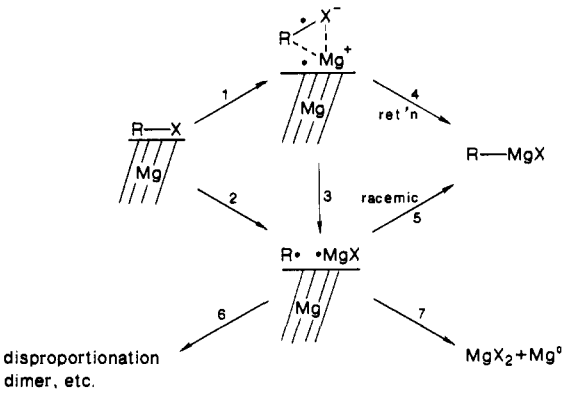
Scheme I. Proposed Mechanism for Grignard Formation

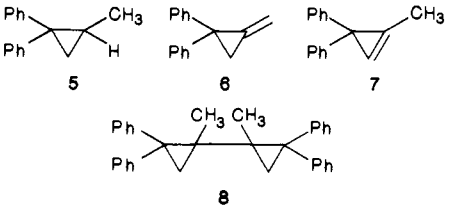


portion of their configuration. It should also be noted that Grignard formations in aprotic solvents give a similar result in that the retention of configuration of **2** (71%)[10] > **1** (59%)[7] > 2-bromooctane (50%; racemization).[16] This is consistent with the observation of Buske and Ford[17] who report that *syn*- and *anti*-7-bromobenzonorbornadiene (the 7-benzonorbornadienyl radical is a σ-radical[18]) yield Grignard reagents with high retention of configuration.

As one might expect the more reactive σ radicals are adsorbed more strongly than π radicals on the magnesium surface. If there is no surface available and the radicals are produced in solution, then the result is complete loss of configuration. Thus, the tri-*n*-butyltin hydride radical chain reductions of **1**, **3**, and **4** gave racemic products, and both *syn*- and *anti*-7-bromobenzonorbornadiene gave an identical mixture of deuterated product when reduced with tri-*n*-butyl deuteride. Since our early work[7] others[17,19-21] have also adopted the radical surface mechanism for Grignard formation which is depicted in Scheme I.

Electron transfer from the magnesium surface into the σ^* orbital of the carbon–halogen bond leads to a tightly held anion radical magnesium cation radical pair at the surface where magnesium insertion can occur to produce the Grignard reagent with retention of optical activity and configuration (pathways 1 and 4). If before magnesium insertion, the anion radical magnesium cation radical dissociates at the surface to the radical magnesious halide radical pair, this would, upon recombination, lead, mainly but not exclusively,[22] to the formation of racemic Grignard reagent (pathways 3 and 5). This same radical pair could be formed directly by electron transfer from the surface to the carbon–halogen bond, and the proportion of anion radical magnesium cation formation (pathway 1, retention) vs radical pair formation (pathway 2, largely racemization) would, as observed, be halogen dependent.[4,7,17] Support for the surface nature of the reaction can also be found in the kinetic analysis of the reaction by Molle, Bauer, and Dubois[21] as well as in their XPS studies.[21] The work of Vogler[23] and Whitesides[20] is also consistent with intermediate species such as a radical magnesious halide radical pair (pathway 2) or an anion radical magnesium cation radical adsorbed on the surface of magnesium (pathway 1).

The highly reactive σ radicals will remain largely at the surface, and there they can dimerize or disproportionate (pathway 6) so that in the reaction of **1** in ether[4,7] one observes the formation of **5**, **6**, **7**, and **8**. Of significance is the formation of **8** which is only



observed in surface[4,7] or surface-like reactions[24] but never when the precursor radical is produced in solution.[25] Compounds **5**, **6**, and **7** are the result of disproportionation of the radical intermediate on the surface.[7] However, some of the radicals leave the surface and react with solvent to form **5**. Using ether-d_{10} as the solvent it was shown that **5** had 6% deuterium incorporated, and in THF-d_8 **5** contained 28% deuterium.[7] Ashby[26] has recently shown that in the reaction of 6-bromo-1-hexene with magnesium, at most, only 25% of the radicals leave the surface.[27] This demonstrates that even a stable π radical remains largely at the surface.

