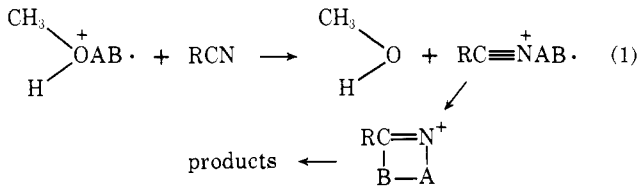
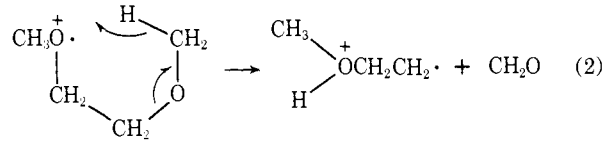
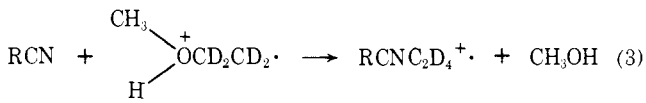
tion.[12] The proposed reaction must therefore fit the outline of eq 1.



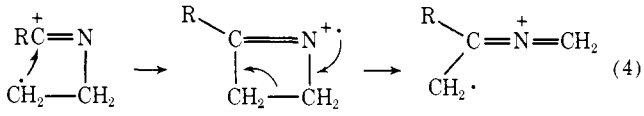
The simplest model of the reagent ion is the m/z 60 ion produced by loss of CH_2O from 1,2-dimethoxyethane (eq 2).[13]



In experiments using a modified Varian 5900 ion cyclotron resonance spectrometer, a range of simple aliphatic nitriles up to C_6 and aromatic nitriles without other functional groups was studied. Labeling experiments confirm the transfer of the central ethylene unit (eq 3). However, it does not transfer



$C_2H_4^{+}\cdot$ to any alkanes, alkenes, alkynes, aromatic hydrocarbons, alcohols, ethers, acids, esters, ketones, aldehydes, amines, Schiff bases, alkyl halides, sulfides, sulfoxides, sulfones, isonitriles, thiocyanates, isothiocyanates, cyanates, or nitro compounds which we have examined,[14] except for a slow reaction with acetone observable above 10^{-5}-Torr pressure which is not paralled by reactions with other ketones. This reaction is at least an order of magnitude slower than the reaction with nitriles. We estimate the latter as being on the order of 8×10^{-10} cm^3/molecule s, that is, reaction after most collisions. The reaction is sometimes faster than proton transfer and, within the compounds studied, never less than one fourth as rapid; it will be interesting to study nitriles containing other functional groups. This specificity and facility indicate that further internal reaction with the nitrile function probably leads to an easily accessible, stable product ion isomeric with the initially formed M + 28 ion. An obvious choice is shown in eq 4; in it the product is stabilized by formation of both a



center analogous to the $CH_2{=}N^{+}{=}CH_2$ ion observed in the spectra of appropriate amines[15] and also an allylic radical. Since the ion is stable to other ion–molecule reactions in mixtures of nitriles and 1,2-dimethoxyethane, this postulation remains only speculation for the moment.

We have demonstrated here that the body of information[16,17] on ion–molecule reactions has become sufficiently large that one can apply the chemistry to the *design* of specific ion–molecule reagents. In the future it will be possible to expand the collection of these reagents to confirm structural detail of new molecules by mass spectrometric analysis using specifically designed ion–molecule reactions.

