Three-Phase Test.[1a] Detection of Free Cyclobutadiene

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Abstract: A new method for the detection of reaction intermediates is described. The technique involves the generation of an intermediate from an insoluble, polymer-bound precursor and its trapping by a second solid phase suspended in the same reaction mixture. The preparations of a polymer-bound *o*-phenanthroline derivative of cyclobutadieneiron carbonyl and a polymer-bound maleimide derivative are described. Oxidation of the former in the presence of the latter is shown to proceed with 96% transfer of free cyclobutadiene between the two polymers.

Chemical reactions on solid supports have provided chemistry with several important disciplines. Heterogeneous catalysis, ion exchange and adsorption chromatography are general examples of reactions and interactions at solid surfaces which are frequently encountered in even the most introductory chemistry laboratories. More specialized applications of insoluble, polymeric supports for organic, inorganic, and biochemical reactions have emerged since the introduction of solid-phase peptide synthesis by Merrifield in 1963.[2] These applications have emphasized either the ease of phase separations or the potential benefits of matrix isolation available for reagents bound to solid phases.[3]

Our attention has focused on a different type of insulation afforded by solid phases; viz., functional groups bound to one polymer bead cannot interact with functional groups bound to another bead. This aspect is based on Merrifield's[4] demonstration that polystyrene beads can be functionalized throughout their interiors. Since only a negligible fraction of the sites are at the surface of the bead, *any observed reactions between two solid phases require the existence of free intermediates.*

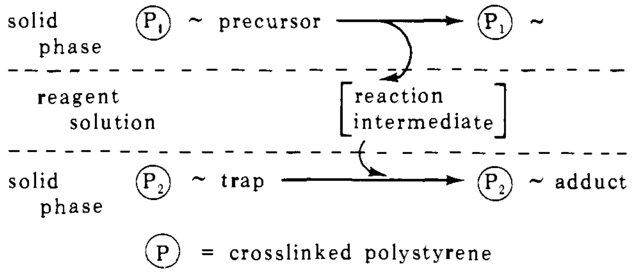


Figure 1.

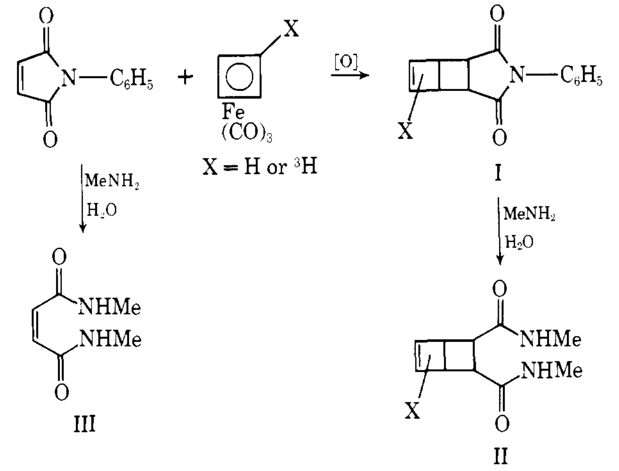
Figure 1 depicts the operation of the three-phase test for the detection of reaction intermediates: a reagent solution liberates the intermediate from a polymeric precursor, and a second solid phase, suspended in the same solution, is used to trap the intermediate.

The applicability of the three-phase test was expected to be limited by the lifetime of the intermediate under study. Intermediates of the reactivity of free methylene are not likely to be suitable substrates for this method, while intermediates with lifetimes on the order of minutes may be studied more conveniently by direct observation. Free cyclobutadiene has been observed only at 8 K,[5] but evidence has recently been presented that oxidation of its iron carbonyl derivatives gives free cyclobutadiene in solution near room temperature.[6] We felt that successful trapping of this reactive species with the three-phase test would establish the viability of the technique and serve to demonstrate the new method's complementarity to kinetic methods and direct observation.

Reference Compounds

The reference diamide IIa (Scheme I) was prepared from the known cyclobutadiene-maleanil[7] adduct by methaminolysis in 40% aqueous methylamine. The diamide III was similarly prepared. Mixtures of II and III were readily separable by preparative TLC on silica using EtOH-Et_2O as eluent.

