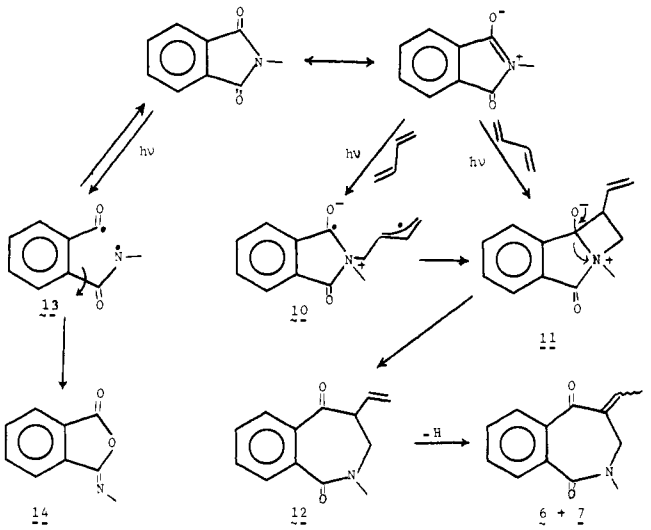
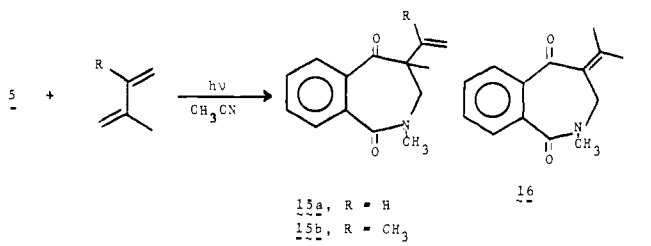
shown, which meets these general criteria, involving diene addition to give dipolar biradical **10,** subsequent closure to azetedine **11,** and opening to **12.** An alternative mechanism would involve α cleavage to biradical **13** which could add diene to afford **12** directly. However, if rotation around the C-C(O) bond in **13** is competitive with reclosure to **5,** a reasonable expectation in view of the fact that diene addition must compete with reclosure, we would expect to see the formation of the isomeric imine **14** in the absence of diene. Irradiation of **5** in pure acetonitrile or *tert*-butyl alcohol gave only unreacted **5.**



The addition reaction occurs with several other dienes with comparable efficiency. Thus, irradiation of **5** in the presence of isoprene affords a 45:55 mixture of **15a** and **16** in 49% isolated yield. The ^{1}H NMR spectrum of **15a** showed δ 1.45 (s, 3 H), 3.2 (s, 3 H), 3.30 and 3.83 (AB pattern, J = 14 Hz), 5.0-5.6 (m, 3 H), 7.3-7.9 (m, 4 H); IR (CCl_4) 1698, 1655 cm^{-1}; *m/e* 229 (9). The spectra of **16** showed δ 2.15 (s, 3 H), 2.35 (s, 3 H), 3.25 (s, 3 H), 4.30 (s, 2 H), 7.5-8.0 (m, 4 H); IR (CCl_4) 1670, 1655 cm^{-1}; *m/e* 229 (100). Similarly, 2,3-dimethylbutadiene gave a 50% isolated yield of **15b:**[20] δ 1.4 (s, 3 H). 1.7 (s, 3 H), 3.18 (s, 3 H), 3.3 and 4.0 (AB pattern, J = 15 Hz), 4.7-4.9 (m, 2 H), 7.2-7.7 (m, 4 H); IR (CCl_4) 1695, 1655 cm^{-1}; *m/e* 243 (9). The photoaddition occurs with 1,3-pentadiene to give the expected products from initial addition of the N atom in **5** to the 1 and 4 positions in the diene.[21] However, we were unable to detect any product formation when **5** was irradiated in the presence of either cyclopentadiene or 2,5-dimethyl-2,4-hexadiene.[22] Experiments with isoprene and phthalimide and *N*-phenylphthalimide also afforded no product suggesting that the reaction is sensitive to electronic effects. Research on the scope and mechanism of these reactions is continuing.



Acknowledgment. This research was partially supported by a grant from the National Institutes of Health (DA 01366).

