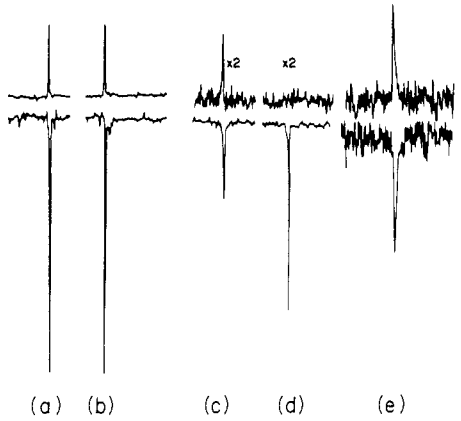


Figure 1. The coupled (upper) and proton broad band noise decoupled (lower) $^{15}N$ NMR spectra of the glycyl-$^{15}N$-glycine (a) amino nitrogen, pH 12.3; (b) amide nitrogen, pH 12.3; (c) the amino nitrogen, pH 8.3; (d) the amide nitrogen, pH 8.3; and (e) the glycine-$^{15}N$ amino nitrogen, at pH 9.3. The spectra were obtained from 32 90° pulses collected on 4 K data points over a spectral range of 3000 Hz, using a 10-sec delay between pulses. No exponential time constant was used. NOE enhancements were measured from the integrated intensities.

vial with 0.25 ml of settled Chelex-100 for a few minutes. The suspension is then filtered through a cotton plug directly into an NMR tube. Addition of HCl can be used to adjust the pH of the filtered solution below pH 5 if desired. It should be noted that glycine and glycylglycine bind to the resin to some extent.

The NOE enhancements and $T_1$ times for non-purified and purified glycylglycine are compared in Table I. Chelex-treated solutions of glycyl-$^{15}N$-glycine-$^{15}N$ (0.5–1 $M$) display an amino resonance, whose line width (2 Hz) was pH independent and whose NOE's were −3.5, −2.8, −3.8, at pH 5.1, 8.3 (Figure 1c), and 12.3 (Figure 1a), respectively. The $T_1$ relaxation time of the amino resonance was 4.6 sec at pH 7.0, compared to 4.7 sec for the amide resonance. The amide resonance displayed NOE enhancements of −3.7, −3.9,[15] and −3.8 at pH 5.0, 8.3 (Figure 1d), and 12.3 (Figure 1b), respectively. The NOE enhancement and line width of a Chelex-100 treated aqueous solution of glycine-$^{15}N$ (~0.5 $M$) were measured at its p$K_a$'s (pH 9.3) and were found to be −1.7 and 7 Hz, respectively (Figure 1e). The fact that total NOE enhancements of the amino group could not be obtained for glycylglycine and glycine at their p$K_a$ values results, we believe, from the very high affinity of these compounds near their p$K_a$ values for the trace amounts of $Cu^{2+}$ which could not be totally removed by Chelex, rather than from some physical effect associated with the zwitterion–anion transition. This is demonstrated not only by the loss of the NOE but also by the extreme line broadening observed in nontreated samples.

It is interesting to note that dipolar relaxation dominates the amino nitrogen spin–lattice relaxation process, even at pH 12.5, at which the rate of proton exchange is between $10^9$ and $10^{10}$ $sec^{-1}$.[6] The concept that the $^{15}N\{^1H\}$ NOE is attenuated by proton exchange, which has so permeated the $^{15}N$ NMR literature,[1,8,14] should be revised.

**Acknowledgments.** We wish to acknowledge the generous supply of $H^{15}NO_3$ provided by the Isotope Separation Plant of the Weizmann Institute. This research was supported by National Institutes of Health Grant No. HL14678-03.

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(15) Even though the nondecoupled amide resonance at pH 8.3 is considerably broadened by chemical exchange of the amide proton, and is observed (Figure 1d) as a very weak broad signal, its intensity was readily measurable in the integration mode and was the same as the nondecoupled amino resonance.