References and Notes

(1) M. L. Gross, P. H. Lin, and L. J. Franklin, *Anal. Chem.*, **44**, 974 (1974).
(2) J. M. S. Henis, *J. Chem. Phys.*, **52**, 282 (1970).
(3) A. J. V. Ferrer-Correia, K. R. Jennings, and D. K. Sen Sharma: (a) *J. Chem. Soc., Chem. Commun.*, 973 (1975); (b) *Org. Mass Spectrom.*, **11**, 867 (1976).
(4) P. J. Ausloos, S. G. Lias, and J. R. Eyler, *Int. J. Mass. Spectrom., Ion Phys.*, **18**, 261 (1975).
(5) J. R. Hass, W. B. Nixon, and M. M. Bursey, *Anal. Chem.*, **49**, 1071 (1977).
(6) R. Yamdagni and P. Kebarle, *J. Am. Chem. Soc.*, **98**, 1320 (1976).
(7) J. F. Wolf, R. H. Staley, I. Koppel, M. Taagepera, R. T. McIver, Jr., J. L. Beauchamp, and R. W. Taft, *J. Am. Chem. Soc.*, **99**, 5417 (1977).
(8) J. I. Brauman, J. M. Riveros, and L. K. Blair, *J. Am. Chem. Soc.*, **93**, 3911 (1967).
(9) J. I. Brauman and L. K. Blair, *J. Am. Chem. Soc.*, **90**, 5636 (1968).
(10) D. Holtz, J. L. Beauchamp, and S. D. Woodgate, *J. Am. Chem. Soc.*, **92**, 7484 (1970).
(11) H. H. Jaffé and S. Billets, *J. Am. Chem. Soc.*, **92**, 6965 (1970).
(12) M. T. Bowers, D. D. Elleman, R. M. O'Malley, and K. R. Jennings, *J. Phys. Chem.*, **74**, 2583 (1970).
(13) T. H. Morton and J. L. Beauchamp, *J. Am. Chem. Soc.*, **97**, 2355 (1975).
(14) Some proton transfers and other reactions are observed.
(15) F. W. McLafferty, *Adv. Chem. Ser.*, **No. 40**, 21 (1963).
(16) T. A. Lehman and M. M. Bursey, "Ion Cyclotron Resonance Spectrometry", Wiley, New York, N.Y., 1976, Chapter 3.
(17) R. C. Dunbar, "Physical Methods of Modern Chemical Analysis", Vol. 3, T. Kuwana, Ed., Academic Press, New York, N.Y., in press.

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Characterization of Surfactant Vesicles as Potential Membrane Models. Effect of Electrolytes, Substrates, and Fluorescence Probes

Sir:

This communication reports properties of surfactant vesicles, prepared from aqueous dioctadecyldimethylammonium chloride (DODAC)[1] dispersions by sonication, which render them to be the simplest functional membrane model investigated to date.

Sonication dispersed DODAC initially to a turbid and ultimately to an optically transparent solution.[3] In agreement with analogous recent data,[2,4,5] electron micrographs[6] confirmed the presence of closed vesicles. The turbid solution, obtained by sonicating the DODAC dispersion for 30 s,[3] contained bilayer vesicles of 2500–4500 Å in diameter. The optically transparent solution, obtained by sonicating the DODAC dispersion for 15 min,[3] consisted of single compartment vesicles whose diameter, read off from electron micrographs, ranged between 1000 to 1500 Å.[7] All of the experiments described below were carried out on optically transparent solutions of single compartment 1000–1500-Å-diameter bilayer DODAC vesicles.

Aqueous solutions of DODAC vesicles were found to be stable for weeks in room temperature. Absence of unsaturated carbon atoms in the surfactant obviates degradation, a common problem with liposomes.[10] Surfactant vesicles, like liposomes,[10] were lysed upon the addition of 80% alcohol. Effects of electrolytes, however, were found to be more pronounced on surfactant vesicles than on liposomes. Sonication of the "typical" DODAC dispersion[3] in 0.10 M NaCl, for example, resulted in gel formation. Turbidity and subsequent flocculation in 0.10 M NaCl was observed even when the amount of DODAC was reduced by almost tenfold (i.e., 1.5 mg of DODAC in 2.0 mL of 0.10 M NaCl). Conversely, *diluted surfactant vesicles* (fourfold dilution of the "typical preparation" [3] by distilled H_2O) remained stable, albeit turbid, in 0.10 M NaCl. Analogous behavior has been observed for other electrolytes. Increase in turbidity upon the addition of electrolytes corresponds to osmotic shrinkage of the vesicles. It appears, however, that the range of electrolyte concentration

