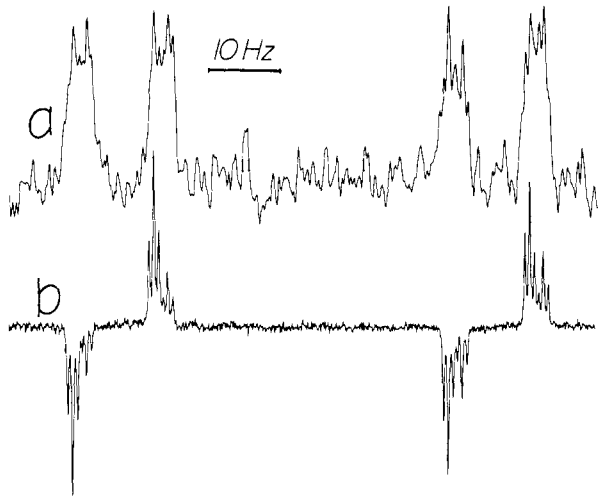
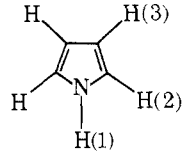


Figure 2. Natural abundance coupled ^{15}N FT NMR spectra of 2-fluoropyridine, **2** (85% v/v in acetone-d_6). (a) Normal coupled spectrum (no gated decoupling, 15 000 transients, 0.35-Hz line broadening). (b) $^{15}N\{H(6)\}$ SPT spectrum obtained after 2000 transients (no line broadening; $\gamma_{H_2}/2\pi$ = 0.5 Hz and τ = 1.0 s).

can often be observed from a single transient. The SPT π-pulse method is a powerful technique for intensity enhancement, especially with spin systems having degenerate ^1H transitions, as well as for sign determination of coupling constants and for spin-lattice relaxation studies.[9-12] In $^{15}N\{^1H\}$ SPT experiments, the ratio $|\gamma_{^1H}/\gamma_{^{15}N}| \approx 10$ is very favorable for intensity enhancements. For example, a ^{15}N nucleus coupled to six equivalent hydrogen nuclei would show a septet containing lines of which six may be enhanced, if the relaxation times and acquisition conditions are favorable,[8,9,13,14] by ratios of 20 to 40. A great advantage of the SPT method is that, for repetitive experiments, the *^{15}N NMR spectrum may be sampled with a repetition rate determined by the usually much greater relaxation rate of the irradiated protons rather than by the often very slow relaxation of the observed ^{15}N nucleus.* In fact, this experiment has several of the advantages of the proton-enhanced nuclear spectroscopy method introduced by Pines et al.[15] to observe rare spins in solids and recently also applied to liquids.[16] Natural abundance ^{15}N spectra for pyrrole (**1**) and 2-fluoropyridine (**2**) in Figures 1 and 2 demonstrate the power of the SPT method.



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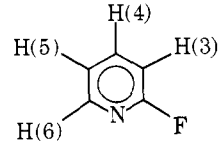

2

Spectra were obtained at 10.15 MHz on a Varian XL-100-15 spectrometer equipped with a Varian Gyrocode decoupler (used to generate the SPT π pulses at 100.06 MHz), Nicolet computer system, MONA multinuclear assessory, and 18-mm probe (however, without quadrature detection or single side-band filter). Free induction decays were stored into 16K data points using a spectral width of 1000 Hz and an acquisition time of 8 s. A 90° flip angle (28 μs) was used for $^{15}N\{^1H\}$ SPT π-pulse spectra and a 40° flip angle for normal spectra. All sample solutions were 85% v/v solutions in acetone-d_6 or benzene-d_6.

For the natural abundance ^{15}N NMR spectrum of pyrrole (**1**) in Figure 1, the S/N ratio is ~1.5 times that of a published spectrum[3] obtained from neat 96% ^{15}N-enriched pyrrole in a 5-mm-o.d. tube using the same number of transients (512) and the same acquisition time (8 s). With different irradiation frequencies and lower power, we have established that $^2J_{^{15}N\text{-}H(2)} \times {}^3J_{H(1)\text{-}H(2)} < 0$ and $^3J_{^{15}N\text{-}H(3)} \times {}^4J_{H(1)\text{-}H(3)} < 0$, thus confirming earlier observations and assumptions.[17] In Figure 2 the usual coupled ^{15}N spectrum of 2-fluoropyridine (**2**) obtained with 15 000 transients in 37 h is compared with an SPT spectrum obtained with 2000 transients in 5 h.