Scheme I



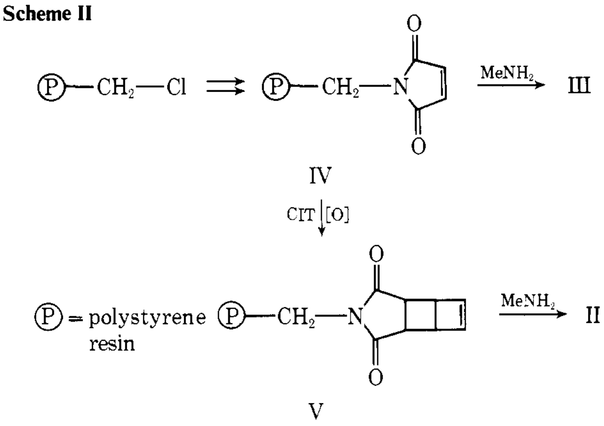
Tritiation of cyclobutadieneiron tricarbonyl (CIT) was accomplished by dissolving CIT in tritiated trifluoroacetic acid. Since the tritiated CIT was difficult to purify, the activity of the cyclobutadiene moiety was assessed by conversion of a sample to the crystalline I, specific activity, 3.60 μCi/mmol.

Reactions on the Solid Phase

The ease with which polymer-bound reagents may be separated from reagents in solution has been frequently discussed.[3] It should be emphasized, however, that one of the advantages of solid-phase synthesis (that intermediates need not be isolated) is frequently offset by the disadvantage that intermediates on the solid phase cannot be isolated or purified. As in solid-phase peptide synthesis, best results are obtained when reactions known to proceed cleanly in solution are employed, and excess reagents in solution are used to ensure that reactions of the polymer-bound component are driven to completion. Infrared spectroscopy provides the most convenient method for monitoring reactions on the solid phase. When resin loadings are in the 0.1 to 1 mequiv/g range, about six beads, made up into a KBr pellet in a minipress, suffice to give beautifully distinct ir spectra. While absorptions in the fingerprint regions must be assigned with caution, the carbonyl and hydroxyl vibration regions are easily interpreted. More quantitative determinations are provided by combustion analysis and, where appropriate, titration of the resin or the solutions resulting from the solid-phase reactions. Changes in resin weight can frequently give a good measure of the extent of reaction.

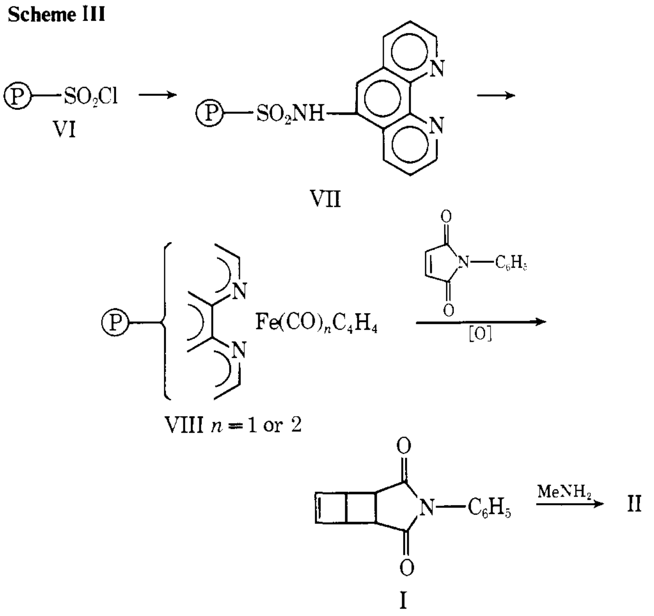
Gabriel synthesis[8] was used to convert chloromethylated 2% cross-linked polystyrene (Merrifield's resin) to the benzylamine. The amine was acylated with maleic anhydride in benzene then cyclized to the imide IV (Scheme II) with NaOAc in hot acetic anhydride. A suspension of IV in ethanol containing excess CIT was oxidized with ceric ion to give V, which on stirring with 40% methylamine yielded II, identical with the reference compound. Pyridine *N*-oxide also proved to be an effective oxidant.[9]