Thus, the evidence that the magnesium surface plays a major role in the Grignard formation reactions is conclusive and is not compatible with intermediate radicals that "diffuse freely in solution at all times".

Acknowledgment. Dr. J. E. Ollman provided some early experiments and also helped design the CO_2 experiments.

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(22) Racemization of the radical occurs when it can rotate 180° before combining with magnesious halide. If the radical combines before rotation this would lead to some retention. Cage reactions have been shown to lead to some retention of configuration, see: Greene, F. D.; Berwick, M. A.; Stowell, J. C. *J. Am. Chem. Soc.* **1970**, *92*, 867. Walborsky, H. M.; Chen, J.-C. *J. Am. Chem. Soc.* **1971**, *93*, 671.
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(27) This is a minimum value since it assumes 100% efficiency for the radical trapping reagent, DCPH.

Temporal Resolution of the Methylation Sequence of Vitamin B_{12} Biosynthesis

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Previous work from Texas, Stuttgart,[1,2] and Cambridge[3-5] has shown that in the overall conversion of uro'gen III to cobyrinic

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(2) Review: Scott, A. I. *J. Heterocyclic Chemistry* **1987**, *24*, Suppl. 9, S-75.
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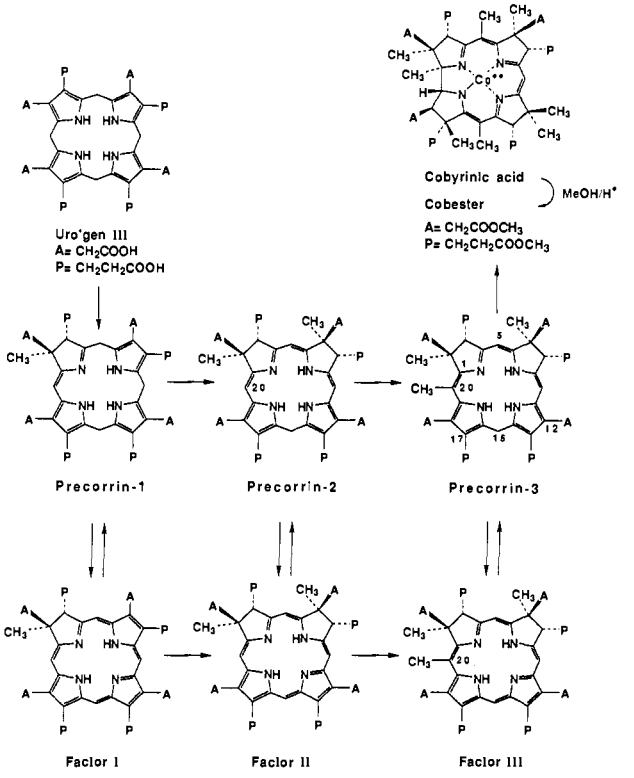
Scheme I



acid (Scheme I) a total of eight methylations are performed upon the uro'gen III template. Isolation experiments[6] have defined the sequence (see Scheme I for numbering system) where uro'gen III is converted to factors I–III (corresponding to methylation at C_2, C_7, and C_{20}, respectively) which are processed biochemically to cobyrinic acid in their *reduced forms* (precorrins **1**–**3**[5]). The identities of the "missing" intermediates between precorrin **3** and cobyrinic acid (Scheme I) are still conjectural, but our earlier work[1,2] showed that the *S*-adenosylmethionine(SAM)-derived methyl groups are inserted at $C_{17} > C_{12\alpha} > C_1$ followed by C_5 and C_{15}, although differentiation at the last two centers proved difficult. Thus, while a series of statistically analyzed pulse experiments using cell-free extracts from *Clostridium tetanomorphum* indicated that the last two methylations take place in the order $C_{15} > C_5$,[5] in this communication we show that in *Propionibacterium shermanii* C-methylation occurs at C_5 before C_{15}.