This method of sensitivity enhancement will also be applicable to larger molecules, which have shorter ^1H and ^{15}N T_1 values. The short proton T_1 values may afford an advantage, for their only effect is to permit more rapid pulsing. Results reported for ^{15}N relaxation times in proteins indicate that these are of the order of several tenths of a second or longer, a time sufficient to permit a substantial portion of the SPT gain to be preserved during acquisition. Furthermore, in the range of longer correlation time, it is not possible to increase sensitivity by utilizing the Overhauser effect, for the enhancement factor lies between 0 and −1, so that the SPT method should be of special advantage.

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A Time-Dependent Cyclodextrin Induced Perturbation of Ionic Equilibria across a Carbohydrate Membrane[1]

Sir:

Molecular transgression across heterogeneous boundaries is currently a focal issue in many areas of chemistry.[2] Explo-

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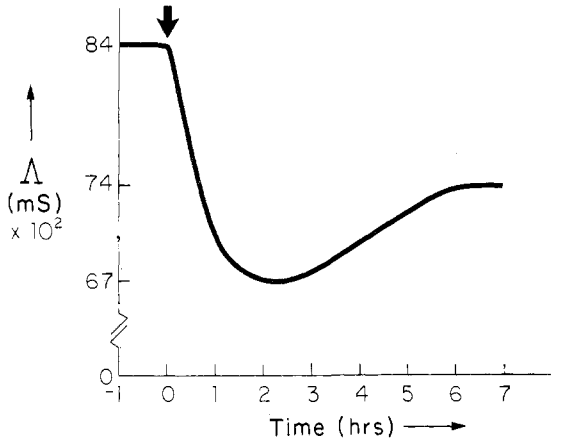

Figure 1. Observed conductance vs. time. Arrow indicates time when PNP and PNP/αCD solutions are brought in contact with the membrane.

ration of model processes is of interest for understanding the chemical basis of and driving forces behind this fundamental phenomena. We herein report kinetic studies of a biomodel for active transport of ions through permeable membranes. Our model system consists of a simple carbohydrate film used to separate aqueous solutions of potassium *p*-nitrophenoxide (PNP) and cyclodextrin (αCD), a neutral nonionic polysaccharide.[3] The αCD binds to and mediates the transport of PNP through the heterogeneous barrier. This biomodal diffusion process produces a potassium salt gradient across the carbohydrate membrane. The ion gradient is transient (0.5–6 h) and kinetically controlled by a *parallel* concentration gradient of αCD.

The commercially available cellulose membrane is highly permeable to water and other low molecular weight compounds, but retains molecules larger than 12 000 daltons.[4] Inasmuch as the average membrane pore diameter is 48 Å, both αCD and PNP, which have minimal radii of 3 and 7 Å, respectively, can readily migrate through the 8×10^5 Å thickness of the hydrated membrane. In a typical experiment, illustrated in Figure 1, the conductance of a 9 mM PNP solution decreases over a period of ~90 min from 0.84 to 0.67 mS when placed in "contact" with, but separated by the membrane barrier from, a solution that contains 18 mM αCD in addition to 9 mM PNP. Following the initial drop, the conductance slowly rises over a 4-h interval to an ultimate value of 0.74 mS. Thus, the initial and final equilibria, each of which have a zero ion gradient for PNP, have been perturbed at intermediate times. As the αCD migrates down its concentration gradient toward equilibrium, an ion imbalance is created through selective PNP migration up that αCD gradient. Control experiments in which methyl α-D-glucoside was used in place of αCD revealed no significant perturbation of the PNP ionic equilibrium. Hence, we attribute our observations to the structurally unique hydrophobic cavity in αCD.