Scheme II



Macroreticular, highly cross-linked polystyrene beads were chlorosulfonated with hot $ClSO_3H$-CCl_4[10] then boiled in a dioxane solution of 5-amino-*o*-phenanthroline to give the polymer-bound ligand, VII (Scheme III). Combustion analysis indicated not only the 0.32 mequiv of ligand/g, but also iron corresponding to 0.007 mequiv/g. Although the origin of the iron could not be traced with certainty, it may have arisen from solvents or during the preparation of the amino phenanthroline.[11]

Scheme III



The ligand resin failed to incorporate iron when heated with a solution of excess CIT in ethanol. Therefore, a suspension of the ligand resin in ethanol containing an excess of CIT was irradiated at 254 nm at room temperature, and the resulting resin was washed free of CIT with ethanol (the final washes were free of iron detectable by atomic absorption). Combustion analysis indicated 0.29 mequiv of phenanthroline/g and 0.28 mequiv of iron/g. An identical preparation using tritium-labeled CIT was performed, and cyclobutadiene moieties were determined by counting tritium released when a sample was oxidized with pyridine *N*-oxide. This indicated 0.25 mequiv of cyclobutadiene/g. Thus, >96% of the phenanthroline groups bear iron, and 90% of the iron bears cyclobutadiene in the complex VIII.

The complex VIII exhibited two carbonyl absorptions in the ir (2050 and 1960 cm^{-1}). For VIII ($n = 1$), only one would be expected so it is possible that the bulk of the iron in VIII bears two carbonyls and only one of the phenanthroline nitrogens. It is also possible that those irons not bearing cyclobutadiene ligands still bear carbonyls. This latter possibility seems less likely than the former since Chapman[12] has shown that the primary process in the photochemistry of CIT is reversible loss of carbon monoxide rather than loss of cyclobutadiene. Once attached to the solid phase, the carbonyls of the complex VIII ($n = 2$) might be protected by the polystyrene from further photochemical labilization and subsequent chelation. Structural ambiguities of this sort are not infrequently encountered in the chemistry of solid supports; happily, they are relatively harmless in the present case.

It was more important to establish that the iron was strongly bound to the precursor VIII, and that it remained so during oxidation. Some indication that CIT was not merely adsorbed on the ligand resin was already provided by the failure of VII to incorporate iron when heated with CIT. More direct evidence was obtained when VII and VIII, separated by a soxhlet thimble, were suspended in the same ethanol solution for 6 hr. Neither increase in the iron composition of VII nor decrease in the iron composition of VIII could be detected, indicating that the iron of precursor VIII is, for present purposes, immobile. Upon oxidation, the chelate derived from VIII should become even more stable, considering the tenacity with which *o*-phenanthroline clings to iron ions.[13] An adverse equilibrium coonstant of at least 10^{+10} must be overcome if Fe^{2+} is to completely escape the resin. Therefore, as a final control, equal amounts of VII and VIII, separated by a coarse, sintered-glass barrier, were immersed in hot benzene and oxidized with pyridine *N*-oxide. Again the iron composition of the two resins remained unchanged. Thus it may be concluded that the iron precursor VIII does not act as a cyclobutadiene carrier during oxidation.