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(17) This is a general correlation for β protons and methyl groups in α,β-unsaturated ketones and acids: L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed, Pergamon Press, London, 1969, p 222.
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(21) The structures of these products were determined by ^{1}H NMR on isomer mixtures. At present we are unable to separate the isomeric products and pure samples were not available.
(22) This observation is important in that it appears that the reaction is least efficient with those dienes having the lowest ionization potentials. Phthalimides have been shown to form weak charge-transfer complexes with amines and aromatic compounds.[10,23] If either a charge-transfer complex or an exciplex was an intermediate in this reaction, we would expect those dienes with the lowest ionization potentials to form the ground- or excited-state complex and react most efficiently. These points are being investigated presently.
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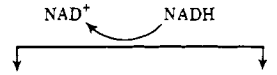
Chemiluminescence Accompanying the Decomposition of 4a-Flavin Alkyl Peroxide. Model Studies of Bacterial Luciferase

Sir:

Flavoenzyme external monooxygenases, in the reduced state (Enz-FlH_2), combine with molecular oxygen and substrate (S) to yield enzyme-bound oxidized flavin (Enz-Fl_{ox}), water, and oxygenated substrate (eq 1).[1] The oxidation of S involves the



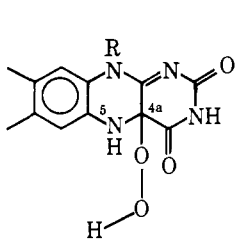
$$\text{Enz-}FlH_2 + O_2 + S \longrightarrow \text{Enz-}Fl_{ox} + H_2O + SO \quad (1)$$

stepwise processes of combination of enzyme-bound dihydroflavin (Enz-FlH_2) with oxygen to provide an oxygenated flavin species (Enz-FlH_2O_2) which then reacts with the bound substrate.[1] It has been suggested that the FlH_2O_2 moiety possesses a 4a-hydroperoxylflavin structure (4a-FlHOOH).[1,2] We have recently reported the synthesis and characterization of 4a-FlC_2H_5OOH and established that its spectrum is almost superimposable upon that of Enz-FlH_2O_2 prepared from *Beneckea harveyi* luciferase.[3] Further, we have established that

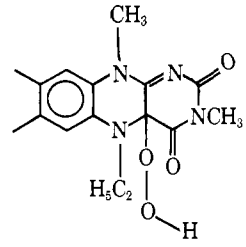
Table I. Chemiluminescence Quantum Yields and Rates of Decomposition Obtained on Mixing Alkyl Hydroperoxides (0.01 M) with $Fl^{+}_{ox}C_2H_5$ (10^{-4} M)[a]

Hydroperoxide	Quantum yield[b]	k, s^{-1}
$C_6H_5CH_2OOH$	3.3×10^{-4}	7.9×10^{-6}
p-$CH_3C_6H_4CH_2OOH$	3.3×10^{-4}	6.7×10^{-6}
$CH_3CH_2CH_2OOH$	5.0×10^{-6}	2.2×10^{-5}
$CH_3(CH_2)_8CH(OH)$-OOH	3.0×10^{-6}	3.5×10^{-3}, 8.2×10^{-5} [c]
$(CH_3)_3COOH$	~0	

[a] Same results are obtained both in anaerobic and O_2-saturated solutions, 30 °C, solvent 4:0.1 t-BuOH-CH_3CN. [b] Based on the standard luminol reaction[10] and corrected for photomultiplier response. [c] The decrease in the light intensity is biphasic.[6]

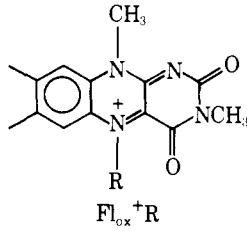


4a-FlHOOH

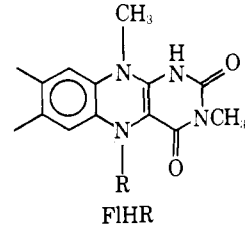


4a-FlC_2H_5OOH

the reaction of either H_2O_2 with compounds of general structure $Fl^{+}_{ox}R$ or the reaction of O_2 with compounds of general structure FlHR provide 4a-FlROOH.[3,4]



$Fl_{ox}{}^{+}R$

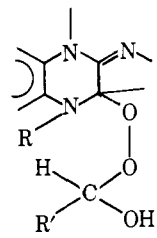


FlHR

It has been shown that the key step of bioluminescence catalyzed by bacterial luciferase (eq 2)

$$\text{luciferase-}FMNH_2 + O_2 + RCHO \rightarrow h\nu + \text{luciferase-FMN} + RCO_2H + H_2O \quad (2)$$

