of an Erlenmeyer flask. After having dried overnight at 10^{-2} Torr (~1 Pa), we resuspended the film by gentle shaking in 1-2 mL of water. Vesicles were formed instantly and were investigated by freeze fracture electron microscopy (EM), video enhanced phase contrast optical microscopy (PCM), gel chromatography, and ^{1}H NMR.

Figure 1 shows a freeze fracture EM micrograph of these vesicles while two PCM micrographs are shown in Figure 2. Most of the vesicles observed by freeze fracture EM (and fractured at their middle) have diameters between 0.5 and 1 μm. These data are supported by observations of completely unperturbed samples in the video enhanced phase contrast optical microscope where rather homogeneous populations of vesicles with diameters slightly below 1 μm were observed. The resolution of our system was ~0.4 μm. (Unfortunately, in reproducing the image on photographic film and paper much of the resolution was lost.) Occasionally vesicles were observed in clusters and a fracture through one of such clusters is shown in Figure 1. This indicates that in the fracture plane, only few vesicles were fractured through their center.

The contamination of vesicles with larger structures (MLV, giant vesicles, or other PL colloid particles) was ruled out by video enhanced PCM. This is an excellent technique for the observation of giant vesicles and liposomes.[9,10] Only a few larger structures, predominantly giant vesicles, occasionally with entrapped smaller vesicles, were observed in these samples.

To check the possible contamination of LUV's with SUV's we have used gel chromatography and ^{1}H NMR. In the gel chromatography experiment vesicles eluted in a symmetric peak in the void volume of Sephacryl S 1000 column (the recovery of PL was not measured) indicating that their minimal size is at least 30 nm.[11] Also, the absence of the ^{1}H NMR high resolution spectrum which is characteristic for SUV's (where the dipolar interaction and anisotropy of absorption lines due to the chemical shift tensor are averaged out due to fast vesicle tumbling and diffusion of PL molecules on the highly curved vesicle surface) indicates that the sample does not contain SUV's within the sensitivity of these two experiments. In addition, SUV's were not observed in the freeze fracture EM micrographs.

The losses of PL due to adsorption on the wafer were no larger than for the case where glass flasks are used as a support. The inconvenience of introducing detergent into the bilayer can be bypassed by doping EYL with ionic PL(s) instead of CTAB. The possible disadvantage of this vesicle preparation method is the relatively low concentration of vesicles obtainable (~1 mg/mL). However, they can be concentrated in a subsequent separate step. After its use, the wafer-bottomed Erlenmeyer flask was washed with $CHCl_3/CH_3OH$ (3:1), rinsed with distilled water, and dried. Several different preparations yielded, within the accuracy of our experimental methods, the same preparations of LUV's.[12]

This method of vesicle preparation was based on the theoretical model of vesicle formation which defines a bilayered PL flake (BPF) as an intermediate structure in the vesicle formation process.[13] Therefore the results of this study, which are in qualitative agreement with this model, also shed some light on the experimentally unproven model of vesicle formation.

We believe that vesicles are formed by the bending and sealing of BPF's which are thermodynamically unstable due to the exposure of hydrocarbon chains to water at their edges. In our experiment the size of BPF's is determined by the topography of the support surface. On swelling they peel off into solution where they are unstable. Each BPF continuously reduces the circumference of its exposed boundaries, i.e., the unfavorable contact interaction at its edges, by bending and finally eliminates it by closing upon itself.

The advantages of this method are its extreme simplicity, rapidity, and avoidance of all potentially harmful treatments. In addition, the LUV's which are formed are larger than vesicles prepared by most other techniques. This and their quick and harmless preparation make them extremely suitable for the encapsulation of drugs and genetic material.

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The Quinuclidine Dimer Cation Radical

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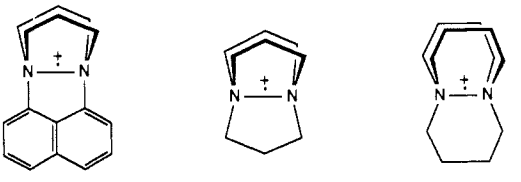
Homonuclear dimer cation radicals containing a three-electron σ bond between two group 15 elements have long been known for phosphines[1] and arsines.[1b,2] Curiously, amines do *not* form such dimer cation radicals. The first apparent exceptions to this generalization are the intramolecular "dimer" cation radicals **1-3**



1 **2** **3**

recently prepared by Alder.[3] Unfortunately, because of the uncertain constraints imposed by their carbocyclic frameworks, these examples shed little understanding on the more common failure of amines to form *inter*molecular dimer cation radicals.[4] We describe herein the preparation and characterization of the first intermolecular amine dimer cation radical and propose a simple rationale to account for its formation.