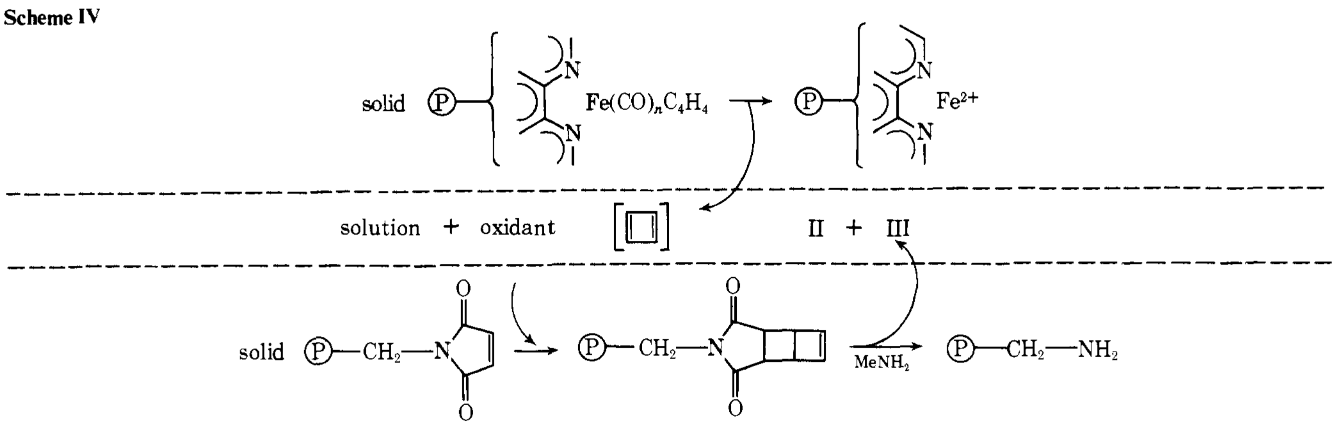
Three-Phase Test

Mixtures of VIII and excess IV were oxidized with ceric ion in ethanol. The beads were readily separable by screening or flotation, and the adduct resin was methaminolyzed to give, after chromatography, a 45% yield of the recrystallized reference diamide (Scheme IV). Pyridine *N*-oxide also proved effective. With this reagent, oxidation of the tritiated precursor, methaminolysis of the adduct, followed by isotope dilution for the reference diamide, a 96% yield for the overall process could be realized.

Conclusions

The present trapping results (in accord with the stereochemical evidence of Grubbs and Schmidt)[6] support the conclusion that oxidation of the iron carbonyl complexes generates free cyclobutadiene and indicate that ions derived from cerium are not required as vehicles for cyclobutadiene transport. Further, the ability of the three-phase test to detect highly reactive intermediates has been demonstrated. The detection of intermediates in acyl and phosphate transfers through application of the three-phase test will be reported shortly.

Scheme IV



Experimental Section

Unless otherwise specified, all solvents and reagents were spectrograde or reagent grade. Elemental analyses were performed by Schwartzkopf Microanalytical Laboratory. Cyclobutadieneirontricarbonyl was purchased[14] or prepared[15] and tritiated as described for its deuteration.[16] Radioactivity was determined by liquid scintillation counting in a Nuclear Chicago Mark II instrument. The counting liquid consisted of 150 mg of 1,4-bis-2-(4-methyl-5-phenyl-oxazoyl)benzene (POPOP), 3.5 g of 2,5-diphenyloxazole (POP), and 50 g of naphthalene in 500 ml of scintillation grade dioxane; efficiencies were determined by internal standardization. Infrared spectra were recorded on a Beckman IR 5A instrument using KBr pellets; atomic absorption spectra were obtained with a Perkin-Elmer 303 instrument. Preparative chromatography was performed on Mallinckrodt "Chromar" sheets, and R_f values refer to this adsorbent in the specified eluent.

Preparation of N,N′-Dimethylbicyclo[2.2.0]hex-5-ene-2,3-dicarboxamide (II). A solution of the cyclobutadiene-maleanil adduct (20 mg) in 2 ml of benzene was treated with 0.5 ml of 40% aqueous methylamine and stirred at room temperature for 1 hr. The mixture was diluted with 4 ml of water and extracted with a 3-ml portion of benzene, then the aqueous phase was evaporated to dryness at reduced pressure. The residue crystallized on trituration with benzene and was recrystallized from ethanol to give 10 mg (55%) of the reference diamide II: mp 196° dec; ir 3400, 1650 cm^{-1}; NMR δ 6.15 (2), 3.35 (2), 2.8 (6), 2.25 (2); R_f 0.8 Et_2O-EtOH 9:1 (v/v). The same procedure was used to convert maleanil to diamide III: mp 208° dec; ir 3309, 1640 cm^{-1}; NMR δ 6.65 (2); R_f 0.35 Et_2O-EtOH 9:1 (v/v).

Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 62.14; H, 6.99; N, 14.40. Found: C, 61.85; H, 7.21; N, 14.43.