A sample of cobyrinic acid labeled equally in all seven methyl groups was isolated from a cell-free homogenate from *P. shermanii* cells (which had previously been fed with [$^{13}CH_3$]-SAM) followed by esterification to give cobester (Scheme I) whose NMR spectrum revealed (Figure 1a) that the intensity of the ^{13}C signal for the methyl group at C_5[7] is 5% lower than that for the C_{15} methyl group (due to small T_1 differences for these centers) in contrast to the edited ^1H spectrum (Figure 1e) in which protons of the C_5 and C_{15} methyls produce equal signal intensities. By studying over 50 temporal variants of the "two-phase" sequence[1] in which precorrin **2** is accumulated in whole cells containing an excess of

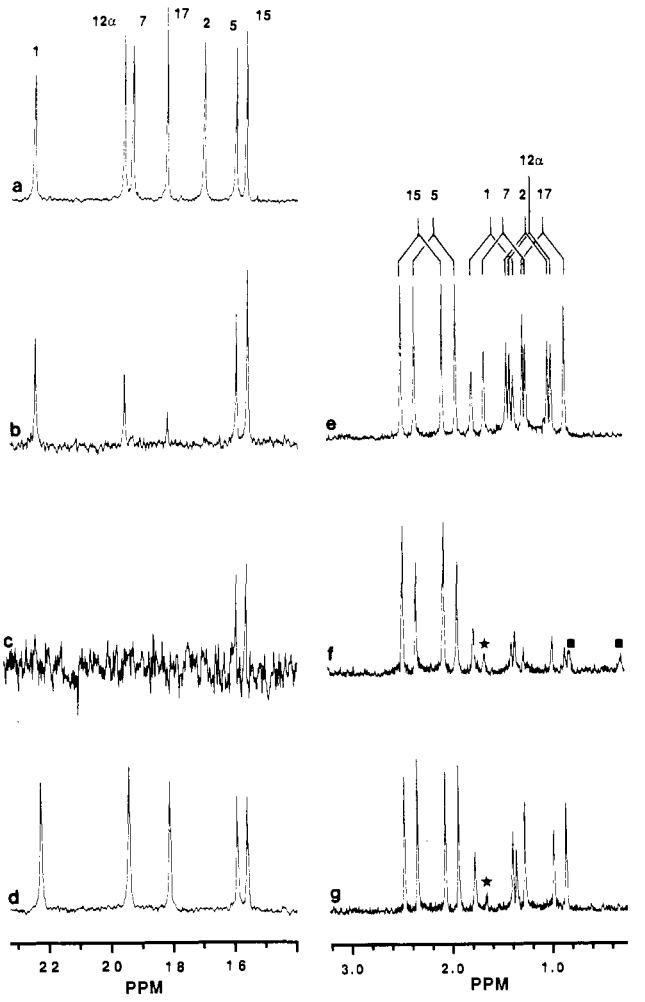


Figure 1. 125 MHz ^{13}C (a–d) and 300 MHz ^1H (e–g) NMR spectra of cobester samples (35–150 μg) dissolved in 0.25 m*L* of KCN-saturated benzene-d_6. ^{13}C NMR spectra were measured with WALTZ-16 proton decoupling with a total recycle time of 1.6 s. Heteronuclear-filtered ^1H spectra were recorded with a total recycle time of 3.1 s and without ^{13}C decoupling, the latter resulting in proton signals appearing as doublets split by $^1J_{CH}$: (a) control experiment, 29 K scans: (b) "normal-pulse" experiment (initial pulse of [^{12}C]-SAM, followed by [^{13}C]-SAM), 65 K scans; (c) [^{13}C]-SAM pulse added during the last hour incubation, 195 K scans; (d) "reverse-pulse" experiment, 40 K scans; (e) control experiment, 1 K scans; (f) "normal pulse", 18 K scans; (g) "reverse pulse", 3 K scans. The (☆) indicates residual water and (■) indicates folded satellites from the solvent. For details of pulse experiments, see text.