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## A New Type of Base Catalyzed Elimination of Hydrogen Halide from Strained Halocyclopropanes

*Sir:*

We recently reported a new general synthesis of chloro- and bromospiro[2.4]heptadienes.[1] These compounds are being investigated as part of a project directed toward the synthesis of spiro[2.4]heptatriene for which a ground state stabilization through spiroconjugation has been predicted.[2] Base catalyzed eliminations of hydrogen halide have been reported as general routes to cyclopropenes.[3] We therefore investigated the reaction of the halospiro[2.4]heptadienes with a variety of bases.

Treatment of 1-chloro-2-*tert*-butylspiro[2.4]heptadiene (cis:trans ratio = 20:80) with excess potassium *tert*-butoxide in *tert*-butyl alcohol at 78° or in tetrahydrofuran at 65° for several hours gave in 74% yield the *trans*-1-*tert*-butoxy-2-*tert*-butylspiro[2.4]heptadiene, isolated by column chromatography (Alumina Grade III, hexane) and by vacuum distillation as a colorless oil, $bp_{0.05}$ 42–45°, mp −3 to −7°. Vapor phase chromatography (6 ft, 0.25 in. OV-17 glass column) and NMR indicated the presence of only one isomer. On the basis of the observed 1,2-coupling constants in the cyclopropane ring of $J_{12}$ = 6 Hz, we assign the trans stereochemistry for the *tert*-butoxy compound.[4] The results are summarized in Table I. Tables II (NMR spectra) and III (mass spectra) appear in the microfilm edition; see paragraph at end of paper regarding supplementary material.

The reaction of 1-chloro-2-*tert*-butylspiro[2.4]heptadiene (cis:trans ratio = 20:80) with excess KO-*t*-Bu and $KSCH_2C_6H_5$ (ratio 1:1) in *tert*-butyl alcohol at 78° for 5 hr gave one single isomer of 1-benzylmercapto-2-*tert*-but-