Polymeric Benzylamine. The procedure of Weinshenker[8] was used. Merrifield's peptide resin (chloromethylated polystyrene; 1.08 mequiv of Cl/g, 200-400 mesh, Cal Biochem), 11.6 g, was suspended in 300 ml of DMF containing 5.9 g (2.5-fold excess) of potassium phthalimide and heated with stirring at 100° for 7 hr. The mixture was filtered and washed with hot DMF (200 ml) and ethanol (50 ml). The ir spectrum of a sample showed the strong 1710 cm^{-1} and weak 1765 cm^{-1} absorptions characteristic of phthalimides. The resin was stirred at reflux in 150 ml of absolute ethanol containing 14 ml of hydrazine hydrate for 9 hr, filtered, and washed with hot 75% ethanol (150 ml), hot 50% ethanol (150 ml), and ether. After drying, the resin weighed 10.98 g and showed no carbonyl ir absorption. Titration indicated 0.89 mequiv of amine/g.

Polymeric Benzylmaleimide (IV). Following the procedure of Dichcard,[17] 8.37 g of the polymeric benzylamine (7.5 mequiv) was suspended in 125 ml of benzene containing 3.5 g of maleic anhydride (fourfold excess) and stirred overnight. The suspension was filtered, and the resin was washed with benzene. A sample showed ir absorptions at 1700 and 3000 cm^{-1}. The remaining resin was suspended in 40 ml of acetic anhydride containing 0.8 g of NaOAc and heated at 80° for 6 hr, during which time the resin became dark brown. After washing with 600 ml of hot water, then 150 ml of hot ethanol, and drying, the brown resin weighed 9.0 g and showed ir 1700 and 1765 cm^{-1} and neither OH nor NH absorptions.

Reaction of IV with CIT in Solution. A chilled 3% ethanolic solution of ceric ammonium nitrate (10 ml) was added dropwise (1 hr) to a stirred suspension of 0.218 g of IV and 57 mg of CIT in 3 ml of ethanol at 0°. After another 1.5 hr of stirring at 0°, the resin was filtered and washed with ethanol, then acetone. The ir of the adduct V showed only subtle changes in the fingerprint region and no longer exhibited the weak 1765 cm^{-1} absorption. The resin was suspended in 4 ml of 40% methylamine[18] and heated with stirring at 70° for 2.5 hr, then filtered and washed with several portions of aqueous ethanol. Evaporation of the combined filtrate and washings gave crystalline diamide **II** identical in melting point, ir and NMR spectra, and TLC behavior with that described above. Similar results were obtained using pyridine *N*-oxide in hot benzene as the oxidant.

Preparation of Polymeric Tosyl Chloride (VI). Macroreticular cross-linked polystyrene (Rohm and Haas XE-305 30 mesh), 11.2 g, was suspended in 36 ml of $ClSO_3H$ and 65 ml of CCl in a 300-ml flask and heated under reflux for 2 hr. The chilled mixture was carefully treated with ice-water, filtered, then washed with CCl_4, acetone, and benzene. The resin weighed 23.8 g after drying and showed intense absorption at 1370 and 1170 cm^{-1}. Samples treated with refluxing aniline released 4.9 mequiv of chloride/g of resin (by potentiometric titration); the increase in weight corresponds to 5.2 mequiv of sulfonyl chloride groups/g of resin.

Preparation of the Ligand Resin VII. The tosyl chloride resin, 140 mg, was suspended in 75 ml of dioxane containing 130 mg of 5-aminophenanthroline[11] and heated under reflux for 2.5 hr. The resin was filtered free of the red solution and washed with dioxane. The brown resin showed new ir absorptions at 2900-3600 and 1650 cm^{-1} and analyzed for 1.35% N (0.32 mequiv of ligand/g) and 0.04% Fe.

Preparation of the Complexed Cyclobutadieneiron Carbonyl Resin VIII. A suspension of 160 mg of VII in 3 ml of absolute ethanol containing 37.6 mg of CIT was stirred in an open beaker and irradiated from above at 254 nm for 1 hr without external cooling. The resin was washed exhaustively with ethanol then pentane to give VIII, ir 2050 (w) and 1960 (m) cm^{-1}, N 1.22% (0.29 mequiv of ligand/g) and Fe 1.58% (0.28 mequiv/g).