SAM in the absence of Co^{2+}, the following protocol gave consistent results. The cells were sonically disrupted, and Co^{2+} was added immediately, followed by a pulse of [$^{13}CH_3$]-SAM (90 atom%) after 4 h. After a further 1.5 h corrin was isolated as cobester. The ^{13}C NMR spectrum of this specimen obtained by the "normal pulse" method[1-5] (Figure 1b) defines the complete methylation sequence, beginning from precorrin **2**, as $C_{20} > C_{17} > C_{12\alpha} > C_1 > C_5 > C_{15}$, with a differentiation of 25% (±5%) in the relative signal intensities for the C_5 and C_{15} methyl groups. Methylation at C_{20} of precorrin **2** to give precorrin **3** is not recorded in the spectrum of cobester[8] since C_{20} is lost on the way to cobyrinic acid, together with the attached methyl group, in the form of acetic acid.[2,6]

The experiment was finely tuned by omitting [$^{13}CH_3$]-SAM until the last 45 min of the incubation whereupon the only observable signals are those of the methyl groups at C_5 and C_{15} (Figure 1c); i.e., sufficient concentrations of intermediates bearing

(5) Uzar, H. C.; Battersby, A. R.; Carpenter, T. A.; Leeper, F. J. *J. Chem. Soc., Perkin trans. 1* **1987**, 1689. The statistical method described by these authors was applied to the "normal" and "reverse" pulse spectra shown in Figure 1 (parts **b** and **d**, respectively). Taking the signal heights for CH_3 at C_1 as 100, subtraction of part b from part d of Figure 1 gives the following difference values (Δ) for the CH_3 groups. The corresponding Δ values for the Cambridge experiments[4,5] are given in parentheses: C_{17} + 71 (+83); $C_{12\alpha}$ + 49 (+13); C_1 0 (0); C_5 – 31 (–14); C_{15}– 68 (–5). The sequence of methylation is given by the descending order of Δ (positive to negative) as $C_{17} > C_{12\alpha} > C_1 > C_5 > C_{15}$. It can be seen that the data sets differ only in the order of C_5 and C_{15} methylation.

(6) For a review, see: Leeper, F. J. *Nat. Prod. Rep.* **1987**, *4*, 441.

(7) Battersby, A. R.; Edington, C.; Fookes, C. J. R.; Hook, J. M. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2265.

(8) In a separate experiment, factor III was prepared from [$^{13}CH_3$]-SAM and precorrin **2** in the presence of the cell-free system and contained one ^{13}C-enriched methyl group at C_{20} (δ 19.2 ppm).