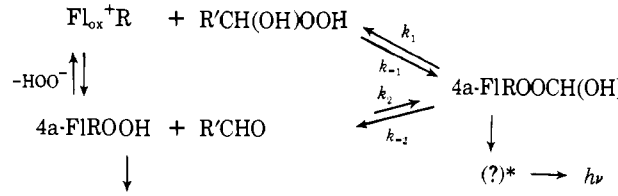
is the reaction of luciferase bound 4a-hydroperoxyflavin mononucleotide (luciferase-$FMNH_2O_2$) with a long chain aldehyde.[2] Further, it has been suggested[5] that the chemiexcitation step involves the breakdown of a mixed peroxide of flavin and aldehyde (4a-FlROOCH(OH)R′, R = H; R′ = long chain alkyl). The reactions of the model compound 4a-FlC_2H_5OOH with a variety of aldehydes have been shown to be accompanied with light emission.[3,6] Evidence for the intermediacy of 4a-



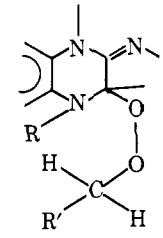
4a-FlROOCH(OH)R′

FlROOCH(OH)R′ (R = C_2H_5; R′ = n-C_9H_{19}) in the model reactions was obtained by comparison of the chemiluminescence resulting from reaction of (i) 4a-FlROOH with R′CHO and (ii) $Fl^{+}_{ox}R$ with R′CH(OH)OOH (Scheme I).[6] In this study we point out the important mechanistic implications of

Scheme I



our finding of chemiluminescence upon primary alkyl hydroperoxide addition to $Fl^{+}_{ox}R$ which provides 4a-$FlROOCH_2R'$.



4a-$FlROOCH_2R'$

Chemiluminescence was observed (λ_{max} of emission ~530 nm) on mixing an acetonitrile solution of $Fl^{+}_{ox}C_2H_5$ with *tert*-butyl alcohol[7] solutions of benzyl, p-methylbenzyl, or n-propyl hydroperoxide.[8] At $Fl^{+}_{ox}C_2H_5$ and hydroperoxide concentrations of $<2 \times 10^{-4}$ M and 1.3×10^{-2} M, respectively, the maximum luminescence intensity was reached in ~4 min. Spectrophotometric studies of the reactions indicated that the equilibrium of eq 3

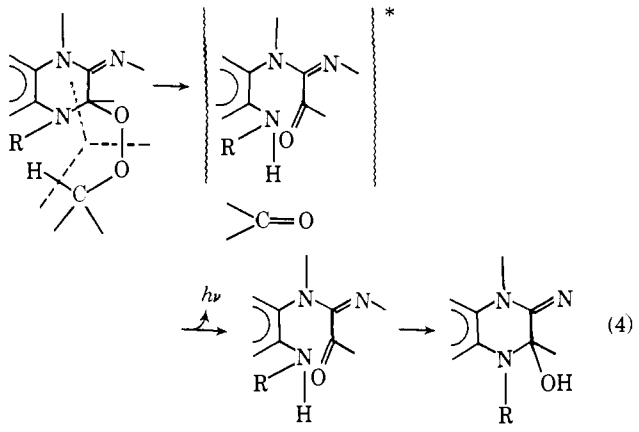
$$Fl^{+}_{ox}C_2H_5 + R'CH_2OOH \rightleftarrows 4a\text{-}FlC_2H_5OOCH_2R' + H^{+} \quad (3)$$