Determination of the Cyclobutadiene in the Precursor VIII. The polymer-bound complex, prepared as above from tritiated (3.60 μCi/mmol) CIT, was powdered and counted; activity was recorded but, in the absence of polymer-bound standards, efficiencies could not be determined. Therefore, 18 mg of the resin was suspended in benzene containing excess (tenfold) pyridine *N*-oxide and maleanil and allowed to stand overnight. The mixture was then heated to reflux, filtered hot, and washed with benzene. The filtrate and washings were concentrated and counted against internal standards giving 0.0165 μCi, corresponding to 0.25 mmol of cyclobutadiene moieties per gram of complex VIII.

Immobility of Iron in the Precursor Resin VIII. A sample of the phenanthroline resin VII (100 mg) was placed in a soxhlet thimble and immersed in a test tube containing an equal weight of the resin complex VIII in 95% ethanol. The tube was stoppered and allowed to stand for 6 hr at 25° with occasional shaking. After drying, VII was analyzed for 0.033% Fe and VIII analyzed for 1.53% Fe.

Immobility of Iron during the Oxidation of VIII. A sample of VIII, 10 mg, in a sintered-glass gas-dispersion tube was immersed in a suspension of VII, 10 mg, in 5 ml of benzene. Pyridine *N*-

oxide (10 mg) was added to the solution, and the system was heated under reflux for 2 hr. The resin derived from VIII was analyzed for 1.7% Fe, while that derived from **VII** showed 0.04% Fe.

Reaction of VIII with Maleanil in Solution. A suspension of VIII, 80 mg, in 1 ml of 95% ethanol containing 20 mg of maleanil was cooled to 0° and stirred as 1.6 ml of a 3% solution of ceric ammonium nitrate in ethanol was added dropwise. After an additional 30 min of stirring, the suspension was filtered and washed with ethanol and water. The combined filtrate and washings were evaporated to dryness, and the residue was extracted with ether. Chromatography of the ethereal solution gave unreacted maleanil and the adduct I, with ir spectrum and TLC behavior identical with that prepared in solution. The residue was examined by atomic absorption and showed only those traces of iron present in the ceric reagent.[19] The oxidized resin showed no carbonyl ir absorptions.

Three-Phase Test. (A) **With Ceric Ion.** A mixture of 600 mg of precursor resin VIII (0.15 mmol) and 3 g of trapping resin IV ($\sim$2.5 mmol) was suspended in 6 ml of 95% ethanol and stirred at 0° as a solution of 0.6 g of the ceric salt *in* 1 ml of H_2O was added slowly (1.5 hr). After an additional hour of stirring, the suspension was filtered and washed with water and ethanol. The resins were mixed with ethanol (the trapping and adduct resins float); the floated resin was drawn off and treated as before with aqueous methylamine. The resulting suspension was filtered, and the filtrate was concentrated. Chromatography gave 14 mg (48%) of recrystallized diamide II, identical in melting point, mixture melting point, and spectroscopic features with the analytical sample.

(B) With Pyridine *N*-Oxide.[9] A mixture of 217 mg (0.054 mequiv) of precursor VIII and 1.4 g ($\sim$1 mequiv) of the trapping agent IV was suspended in 6 ml of benzene containing 100 mg of pyridine *N*-oxide and heated under reflux for 2 hr. After filtration, washing, and separation as in A, the adduct resin was treated with methylamine. The reference diamide II could be isolated and identified by TLC.

(C) **Determination through Isotope Dilution.** A mixture of 189 mg of tritiated VIII (0.047 mequiv of cyclobuta*d*iene) and 719 mg of IV was suspended in 8 ml of benzene containing 45 mg of pyridine *N*-oxide and heated under reflux for 2.5 hr. The resins were filtered, washed, and separated by flotation as in A. The combined filtrate and wash solutions showed 2.5×10^{-3} μCi or 1.5% of the total initial activity, while no activity could be detected on the precursor resin. The adduct resin was treated as before with methylamine, and an aliquot ($^1/_{10}$) of the resulting solution was used to dissolve 9.8 mg of unlabeled II. The diamide II was recovered and recrystallized. The specific activity (0.29 μCi/mmol) indicated 44 μmol in the aliquot (94% yield).

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References and Notes

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