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Table I. Substrate Entrapment and Retention in Single Compartment Surfactant Vesicles

Substrate	% entrapment[a]	% leakage[b]
L-Alanine[c]	1.2	
L-Serine[c]	1.8	22.0, 3 h
8-Azaguanine[c]	34 (1.8)[d]	7.2, 3 h 15.4 (16),[d] 3 h
2-Aminopyridine HCl, 2AP[e]	0.05	
Dansyl-*n*-octadecylamine,[e] DSOA	62 (54)	Not detectable at 20 h

[a] Expressed as percent of entrapped substrate relative to that added prior to dispersion by sonication. In parenthesis are the values found using cationic single compartment dipalmitoyl-DL-phosphatidylcholine liposomes. [b] Expressed as percent of released substrate relative to that entrapped. Leakage was determined by dialysis using a seamless cellulose tubing at 25.0 °C. Rates of leakages followed saturation behavior. [c] Monitored by using C-14 labeled substrates. [d] Taken from ref 12. [e] Monitored spectrophotometrically.

which causes shrinkage of DODAC vesicles is considerably smaller than that observed for liposomes.[10,11]

Single-compartment bilayer DODAC vesicles entrapped and retained a number of substrates (Table I). They were cosonicated with the DODAC dispersions. Gel filtration on Sephadex G-50 separated the free molecules from those entrapped in the vesicles. The emerging pattern (Table I) is quite similar to that observed for entrapments in liposomes.[10,12,13] Long chain dansyl-*n*-octadecylamine is substantially taken up and it is likely to line up along the hydrocarbon backbones of the DODAC vesicles. Entrapments and retention of polar amino acids in surfactant vesicles and liposomes[10,14] follow the same behavior. The ability of surfactant vesicles to encapsulate molecules is perhaps best illustrated by the successful incorporation of the 2-aminopyridine monocation in positively charged DODAC vesicles. Even more impressive is the 34% entrapment of 8-azaguanine (Table I). In cationic single compartment liposomes, the uptake of this molecule is only 1.8%.[12]

Binding of L-serine and 8-azaguanine to the outer surface of DODAC vesicles were also established. These substrates were added to the already formed vesicles (using the "typical preparation").[3] Free substrates were separated from those bound to the vesicles by gel filtration on Sephadex G-50. The charged outer surface of the vesicles attracted 0.8 and 6.2% of the added L-serine and 8-azaguanine, respectively. Comparisons with the amount of entrapped substrates (Table I) indicate that most of the 8-azaguanine is localized in the interior of the vesicle while L-serine is equally distributed between the interior and the outer surface. Subsequent to binding, 52% of 8-azaguanine is released within 90 min from the outer surface of the vesicle. Conversely, the rate of entrapped L-serine leakage is identical with its release from the outer surface of the DODAC vesicle. Apparently, the diffusion of L-serine across the hydrocarbon bilayer of the vesicle is more facile than that for 8-azaguanine.

Dansyl-*n*-octadecylamine, DSOA, 2-methylanthracene, 2MA, 2-aminopyridine hydrochloride, 2AP, were used as fluorescence probes to report their microenvironments. Excitation of DSOA entrapped in DODAC vesicles resulted in an emission maximum of 475 nm with a relative fluorescence efficiency (on the previously reported calibration scale)[15] of 0.7. Using the previously determined correlations,[15] DSOA had an apparent environment in DODAC vesicles somewhat more polar than benzene. Conversely, DSOA in liposomes experiences an environment substantially more polar than chloroform.[15] This difference in the apparent polarities is a consequence of the different location of the dansyl moiety of the probe in DODAC and liposome vesicles. This, in turn, may reflect differences in the bilayer thickness of these vesicles. Using fluorescence polarizations, microviscosity of the environment of 2MA entrapped in DODAC vesicles were determined to be 144 cP. In contrast, 2MA revealed a microviscosity of 291 cP in single compartment dipalmitoyl-DL-phosphatidylcholine.[8] 2AP is electrostatically repelled from the surface of the cationic DODAC vesicle and is located, therefore, well within the aqueous interior of the surfactant vesicle. This postulate is borne out by the identical microviscosity as determined by fluorescence polarization of 2AP in the DODAC vesicle (P = 0.00228) and in bulk water (P = 0.00220). However, the observed emission maximum in the DODAC vesicle (λ_{max} 357 nm) indicated 2AP to be in environment somewhat more polar than ethanol but not as polar as water.[16]