The extent of the observed ion imbalance depends on the magnitude of the αCD gradient. As shown in Figure 2, the difference in conductance of the solutions on either side of the membrane ($\Delta\Lambda$) is 0 if the value of R, the mole ratio of αCD to PNP, is also 0. As R increases to ~2.8, $\Delta\Lambda$ increases to a maximum. At larger R, $\Delta\Lambda$ asymptotically returns to 0. These observations may be explained as a consequence of the well-established formation of a molecular inclusion complex between PNP and αCD. In the absence of any αCD ($R = 0$) no ionic imbalance is possible and $\Delta\Lambda = 0$. At large αCD concentrations, where R approaches infinity, a sufficient quantity of αCD will diffuse across the membrane to bind essentially all of the PNP in the entire system within a short period. Thus, no driving force would exist to generate an ion gradient. At intermediate R values, the rates for diffusion of both αCD and

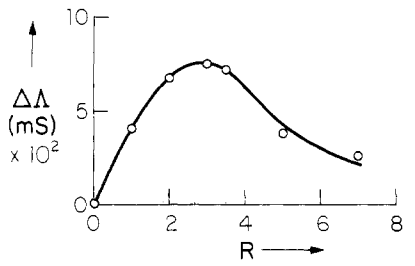

Figure 2. Variation of ion imbalance with concentration of αCD.

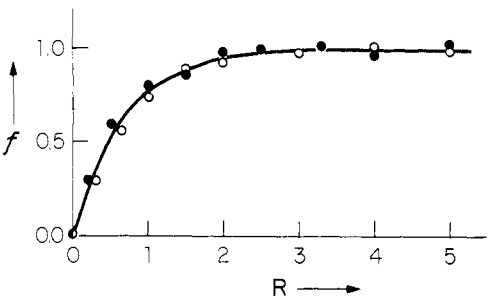

Figure 3. Fraction of PNP bound to αCD as a function of αCD concentration: open circles from conductimetric titration,[7] closed circles from kinetic data,[6,8] solid line calculated from Kd and known concentrations.[3,5,9]

PNP across the membrane are competitive, so that a transient ion gradient can be established with a variable degree of imbalance.

These phenomena depend on the rates for mass transport through a heterogeneous barrier, as governed by both the intrinsic rate constants for that process and, as well, the concentrations of the diffusing species. The exergonic binding of αCD and PNP (ΔG = ca. −4.6 kcal/mol)[5] provides the ultimate source of energy to maintain the ion gradient. Thus, the binding process effectively removes free PNP from one solution, and thereby drives free PNP from the opposite side of the membrane to diffuse across in an attempt to achieve an equal equilibrium distribution of free PNP throughout the entire system at all times.

As shown in Figure 3, the effect of added αCD on the fractional change in either (a) the conductance of a solution of PNP (open circles)[6] or (b) the observed first order rate constant for diffusion of PNP ions across the membrane (closed circles)[7,8] produces a curve that is clearly superimposable on the curve (solid line) for the fractional degree of binding between αCD and PNP, calculated from the known values of substrate concentrations and K_d.[5,9] As an additional indication that the formation of an αCD·PNP complex provides the driving force inherent in these experiments, we note that the maximal value for $\Delta\Lambda$ in Figure 2 occurs at approximately the same concentration of αCD ($R \doteq 2.8$) as is needed to achieve complete binding of PNP, illustrated in Figure 3. The rate constant for diffusion of PNP (2.3×10^{-4} s^{-1}) was measured in the absence of added αCD, while the corresponding value for the αCD·PNP complex (6.4×10^{-5} s^{-1}) was determined from the limit of the observed rate constant at high αCD concentrations.[9] The rate constant for free αCD diffusion across the membrane (9.6×10^{-5} s^{-1}) was determined in the absence of any added PNP.[10]

A minimal kinetic description of the curve in Figure 1 requires two superimposed mutually compensating exponential processes. Analysis of our data in terms of this model reveals that, although the magnitude of the deflection ($\Delta\Lambda$) significantly varies with R, the apparent first-order rate constants, which account for the overall curve shape, 2.8×10^{-4} s^{-1} and 7.1×10^{-5} s^{-1}, are both invarient with R and essentially the same as the independently measured values for diffusion of free

PNP and αCD·PNP complex through the cellulose membrane discussed above. The observed differences in the diffusional rate constants is in accord with that expected from differences in the hydrodynamic radii. The apparent "spherical" radii, estimated from CPK molecular models, for PNP and αCD·PNP are 2.2 ± 0.8 and 6.6 ± 0.5 Å, respectively. The ratio of these radii, 3.0 ± 0.9, is not significantly different from 3.6, the value of the inverse ratio of the observed diffusional rate constants for these species.[11] The greater mobility of αCD relative to the αCD·PNP complex also seems reasonable in view of the solvent access to the interior cavity of the free species.