Scheme II

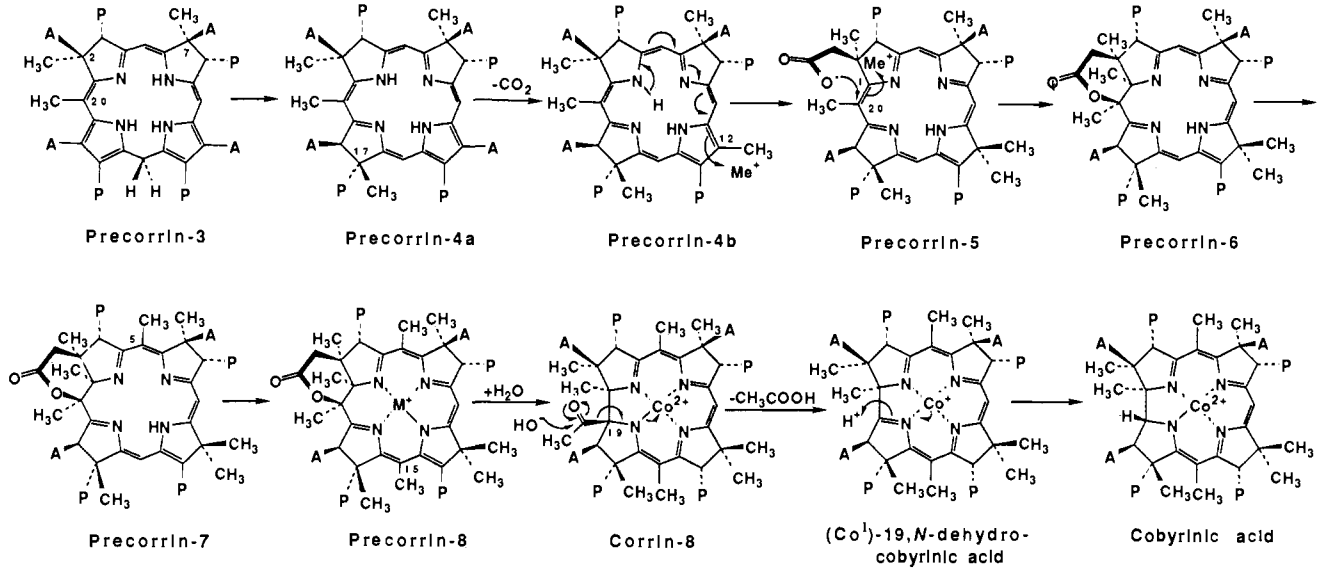


Table I. Relative Yields of Conversion of Precorrins **2** and **3** and of Ring C-Decarboxylated Precorrins **2** and **3** to Cobyrinic Acid

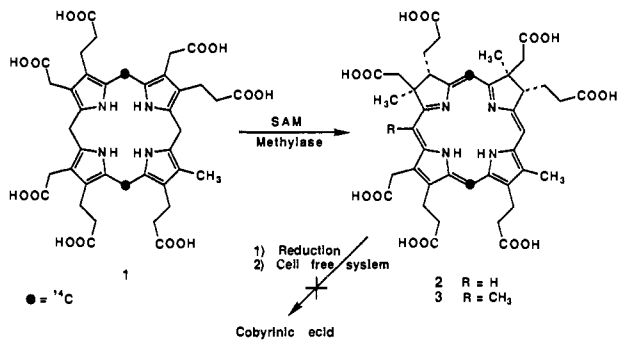
				yield of cobyrinic acid (as cobester)		
expt	substrates	nmol	dpm/nmol[a]	nmol[b]	dpm/nmol	nmol[c]
1	precorrin **2**[d]	400	4880	86	3020	53
2	decarboxy precorrin **2**[e] (**2**)	400	15268	39	<10	0
3	decarboxy precorrin **3**[e] (**3**)	300	15268	35	<10	0
4	precorrin **3**[f]	460	8650[g]	85	1036[g]	10
	(+)-**2**	516	15268	85	10[a]	~0

[a] dpm's are for ^{14}C except for experiment 4 which contains precorrin 3 (^{3}H) and **2** labeled with ^{14}C as in Experiment 2. [b] Endogenous cobyrinic acid is formed in all incubations. [c] Based on radiochemical yield of conversion of labeled substrate to product. [d] Derived from [4-^{14}C]-ALA. [e] Derived from [5,15-$^{14}C_2$]-12-C-decarboxyuro'gen III (**1**).[14] [f] Derived from [2,3-$^{3}H_4$]-ALA and reduced to precorrin **3** level before incubation. [g] ^{3}H.

up to six methyl groups have accumulated at natural abundance to observe only the last two insertions of $^{13}CH_3$. A "reverse pulse"[1-5] experiment ([$^{13}CH_3$]-SAM added at the outset, [$^{12}CH_3$]-SAM after 3 h) inverts the relative intensities of the C_5 and C_{15} methyls (Figure 1d) which are now virtually equal, although this technique involves kinetics which preclude perfect inversion of the "normal pulse" profile[5] (as in Figure 1b). Independent confirmation of these results can be clearly seen in the edited ^{1}H spectra of cobester obtained both by the "normal pulse" technique (Figure 1f) and the "reverse pulse" method (Figure 1g) which reveal the identical order ($C_5 > C_{15}$).

It is now apparent that several discrete methyl transferases are involved in the biosynthesis of cobyrinic acid from uro'gen III, since *enzyme-free* intermediates must accumulate in order to dilute the ^{13}C label. A rationale for these events is given in Scheme II, which takes the following facts into account: (a) the methionine-derived methyl group at C_{20} of precorrin **3**[8] does not migrate to C_1 and is expelled together with C_{20} from a late intermediate (as yet unknown) in the form of acetic acid;[2,6] (b) neither 5,15-norcorrinoids[9] *nor* descobalto cobyrinic acid[10] are biochemical precursors of cobyrinic acid;[11] (c) regiospecific loss of ^{18}O from [1-^{13}C,1-$^{18}O_2$]-5-aminolevulinic acid-derived cyanocobalamin from the ring A acetate[12] occurs, in accord with the concept of lactone formation,[13] as portrayed in Scheme II, where the structures precorrins **5** and **6**[11] summarize *either* direct methylation at C_1 or methylation at C_{20} followed by $C_{20} \rightarrow C_1$ migration and lactonization. If the latter mechanism is operative, the $C_{20} \rightarrow C_1$ migration must be stereospecific, since precorrin **3** labeled at C_{20} with $^{13}CH_3$[8] is transformed to cobyrinic acid with complete loss of label. The temporal resolution of the methylation steps with respect to C_5/C_{15} is in contrast to recent reports that C_{15} is methylated before C_5,[4,5] in extracts of a different B_{12} producing organism, *C. tetanomorphum*, with a differentiation (based on statistical analysis) of 9% (±1%).