Since upon neutralization the fluorescence efficiency of 2AP decreases 20-fold and since the emission maximum is shifted from 367 to 355 nm,[16] this probe provided a fruitful means for monitoring hydrogen-ion concentration at the interior of DODAC vesicles as well as permeabilities of protons and hydroxide ions across the hydrocarbon bilayer. Two different experiments were performed. In the first, 2AP was dispersed with DODAC in 1.0×10^{-2} M HCl. Subsequent to gel filtration, the pH of DODAC entrapped 2AP was adjusted to 10.0 by NaOH. As soon as measurements could be made (within 2 min) the emission spectra indicated the complete neutralization of 2AP. Subsequent to displacing the chloride counterions, hydroxide ions permeate freely, therefore, across the DODAC vesicles.[17] In the second set of experiments, neutral 2-aminopyridine was entrapped into the DODAC vesicle at pH 12. Subsequent to the separation, the pH of the DODAC entrapped probe was adjusted to 2.0 (by HCl). Within the time of measurements, the spectra indicated complete protonation. Apparently, in spite of electrostatic repulsions, the proton permeates freely into the vesicle interior. Conversely, substantial proton gradients can be maintained across cationic liposomes.[8]

This study established surfactant vesicles to be the simplest membrane mimetic agents. They are stable, do not degrade, and are able to entrap molecules. They are sensitive, however, to electrolytes and their bilayers are less dense than those of liposomes or membranes. Consequently, chemical modifications are needed prior to utilizing these surfactant vesicles for permeability studies. Such modifications are the object of our current attention.

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References and Notes

(1) We are grateful to Drs. K. Deguchi, J. Mino, and K. Tsujii of the Kao Soap Company Ltd., Tokyo, Japan, for supplying us with a sample of pure DODAC and for providing information prior to publication.[2]
(2) K. Deguchi and J. Mino, unpublished results, 1977.
(3) Typically, 12 mg of DODAC was dispersed in 2.0 mL of distilled water at 50 °C by means of the microprobe of a Braunsonic 1510 sonifier set at 70 W. Under this condition, dispersion to turbid solution was accomplished within 30 s and optically clear solution appeared 15 min subsequent to the beginning of sonication. Sonication was monitored spectrophotometrically by measuring turbidity at 310 nm. The turbidity decreased exponentially as a function of time.
(4) T. Kunitake, Y. Okahata, K. Tamaki, F. Kumamaru, and M. Takayanagi, *Chem. Lett.*, 387 (1977).
(5) T. Kunitake and Y. Okahata, *J. Am. Chem. Soc.*, **99**, 3860 (1977).
(6) Samples for electron microscopy were prepared by mixing equal volumes of DODAC vesicles with 1.0% uranyl acetate. This mixture was cosonicated in a bath-type sonicator at room temperature for 10 min. A Hitachi HU 11-E electron microscope was used.
(7) It is to be noted that the sizes of single-walled DODAC vesicles, determined in the present work, are larger than those reported by Kunitake for *didodecyldimethylammonium bromide*.[5] Furthermore, Kunitake et al. observed

the formation of a turbid solution containing only lamella upon dispersing DODAC *below 35 °C*.[4] Negatively stained electron micrographs of DODAC vesicles, sonicated at *50 °C* (phase transition temperature of DODAC vesicles were determined to be in the range of 30 to 50 °C),[8] clearly indicated the presence of single-walled vesicles whose diameters are in the range of 1000 to 1500 Å, and which are identical with those obtained by Deguchi and Mino[2] under identical conditions. The size of these vesicles were also confirmed by their appearance in the void volume on gel filtration on Sepharose 2-B.[9] Addition of cholesterol to DODAC decreased substantially the size of DODAC vesicles.[9]