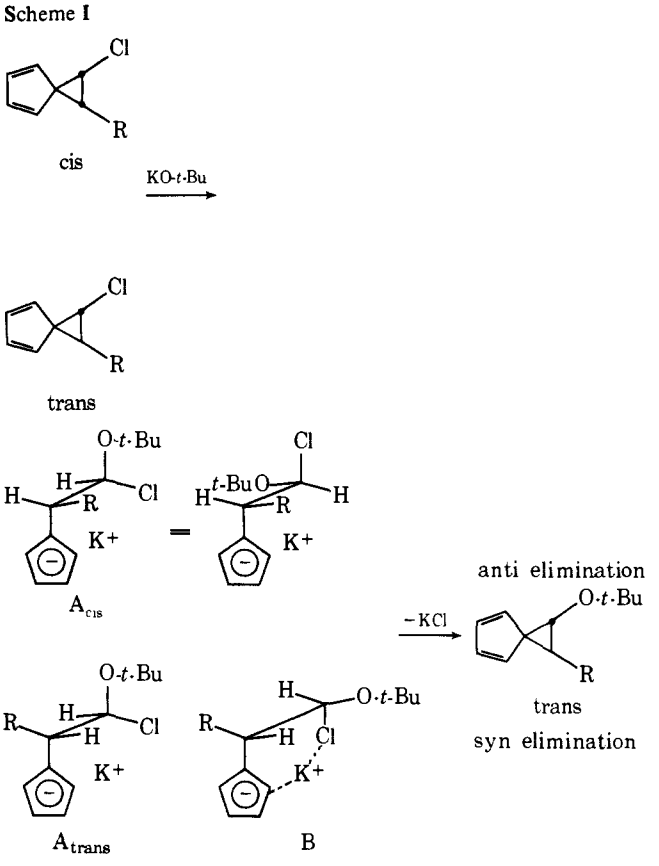
Table I. Results

| Spiro[2.4]heptadiene | *a* | Reagent | Spiro[2.4]heptadiene | % yield | $J_{1,2}$ Hz | Stereochemistry |
|---|---|---|---|---|---|---|
| 1-Chloro-2-methyl- | 83:17 | *t*-BuOK–*t*-BuOH | 1-*tert*-Butoxy-2-methyl- | 72 | 5 | Trans |
| 1-Chloro-2-isopropyl- | 69:31 | *t*-BuOK–*t*-BuOH | 1-*tert*-Butoxy-2-isopropyl- | 70 | 5 | Trans |
| 1-Chloro-2-*tert*-butyl- | 20:80 | *t*-BuOK-*t*-BuOH | 1-*tert*-Butoxy-2-*tert*-butyl- | 75 | 6 | Trans |
| 1-Chloro-2-*tert*-butyl- | 20:80 | *t*-BuOK–*t*-BuOD | 1-*tert* Butoxy-2-*tert*-butyl- | 68 | 6 | Trans |
| 1-Chloro-1-deuterio-2-*tert*-Butyl- | | *t*-BuOK–*t*-BuOH | 1-*tert*-Butoxy-1-deuterio-2-*tert*-Butyl- | 69 | ... | ... |
| 1-Chloro-2.2-dimethyl- | ... | *t*-BuOK–*t*-BuOH | 1-*tert*-Butoxy-2.2-dimethyl | 72 | ... | ... |
| 1-Chloro-2-*tert*-butyl- | 20:80 | *t*-BuOK–$C_6H_5CH_2SK$–*t*-BuOH | 1-Benzylmercapto-2-*tert*-Butyl | 76 | 7.2 | Trans ? |
| 1-Chloro-2-isopropyl- | 69:31 | *t*-BuOK–$C_6H_5CH_2SK$–*t*-BuOH | 1-Benzylmercapto-2-Isopropyl- | 65 | 6.5 | Trans ? |

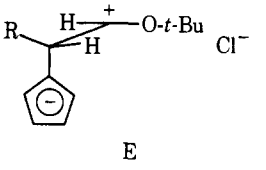
[a] Cis:trans ratios.

ylspiro[2.4]heptadiene as a colorless oil, $bp_{0.05}$ 104–105° (mass spectrum, $M^+$ = *m/e* 270; NMR spectrum, $\delta$ 0.85 (9 H, s), 2.58 (2 H, AB, $J_{AB}$ = 7.2 Hz), 3.39 (2 H, s), 6.3 (4 H, m), 7.06 (m, 5 H)). The unsubstituted chlorospiro-[2.4]heptadiene and the phenyl derivative have so far failed to give the corresponding *tert*-butoxy compounds. In view of the results reported by Wiberg[5] a most likely mechanism for the above reactions appeared to be the elimination of HX yielding the spiro[2.4]heptatrienes, which subsequently add *tert*-butyl alcohol or benzylmercaptan to the strained cyclopropene to give the observed *tert*-butoxy or benzylmercapto compounds. However, the reaction of 1-chloro-2-*tert*-butylspiro[2.4]heptadiene with KO-*t*-Bu in *tert*-butyl alcohol-*d* did *not* result in the formation of 1-*tert*-butoxy-2-deuterio-2-*tert*-butylspiro[2.4]heptadiene; furthermore 1-chloro-2,2-dimethylspiro[2.4]heptadiene upon treatment with KO-*t*-Bu in *tert*-butyl alcohol gave the 1-*tert*-butoxy-2,2-dimethylspiro[2.4]heptadiene. No $\beta$-elimination is possible in the second case. Direct SN2 substitution can be reasonably excluded on the basis of the selective formation of only one isomer, independent of the stereochemistry in the starting materials. The exclusive formation of the benzylmercapto compound in the competition experiments with KO-*t*-Bu and $KSCH_2C_6H_5$ in *tert*-butyl alcohol argues against a SN1 reaction. The alternative mechanistic possibility, $\alpha$-elimination of HCl, followed by nucleophilic addition of *tert*-butyl alcohol to the intermediate cyclopropylcarbene can be excluded on the basis of the following labeling experiments. 1-Chloro-1-deuterio-2-*tert*-butylspiro-[2.4]heptadiene[6] upon treatment with KO-*t*-Bu in *tert*-butyl alcohol gave the 1-*tert*-butoxy-1-deuterio-2-*tert*-butylspiro[2.4]heptadiene with no loss of deuterium. Similarly, 1-chloro-2-*tert*-butylspiro[2.4]heptadiene when treated with KO-*t*-Bu in *tert*-butyl alcohol-*d* gave 1-*tert*-butoxy-2-*tert*-butylspiro[2.4]heptadiene containing no deuterium. On the basis of these results we propose the following mechanism for the conversion of 1-halo-2-alkylspiro-[2.4]heptadienes into 1-*tert*-butoxy-2-alkylspiro[2.4]heptadienes (Scheme I). The nucleophile attacks the cyclopropane ring opening it to the stabilized cyclopentadiene anion A, which undergoes a 1,3-elimination of halide regenerating the spiro[2.4]heptadiene system. Nucleophilic openings of electronically activated cyclopropanes have been reported in the literature.[7] In order to explain the observed formation of only one stereoisomer, it is proposed that the 1,3-elimination of halide from the intermediate $A_{cis}$, formed from *cis*-1-chloro-2-alkylspiro[2.4]heptadiene and KO-*t*-Bu is an anti elimination, while the intermediate $A_{trans}$ yields the same *trans*-1-*tert*-butoxy-2-alkylspiro[2.4]heptadiene through a syn elimination with a transition state resembling B. The transition state for the anti elimination in this case is apparently less favored because of van der Waals repulsion between the substituents. Alternatively, as pointed out by