The general inability of amines to form dimer cation radicals might have two possible origins, termed here, orbital extension and structural reorganization. The first hypothesis recognizes that the nonbonding orbital on nitrogen is considerably contracted vis-a-vis those of the lower elements in group 15. Since the three-electron σ bonds in dimer cation radicals are considerably longer than the corresponding two-electron bonds,[3f,5] it seems plausible that the amine dimer cation radicals would suffer the poorest orbital interpenetration in the series.

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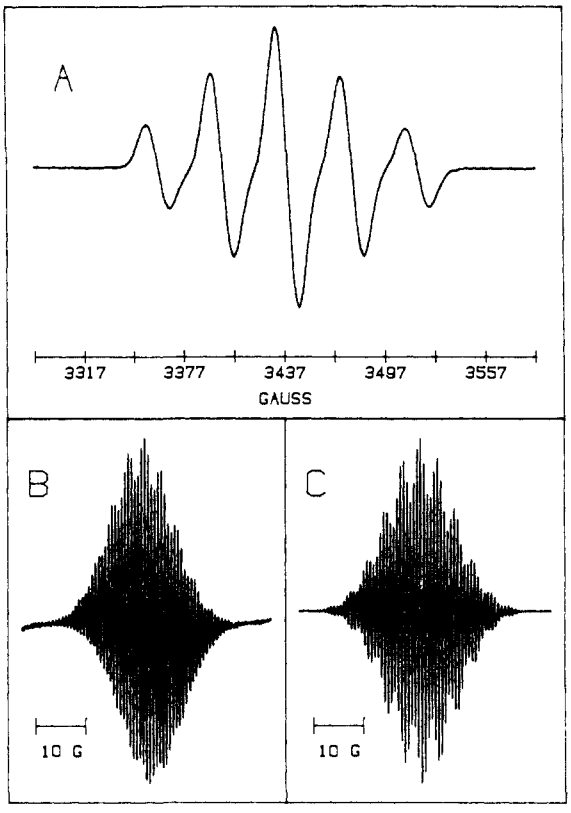
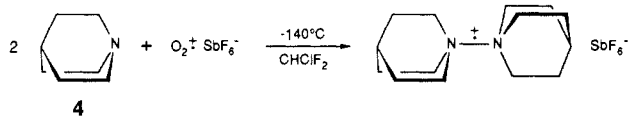


Figure 1. (A) Experimental EPR spectra (9.63748 GHz) under low resolution conditions, $g = 2.0023$; (B) high resolution expansion of the center peak from (A); (C) simulated spectrum using splitting constants and multiplicities in text.

The second explanation is based upon the differences in the structural reorganization energies required for monomer cation radicals to form dimer cation radicals. Evidence from EPR spectroscopy reveals that trialkylamine cation radicals are planar,[6] while the corresponding phosphine[7] and arsine[8] cation radicals are distinctly pyramidal. Thus the structural distortions required for formation of the pyramidal dimer cation radicals should be greatest for the amines.

We reasoned that this latter hypothesis might be tested by preparing an amine cation radical which was constrained to be pyramidal and thus predisposed to form a dimer cation radical. Toward this end we examined the one-electron oxidation of quinuclidine (**4**, 1-azabicyclo[2.2.2]octane). It is worth noting in this context that the quinuclidine monomer cation radical has been previously prepared by photolysis of the amine–chlorine adduct in CF_3SO_3H.[9] Obviously, this method of cation radical generation would have precluded formation of the dimer cation radical. We instead chose to oxidize quinuclidine by using a stoichiometric one-electron oxidant, dioxygenyl hexafluoroantimonate ($O_2^{\bullet+}SbF_6^-$),[10] and in a nonacidic solvent, chlorodifluoromethane.

The reaction of **4** (0.10 mmol) with $O_2^{\bullet+}SbF_6^-$ (0.05 mmol) in $CHClF_2$ (0.6 mL) at −140 °C for 4 h produced a homogeneous crimson solution. The −140 °C EPR spectrum of the solution



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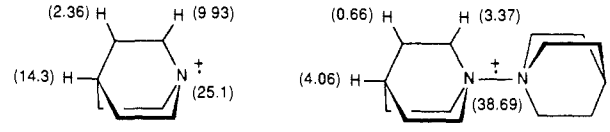
is shown in Figure 1. Above −100 °C this spectrum as well as the color rapidly decayed. The most characteristic feature of the EPR spectrum is the large splitting constant of 38.69 G. Its multiplicity and the peak intensity ratios are consistent with a coupling to two equivalent nitrogen atoms. This strongly recommends the dimer cation radical structure.