(8) K. Kano, unpublished data, 1977.
(9) P. L. Klahn, A. Romero, C. D. Tran, and J. H. Fendler, unpublished work.
(10) A. D. Bangham in "Progress in Biophysics and Molecular Biology", Vol. 18, J. A. V. Gutler and D. Noble, Ed, Pergamon Press, Oxford, 1968, p 19; D. Papahadjopoulos and K. K. Kimelberg, *Prog. Surface Sci.*, **4**, 141 (1973); A. D. Bangham, M. W. Hill, and N. G. A. Miller in "Methods in Membrane Biology", Vol. 11, E. D. Korn, Ed., Plenum Press, New York, N.Y., 1974, p 38.
(11) M. C. Blok, L. L. M. Van Deenen, and J. de Grier, *Biochim. Biophys. Acta*, **433**, 1 (1976).
(12) J. H. Fendler and A. Romero, *Life Sci.*, **18**, 1453 (1976).
(13) J. H. Fendler in "Liposomes in Biological Systems", G. Gregoriadis and A. C. Allison, Ed, Wiley, New York, N.Y., 1978.
(14) R. A. Klein, M. J. Moore, and M. W. Smith, *Biochim. Biophys. Acta*, **233**, 420 (1971).
(15) A. Romero, J. Sunamoto, and J. H. Fendler, *Colloid Interface Sci.*, **V**, 116 (1976).
(16) A. Weisstuch and A. C. Testa, *J. Phys. Chem.*, **72**, 1982 (1968); S. Babiak and A. C. Testa, *ibid.*, **78**, 201 (1974).
(17) In the presence of cholesterol, rates of OH^- permeabilities become measurable.[9]

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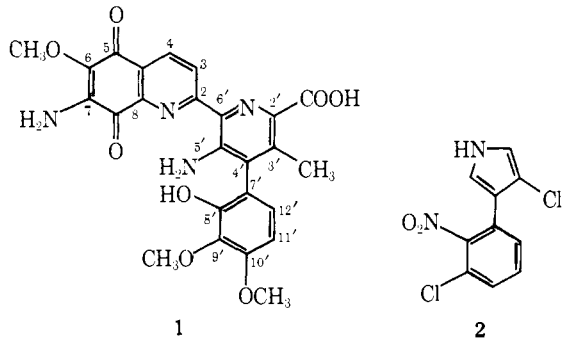
Studies of Nitrogen Metabolism Using ^{13}C NMR Spectroscopy. 1. Streptonigrin Biosynthesis

Sir:

We describe herein the use of ^{15}N–^{13}C couplings in ^{13}C NMR spectroscopy as an efficient tool for the study of nitrogen metabolism. Since there is no long-lived radioactive nitrogen isotope, the study of secondary nitrogen metabolism has lagged far behind those of carbon and hydrogen. While the fate of nitrogen-15 enriched precursors in secondary metabolism has been determined in a few instances by mass spectrometry,[1] the presence of more than one nitrogen in a metabolite leads to ambiguous results unless useful fragmentation patterns can be clearly defined; frequently some chemical degradation is necessary. Bycroft and co-workers have reported[2] the use of ^{15}N NMR in the study of penicillin biosynthesis using a high-producing *Penicillium* strain that afforded a percent incorporation of precursor unusually high even for microbial metabolites.