Scheme I



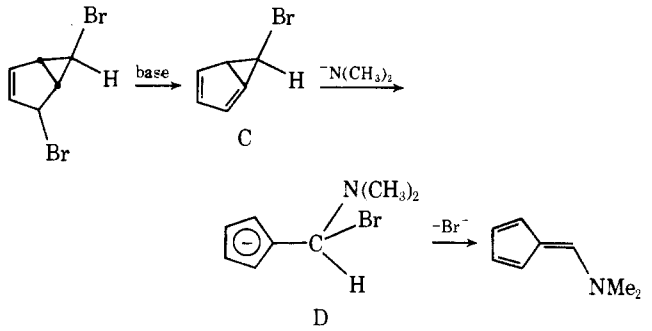

the referees, intermediates $A_{cis}$ and $A_{trans}$ can react in an SN1 reaction through the same planar oxygen stabilized carbonium ion E (as ion pair) leading to the more stable trans products, as observed.




E

This nucleophilic ring opening of a strained cyclopropane leading to a substituted cyclopentadienide anion may also be operative in a recently reported reaction, proposed to proceed through *m*-benzyne.[8] In this paper, the reaction of *exo,exo*-2,6-dibromobicyclo[3.1.0]hexene with dimethylamine and KO-*t*-Bu, giving 6-dimethylaminofulvene and 6-*tert*-butoxyfulvene, has been interpreted to proceed through 1,3-benzyne (bicyclo[3.1.0]hexatriene). In view of our results we should like to suggest an alternative mechanism (Scheme II). Nucleophilic attack at the bromobicyclo-

Scheme II




[3.1.0]hexadiene, C, opens the highly strained cyclopropane ring to give the intermediate D. Rapid elimination of $Br^-$ yields the 6-substituted fulvene.

**Acknowledgments.** We thank Professor F. Mallory for suggesting the nucleophilic attack at the cyclopropane ring. We are grateful to the Research Foundation of the City University of New York for financial support of this work (FRAP Award No. 10596).

**Supplementary Material Available.** Tables II (NMR spectra) and III (mass spectra) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JACS-75-5946.