Scheme III



It has also been possible to define the point in the biosynthetic sequence where ring C-decarboxylation occurs by using synthetic [5,15-$^{14}C_2$]-12-decarboxylated uro'gen III[14] (**1**) as a substrate for

(9) Nussbaumer, C. Dissertation No. 7623, E.T.H. Zurich, 1984. The norcorrinoids are demethylated at positions 5 and 15 in cobyrinic acid.

(10) Podschun, T. E.; Müller, G. *Angew. Chem.* **1985**, *97*, 63.

(11) Scheme II employs the recently suggested[5] nomenclature for B_{12} precursors. Whilst the "classical" names factor I–III for the oxidized (isolated) forms of the intermediates are retained, the species undergoing biotransformation are designated as precorrins, followed by a digit indicating the number of methyl groups derived from SAM. The hypothetical intermediates in Scheme II have been named, although none has yet been isolated.

(12) Kajiwara, M., personal communication.

(13) Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 5.

(14) Scott, A. I.; Georgopapadakou, N.; Ho, K. S.; Klioze, S.; Lee, E.; Lee, S. L.; Temme, G. H.; Townsend, C. A.; Armitage, I. M. *J. Am. Chem. Soc.* **1975**, *97*, 2548. Compounds **2** and **3** were prepared by incubation of [5,15-$^{14}C_2$]-**1** (15300 dpm/nmol) and SAM with cell-free extracts of *P. shermanii* in yields of 5% and 1.2%, respectively, and exhibited the appropriate spectroscopic properties (Müller, G.; Savvidis, E., to be published) virtually identical with those of factors II and III except for ring C($CH_2CO_2H \rightarrow CH_3$).

the nonspecific methylases of *P. shermanii* to prepare the 12-methyl analogs **2** and **3**, of factors II and III, respectively (Scheme III). Reduction and incubation of these possible intermediates with the corrin-synthesizing cell-free system in the presence of SAM afforded (after esterification) samples of cobester whose specific activities are compared in a control experiment with those of cobester derived from [^{14}C]-precorrin **2** and from a mixture of [^{3}H]-precorrin **3** and [^{14}C]-**2**. As shown in Table I, these ring C-decarboxylated analogues are not substrates for the enzymes of corrin biosynthesis (experiments 2–4), leading to the conclusions that (a) in normal biosynthesis, precorrin **3** is *not* the intermediate which is decarboxylated and (b) decarboxylation occurs at some stage after the fourth methylation (at C_{17}) and by mechanistic analogy, before the fifth methylation at C_{12}. Hence, two isolable *pyrrocorphin* intermediates viz. precorrins **4a**, **4b**[11] should intervene between precorrins **3** and **5**; i.e., precorrin **3** is C-methylated at C_{17} to give precorrin **4a** followed by decarboxylation (→**4b**) and subsequent C-methylations at C_{12a}, C_1, C_5, C_{15}, in that order. Although this sequence differs in the penultimate stages from that reported elsewhere,[4,5] consensus over the timing of decarboxylation in ring C has been reached since identical conclusions have recently been reported by Blanche et al.[15] using *Pseudomonas denitrificans*. The foregoing evidence provides the necessary impetus for the successful isolation of several of the remaining intermediates of the B_{12} pathway proposed in Scheme II.

Acknowledgment. We thank the N.I.H (DK32034), The Robert A. Welch Foundation, D.F.G. and N.A.T.O. for generous support of this work and M. Höhn, Finnegan MAT, Bremen for FAB-MS spectra of compounds **2** and **3**.