Further structural confirmation was obtained by an analysis of the smaller ^1H hyperfine splittings. These were extracted from the experimental spectrum by a cepstral analysis.[11] The resulting cepstrum revealed three unique hyperfine splitting constants: 4.06, 3.37, and 0.66 G. The 3.37 G splitting was assigned to the 12 protons on the α-carbon (to nitrogen) based upon the EPR spectrum obtained from the oxidation of quinuclidine-*2,2,6,6,7,7-d*$_6$. This compound was prepared by two successive deuteriations of quinuclidine (1 M in D_2O) with Raney nickel[12] (0.5 g/mL) at 100 °C for 40 h. ^1H and ^2H NMR analysis showed that it was ≥99.7% D and that ≤1.0% deuterium was incorporated at the β or γ carbon atoms. The two remaining hyperfine assignments were easily made by simulation of the EPR spectrum. Assignment of the 4.06 and 0.66 G splittings to 2 H and 12 H, respectively, gave a simulated spectrum in good agreement with the experimental one (see Figure 1); the alternative assignment was unsatisfactory. Finally, the yield of the cation radical salt (12%) was measured by its paramagnetic susceptibility in $CHClF_2$ by using the Evans NMR shift method.[13]

The combined ^{14}N and ^1H hyperfine multiplicities limit the dimer structure to effective D_{3h} or D_{3d} symmetry. Doubtless the latter is preferred on steric grounds.

Shown below is a comparison of the hyperfine splitting assignments for the monomer and dimer cation radicals. The



structural assignments of the ^1H splittings follow the same trend for both cation radicals although their magnitudes are, as expected,[1f] considerably smaller for the dimer cation radical. The much larger ^{14}N splitting for the dimer is perhaps more surprising considering that the unpaired electron must now be shared between two nitrogens. Its magnitude exceeds even that of **3** ($a(^{14}N)$ = 35.9 G) and suggests that the geometry at nitrogen for the monomer cation radical is considerably flattened relative to the dimer cation radical.

The reorganization energy hypothesis is sufficient to explain the unique ability of quinuclidine to form an intermolecular amine dimer cation radical. It is worth pointing out, however, that a kinetic-based argument might also accommodate our results. The α-carbon atom of the quinuclidine cation radical is Bredt's rule protected from proton or hydrogen atom abstraction, two commonly proposed pathways of amine cation radical decomposition. A decrease in the rate for either of these two processes might permit dimerization to compete more effectively. Thus one might predict that the quinuclidine monomer cation radical would form a dimer cation radical, while an unblocked amine cation radical would react at the α-carbon more rapidly than it forms a dimer cation radical. This argument assumes, of course, that formation of a dimer cation radical would be energetically favorable, even for unconstrained amines.

However, the kinetic argument does not accommodate recent results with the ammonia dimer cation radical, prepared by γ-irradiation of hydrazinium sulfate at 77 K.[14] Importantly, warming the dimer cation radical produces the ammonia monomer cation radical and (presumably) ammonia, in an *irreversible*

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reaction. This latter observation requires a negative bond dissociation energy for the dimer cation radical[15] and, as such, invalidates the basic assumption of the kinetic argument. On the other hand, one need only make the reasonable assumption that the reorganization energy of the ammonia dimer cation radical is larger than that of quinuclidine, for the reorganization energy hypothesis to unify the ammonia and the quinuclidine dimer cation radical results in a single principle.

In summary, the previously anomalous behavior of amines toward group 15 dimer cation radical formation can now be explained by their uniquely planar monomer cation radical structures and the distortion energy required to form a nonplanar dimer cation radical.