Studies are in progress to further characterize and extend this oscillatory kinetic phenomena, as a model for active transport in biological membranes.

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(8) Good first-order kinetics were observed through >90% of each equilibration reaction. Measurements were made in an apparatus consisting of two chambers separated by a 13.75 cm^2 (2.5 × 5.5 cm) section of membrane, thermostated at 28.0 °C. Control experiments in which the membrane surface area was varied over a factor of four revealed no significant effect on the observed rate constants.
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Evidence for a Radical-Anion Pathway of a Phenylcyclopropyl Ring Cleavage in the Presence of Potassium *tert*-Butoxide

Sir:

2,3,4-Triphenyl-*endo*-tricyclo[3.2.1.$0^{2,4}$]octane (**1**) has been reported to undergo ring opening when treated with potassium *tert*-butoxide (KO-*t*-Bu) in dimethyl sulfoxide to give after workup 2,3,4-triphenylbicyclo[3.2.1]oct-2-ene (**2**).[1,2] This reaction was presumed to proceed via a forbidden disrotatory ring opening of the cyclopropyl anion formed by deprotonation and is the only example of such a reaction which proceeds facilely. Although **1** is probably more strained than a simple cyclopropane,[4] the known high-energy, symmetry-imposed barrier for disrotatory cyclopropyl anion openings[5] and the short lifetime expected for the cyclopropyl anion in Me_2SO[6] cast doubt on the validity of the proposed mechanism and suggested that further study of the reactions of **1** with strong bases should prove interesting. We report herein the results of our studies from which we infer that conversion of **1** to **2** occurs by a process involving reduction of **1**, radical-anion cyclopropyl bond cleavage, rearrangement, and oxidation of an intermediate to give **2**. Our interpretation requires that KO-*t*-Bu/Me_2SO and related base solutions can act as electron donors and suggests that other reactions related to the conversion of **1** to **2** may proceed by radical-ion pathways which were not previously considered.

Treatment of **1** with KO-*t*-Bu/Me_2SO at 70 °C as described by Mulvaney[1] or at 25 °C for 20 h gave **2**. Similarly **1** was converted to **2** by treatment with KO-*t*-Bu/hexamethylphosphoramide (HMPA) at 25 °C for 24 h or by dimsylpotassium (from KH and Me_2SO) in Me_2SO at 70 °C for 24 h. However, treatment of **1** with several other strong bases failed to produce **2**.[8] When **1** was treated with *n*-butyllithium–tetramethylethylenediamine complex in hexane at 25 °C, a purple solution (λ_{max} shoulder at 510–520 nm) was formed. Addition of deuterium oxide to this solution gave **1** which contained from zero to four deuterium atoms by mass spectrometry.[10] It is most likely that **3** was formed in this reaction since 1-lithio-1,2-diphenylcyclopropane (**4**) has λ_{max} at 490 nm.[11] Polydeuterated **1** could be formed by initial deuteration on an ortho or para position of the phenyl ring at C-3 to give, for example, **5** which should exchange protons readily.[12] The ^1H-decoupled ^{13}C NMR spectrum of polydeuterated **1** showed, among other minor changes, a greatly diminished intensity for the signal assigned to the para carbon atom of the phenyl ring on C-3 of **1** which is consistent with loss of Overhauser enhancement due to significant deuterium substitution.[13] Since **3** is stable, a cyclopropyl anion cannot be an intermediate in the pathway for conversion of **1** to **2**.

We conclude that **1** is converted to **2** by the mechanism shown in Scheme I. Electron transfer, presumably initially from base, to **1** gives radical anion **6** which cleaves to **7**. Subsequent rearrangement of **7** gives **8** which resembles a stilbene radical anion. Transfer of an electron from **8**, possibly to another molecule of **1**, produces **2**. In addition to the evidence

Scheme I

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