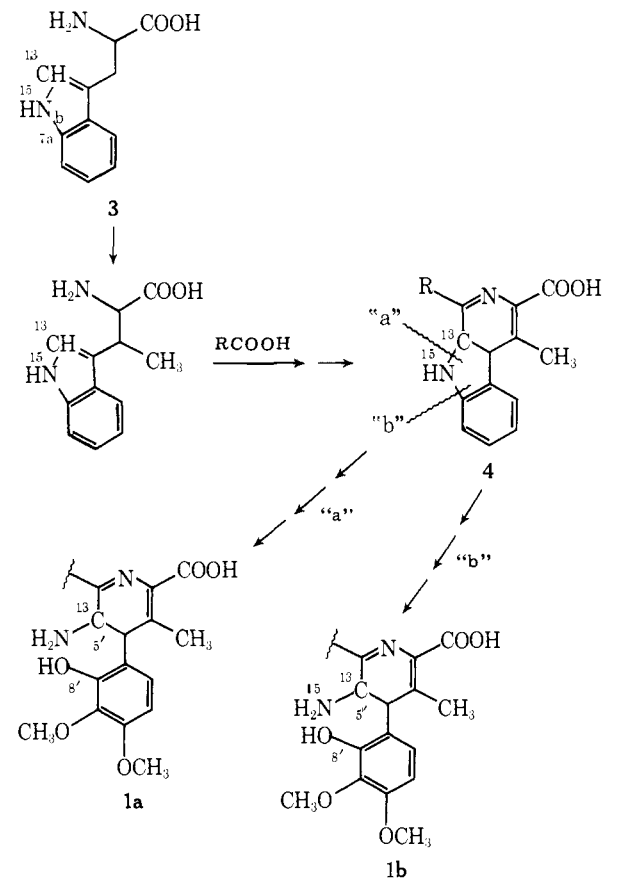
In the last few years homonuclear couplings (^{13}C–^{13}C)[3] and heteronuclear couplings (^{2}H–^{13}C)[4] in ^{13}C NMR spectra have been used to study biosynthetic pathways. They provide relatively direct indications of bond-breaking and bond-forming reactions without recourse to time-consuming and often impractical chemical degradations. The investigation of ^{15}N–^{13}C couplings seemed a logical extension which might simplify the study of nitrogen metabolism. Rinehart and co-workers have recently used this technique to determine the origin of the carbamate residue in geldanamycin.[5]

We recently reported[6] that in the biosynthesis of streptonigrin (**1**),[7] an antibiotic produced by *Streptomyces flocculus* ATCC 13257, the 4-phenylpicolinic acid moiety is derived from tryptophan, suggesting a new pathway for the formation of pyridine rings[8] and a new metabolism of tryptophan.[9] This apparent biosynthesis required cleavage of the intact indole ring at a C–N bond. Only one example of such a cleavage has previously been demonstrated; the biosynthesis



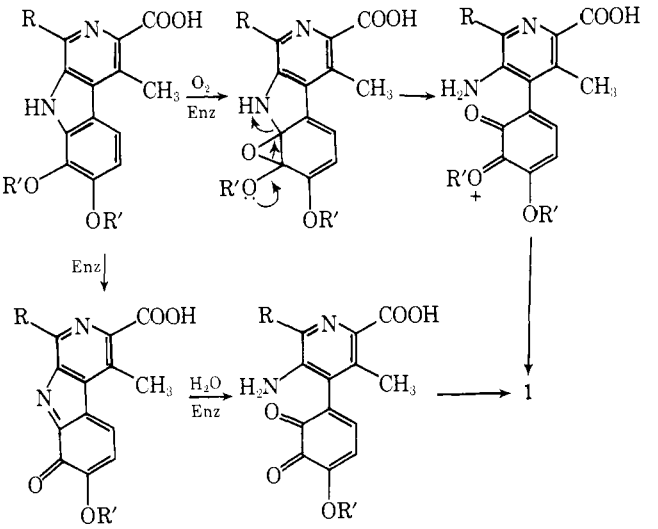
1 2

Scheme I



1a 1b

Scheme II[a]



[a] R' = H or CH_3. The exact timing of the methylations has not yet been determined.

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