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A Study of the [1,7]-Sigmatropic Shift of a 1-Hydroxylated 3-Desoxy Previtamin D to Vitamin D: Observation of a Modest Primary Deuterium Kinetic Isotope Effect[1]

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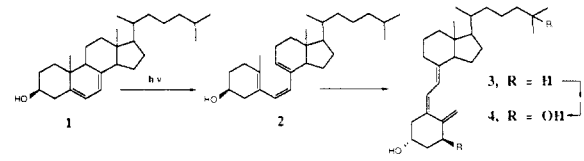
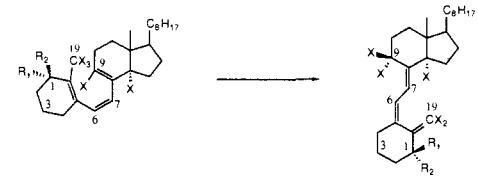
The primary metabolic pathway (Scheme I) leading to the active form of vitamin D, namely 1α,25-dihydroxyvitamin D_3 (**4**),[2] formally incorporates two classical pericyclic processes. These include the photochemical electrocyclic ring opening of 7-dehydrocholesterol (**1**) to previtamin D_3 (**2**) and then the thermal transformation (a [1,7]-sigmatropic hydrogen shift) of previtamin D_3 to vitamin D_3 (**3**). The latter transformation in solution has been well studied.[3] In 1965, Akthar and Gibbons firmly established the pathway of the thermal transformation through studies using C-19 tritium-labeled materials.[4] In 1979, Mazur and co-workers synthesized 19,19-dideuteriovitamin D_3 and reported that the transformation of previtamin D_3 to vitamin D_3 occurs with an exceptionally large primary deuterium kinetic isotope effect (k_H/k_D) of ~45 at 80 °C.[5] Our interest in studies of

Scheme I



Scheme II



5a, R_1=OH; R_2=X=H
b, R_1=OH; R_2=H; X=D

6a, R_1=OH; R_2=X=H
b, R_1=OH; R_2=H; X=D

7a, R_1=H; R_2=OH; X=H
b, R_1=H; R_2=OH; X=D

8a, R_1=H; R_2=OH; X=D
b, R_1=H; R_2=OH; X=D

Table I. Summary of Kinetic Data for **5** and **7**

substrate	T,[a] °C	$k^b \times 10^4$, s^{-1}	k_H/k_D[c]
5a (1*S*-d_0)	80.35	7.67 (±0.34)	6.06 ± 0.02
5b (1*S*-d_5)	80.35	1.25 (±0.04)	
7a (1*R*-d_0)	80.35	6.20 (±0.15)	6.13 ± 0.21
7b (1*R*-d_5)	80.35	1.02 (±0.06)	

[a] ±0.05 °C. [b] The errors are maximum errors (absolute deviations from the mean). [c] For the previtamin D to vitamin D transformation at 80.35 °C.

19,19,19-trideuteriated derivatives of previtamin D_3 and their various 1-hydroxylated counterparts stems from the possibility of utilizing previtamins as biochemical or chemical research tools. It was anticipated that a heavy isotope at C-19 would attenuate the rate of the [1,7]-sigmatropic shift so as to facilitate handling of the thermally unstable previtamins. Thus, in the case of 1α,25-dihydroxyvitamin D_3 (**4**), its previtamin form might be anticipated to exist in nature, and its more stable 19,19,19-trideuterio counterpart would facilitate evaluation of its biological profile. Moreover, the latter might have practical application as a "slow release" source of the highly potent, and potentially toxic, natural hormone **4**. In this communication we describe our initial studies in this area through kinetic investigations of the isomerization of 3-deoxy-1-hydroxyprevitamin D_3 epimers **5** and **7** (Scheme II),[6] a [1,7]-sigmatropic shift model for the previtamin form of the natural hormone.

The synthesis of the previtamins used in this study is outlined in Scheme III. Grundmann's ketone **9a** (or **9b**; available by three cycles of base-catalyzed exchange of the acidic protons of **9a** with $NaOCH_3$, CH_3OD) was reacted with the monoanion of bis(trimethylsilyl)acetylene and subsequently benzoylated to give **10a** (or **10b**). Flash vacuum pyrolysis (FVP) of the benzoate **10a** (or **10b**) yielded the CD-ring fragment **11a** (or **11b**), which was coupled to A-ring fragment **12a** (or **12b**).[7] Subsequent Lindlar hydrogenation of the resulting **13a** (or **13b**) gave the previtamin ketone **14a** (or **14b**), which was reduced to the previtamins **5a** and **7a** (or **5b** and **7b**). The epimeric previtamins were separated and then stored at −80 °C.

Overall deuterium incorporation was measured at each stage by mass spectroscopy and was found to be >98% complete. Site specific deuterium incorporation was checked by ^1H NMR and proved to be complete within the limits of detection. The kinetic studies were performed in a manner previously described.[8] Stock

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