. Before proceeding with the analysis of the derivatives, the spectrum of cholesterol itself in the presence of a shift reagent was examined. The present communication reports the findings of this experiment.

A 0.131 *M* solution of cholesterol in $CDCl_3$–TMS was prepared volumetrically. The shift reagent tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione)europium(III) was weighed into the volumetric flask before bringing the solution to final volume. The amount added was such that the final solution was 0.128 *M* in the shift reagent. Thus, the molar ratio of shift reagent to steroid, *f*, was 0.98 in this solution.

An aliquot of the solution was used to obtain its spectrum at 100 MHz.[4] After removal of the aliquot, the remaining solution was weighed and a further solution *with identical value of f* was prepared by dilution. By repeating this process, spectra of cholesterol in the presence of shift reagent were obtained for various concentrations of cholesterol, all at identical molar ratios of shift reagent:cholesterol.

Assignments of resonances were made by comparison with Hinckley's data[1] and by consideration of the expected coupling patterns for the methylene protons of ring A of cholesterol. A plot of the chemical shifts, δ, *vs.* molar concentration of cholesterol, *C*, is shown in Figure 1. It is seen that the variation of δ with *C* is not linear, even though *f* is constant throughout the entire range of *C* examined. Furthermore, it is obvious that even at the highest concentration of cholesterol investigated, the value of δ is still increasing. A similar observation of nonlinearity of δ with respect to concentration of substrate has been reported by Tomic, *et al.*, in a study of cyclopropylmethanols and the shift reagent tris(dipivalomethanato)praseodymium(III).[5]

The nature of this variation of δ with *C* is reminiscent of the variation of δ OH with concentration of alcohols in solution, which is attributed to the rapid equilibrium of hydrogen bonding in such cases.[6] If it is assumed that in the present case complex formation between substrate S and europium chelate E involves a rapid equilibrium with the complex SE (as in (1)), the equi-

$$S + E \rightleftharpoons SE \quad (1)$$

(1) C. C. Hinckley, *J. Amer. Chem. Soc.*, **91**, 5160 (1969).

(2) P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *ibid.*, **92**, 5737 (1970).

(3) C. C. Hinckley, M. R. Klotz, and F. Patil, *ibid.*, **93**, 2417 (1971).

(4) The cholesterol used in this experiment was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. The shift reagent was obtained from Alfa Inorganics, Beverly, Mass., and was kept in a sealed ampoule in a desiccator until used. Spectra were obtained on a Varian Associates HA-100-15 spectrometer using a 5-mm sample tube. In some cases spectra were recorded after several scans using a time-averaging computer. The temperature at which the experiment was performed was 37 ± 0.5°.

(5) L. Tomic, Z. Majerski, M. Tomic, and D. E. Sunko, *Chem. Commun.*, 719 (1971).

(6) J. W. Emsley, J. Feeney, and L. H. Sutcliff, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 1, Pergamon Press, New York, N. Y., 1965, Chapter 9, and references therein.