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## Ferrous Porphyrin–Mercaptide Complexes. Models for Reduced Cytochrome P-450

*Sir:*

Cytochrome P-450, a protoheme-containing monooxygenase system gives, upon reduction in the presence of CO, an anomalous Soret band at about 450 nm. During the enzymatic cycle of cytochrome P-450 ferric cytochrome P-450 first combines with a substrate, followed by a one-electron reduction to form a ferrous cytochrome P-450–substrate complex which can bind either oxygen or CO reversibly.[1-3] It is suggested that the "activated" oxygen, formed after the addition of the second electron to the $O_2$–P-450 complex, interacts with the substrate to give rise to hydroxylated product, water, and ferric cytochrome P-450. Thus, cytochrome P-450 not only functions as an electron transporter but resembles the oxygen carriers hemoglobin and myoglobin, in terms of its capability toward $O_2$ binding.

The axial ligands of the heme iron in cytochrome P-450 are of great interest, since they hold the key to our understanding of the enzymic function and the underlying principles that enable the single complex protoheme to perform various functions ranging from oxygen transport, oxidation catalysis, to electron transport. The possibility of axial sulfur ligation in cytochrome P-450 has been repeatedly expressed in the literature based on EPR evidence.[3-5] Although well-characterized five-coordinate thiolates of ferric protoporphyrin dimethyl ester ($Fe^{III}$PPDME) and *meso*-tetraphenylporphyrin ($Fe^{III}$TPP) have been prepared and shown to exhibit similar EPR parameters to those of high-spin ferric cytochrome P-450,[6,7] model studies on sulfur ligated ferrous porphyrins have been scarce, and the CO-P-450 type spectrum is difficult to duplicate.[8,9] Recently, Stern and Peisach[10] reported that the combination of reduced heme, thiol, CO, and a strong base under stringent mixing procedure could result in the partial appearance of a 450-nm Soret peak, thus implying a mercaptide anion at the fifth coordination site. However, the failure to produce a complete 450-nm peak as well as the inability to duplicate similar results by sequential addition of reagents was puzzling and has been attributed to the "transient nature" of the intermediate species. We chose to study the interaction between ferrous porphyrins and thiols in order to assess the role of sulfur ligands in cytochrome P-450.

Under basic conditions, thiols readily reduce Fe(III) porphyrins to Fe(II) porphyrins. When a protohemin–DMSO solution (~10 $\mu M$) was mixed with an equal volume of a solution of *n*-BuSH (1 *M*)–$NMe_4OH$ (2 *M*) in EtOH, under CO, the visible spectrum exhibited two Soret peaks at 450 (50%) and 412 nm (50%). The ratio of the two was independent of the mode or temperature of mixing but somewhat dependent on the concentration of thiol or base at low concentrations. With the same procedure, mesoheme gave predominantly the normal CO–mesoheme Soret peak at 408 nm (95%) and a small shoulder at about 440 nm, while 2,4-diacetyldeuteroheme resulted in an intense peak at 470 nm with less than 10% of the normal peak at 430 nm.[11] DMSO as solvent appears to be important to bring about the long wavelength Soret peak.[12] We have used several other bases and find that their ability to produce this spectrum follows the order: KH > NaH, $NMe_4OH \gg$ *n*-BuLi, 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU), 1,8-bis(dimethylamino)naphthalene (Proton Sponge). These findings suggest that the interaction between $RS^-$ and Fe(II) porphyrin is weak and occurs only under the most thermodynamically favorable conditions, when interaction between the mercaptide and counterion is minimal. This situation can be likened to the $CN^-$–Fe(II) porphyrin interaction in which the repulsion between the negatively charged ion and the high charge density at the iron engenders extremely low binding affinity. It is expected that the peripheral electronic effect of porphyrin and the solvation and/or ion dissociation of mercaptide salt in solution become very crucial here.

To enhance the activity of the mercaptide anion, crown ether complexes[13,14] of potassium *n*-butyl mercaptide were used. Under these conditions we observed 100% conversion to the long wavelength Soret band with protoheme and 2,4-diacetyldeuteroheme and about 95% with mesoheme in DMSO as solvent. The reagent was prepared by stirring the mercaptide salt (~0.5 *M*) in DMSO under argon or CO and then adding dibenzo-18-crown-6[15] (~0.2 *M*). The CO binding to the heme is reversible and can be achieved in