(15) Blanche, F.; Handa, S.; Thibaut, D.; Gibson, C. L.; Leeper, F. J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1988**, 1117.

Excited-State Proton-Transfer Polymerization of Amorphous Formaldehyde

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Received October 17, 1988

Fundamental investigations of photochemical reactions in the solid state are of current interest because they represent a method of inducing localized changes in the chemical composition of solids with use of a high degree of spatial control (e.g., using lasers). A detailed understanding of these processes is important for developing new materials with useful optical and chemical properties (e.g., holographic gratings, photoresists, and high density optical memories).

Crystalline formaldehyde is known to polymerize in the solid state when exposed to ionizing radiation.[1-4] Recently, we reported that polymerization also occurs in amorphous solid films in the presence of a strong acid such as HCl.[5] The films were formed by vapor deposition of chlorine and formaldehyde onto a CsI optical window mounted at the cold tip of a cryogenic refrigerator (10–77 K). Chlorine atoms formed by photodissociation of Cl_2 reacted with CH_2O to form HCl and CHClO. Proton transfer from the HCl to another formaldehyde molecule then initiated oligomerization of nearby formaldehyde molecules to polyoxymethylene (POM). Quantum yield measurements made by infrared absorption spectroscopy showed that the average length of the oligomers was 6.4 ± 1.3 monomer units. This is much lower than the chain lengths of 10^3–10^6 reported for the crystalline solid,[1] and is one consequence of molecular disorder in the amorphous state.

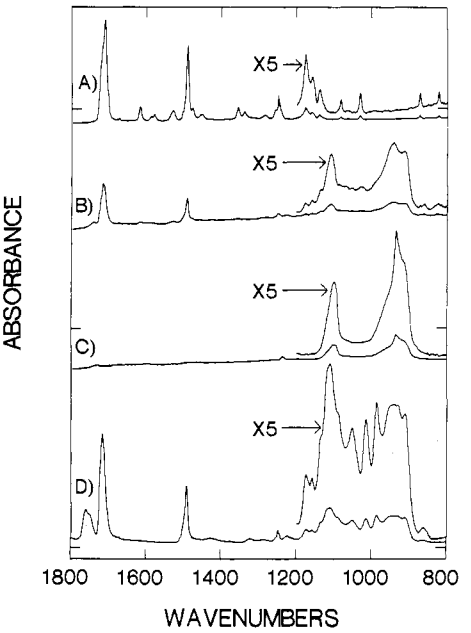

Figure 1. Infrared spectra of solid films of doped formaldehyde deposited on a 77 K CsI window under various conditions. Each tick mark on the vertical axis represents one absorbance unit (base 10): (A) 20:1 mixture of formaldehyde:2-nitrophenol before photolysis, (B) same sample after photolyzing with 4000 pulses at 308 nm (1.06 mJ cm^{-2} pulse^{-1}), (C) same sample after warming to room temperature under vacuum, (D) 11:1 mixture of formaldehyde:chlorine after photolyzing with 15 000 pulses at 308 nm (1.0 mJ cm^{-1} pulse^{-1}).

In this communication, we report that chain polymerization of formaldehyde can also be initiated by excited-state proton transfer from 2-nitrophenol. This molecule has a pK_a of 9.1 in the ground state, but upon absorption of a photon the molecule is estimated to have a pK_a of about −0.6 in the S_1 state[6] excited at 308 nm. We believe this to be the first example of a solid-state chain reaction which is initiated by proton transfer from an electronically excited dopant molecule. The technique is extremely powerful because it provides a way of temporarily generating a strong acid within a solid for the purpose of inducing transient or irreversible chemical reactions. Photochemical generation of acids has previously been shown by other workers to be useful for photolithography by inducing depolymerization[7] and/or dissolution[8-10] of polymeric photoresists. Photoacids are also known to induce cationic polymerization of several different monomers in liquid solutions.[11] All of the previous work has involved chemical rearrangements which form the acid in its ground state.

Transmission infrared spectra of a 20:1 mixture of CH_2O:2-nitrophenol before and after laser photolysis are shown in Figure 1. Photolysis reduces the intensities of IR bands attributable to formaldehyde[12,13] and 2-nitrophenol.[14] The POM product appears

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