was established within this time. For example, within a few minutes after the initiation of the reaction of 10^{-4} M $Fl^{+}_{ox}C_2H_5$ with 1.0×10^{-2} M benzyl hydroperoxide, ~60% of the flavin was as 4a-$FlC_2H_5OOCH_2C_6H_5$ (λ_{max} ~ 370 nm) while 40% was as $Fl^{+}_{ox}C_2H_5$ (λ_{max} 550 nm). The initial intensity of chemiluminescence was found to be proportional to the concentration of $Fl^{+}_{ox}C_2H_5$ below 5×10^{-5} M ($[C_6H_5CH_2OOH] = 1.3 \times 10^{-2}$ M). At $Fl^{+}_{ox}C_2H_5$ concentrations $>10^{-4}$ M, the initial intensity was found to level off in accordance with the equilibrium of eq 3. The decrease in the intensity of light emission was found to obey first-order kinetics to >4 half-lives with all three primary hydroperoxides tested. The first-order rate constants obtained from light emission measurements were found to be identical with those obtained by monitoring flavin peroxide disappearance spectrophotometrically at 370 nm. These rate constants, as well as the quantum yields obtained, are shown in Table I, which also includes data obtained with 1-hydroxydecyl hydroperoxide and *tert*-butyl hydroperoxide. Inspection of Table I reveals that the -OH group in $CH_3(CH_2)_8CH(OH)OOH$ is not required for the chemiluminescent reaction. Thus, with $CH_3CH_2CH_2OOH$, the quantum yield is about the same as that obtained with $CH_3(CH_2)_8CH(OH)OOH$. With $C_6H_5CH_2OOH$ and p-$CH_3C_6H_4CH_2OOH$, on the other hand, the quantum yields are ~100-fold greater than with $CH_3(CH_2)_8CH(OH)OOH$ (Table I). When the N^{10}-methyl group of $Fl^{+}_{ox}C_2H_5$ was replaced by -$CH_2CH_2C_6H_4$-m-OCH_3,[9] the quantum yield was found to increase by ~3-fold (with p-$CH_3C_6H_4CH_2OOH$, quantum yield = 10^{-3}, a value ~10^2 less than seen in the enzymatic reaction). Since the emitter has yet to be identified, the yield of excited states cannot be calculated.

The reactions of $Fl^{+}_{ox}C_2H_5$ (10^{-4} M) with

$C_6H_5CD_2OOH$[11] (5.7×10^{-3} M to 4.0×10^{-2} M) and $C_6H_5CH_2OOH$ (5.7×10^{-3} M to 4×10^{-2} M) were compared. The time course of light emission was found to be identical with both hydroperoxides (i.e., no kinetic isotope effect). However, the total amount of light emitted with $C_6H_5CH_2OOH$ was two times greater than that obtained with $C_6H_5CD_2OOH$. The finding of a product isotope effect of 2.0 indicates that the C-H(D) bond is breaking in the chemiexcitation step and the absence of a kinetic isotope effect establishes that the rate of the chemiexcitation step is slower than that of a competing nonchemiluminescent and kinetically controlling process.[12] Similar deuterium isotope effects upon the quantum yield and in the absence of kinetic isotope effects were previously obtained for the reaction of $Fl^+{}_{ox}C_2H_5$ with $CH_3(CH_2)_8CH(OH)OOH/CH_3(CH_3(CH_2)_8CD(OH)$-$OOH$[6] and for the reaction of 4a-FlC_2H_5OOH with H_2CO/D_2CO.[3]

Since compounds of structures 4a-FlROOCH(OH)R′ and 4a-$FlROOCH_2R'$ provide chemiluminescence while 4a-$FlROOC(CH_3)_3$ does not (Table I), we may conclude that the minimal structural requirement for the chemiluminescent reaction is represented by 4a-$FlROOCHR_1R_2$. The mixed peroxide structures, as well as the observation of primary deuterium isotope effects, reasonably dictate that chemiluminescence accompanies the breaking of both O-O and C-H(D) bonds. At least six detailed mechanisms have been proposed for bacterial luciferase within the last 5 years. With the reasonable presumption that our studies (this and ref 3 and 6) relate to the enzyme-catalyzed reaction, we may conclude that these six proposals of mechanisms are incorrect. Four[13-16] are inconsistent with the intermediacy of the mixed peroxide 4a-FlHOOCH(OH)R′ in the bioluminescent reaction and cannot, in any event, be applied to N^5-alkylated flavins. In addition, three of these mechanisms,[13,14,16] as well as another one,[17] cannot be applied to the reaction of $Fl^+{}_{ox}C_2H_5$ with primary alkyl hydroperoxides since they require the hydroxyl group of 4a-FlROOCH(*OH*)R′. Finally, chemiluminescence via the breakdown of 4a-FlROOCH(OH)R′ by a Baeyer-Villiger mechanism, as suggested by Eberhard and Hastings,[5] would require that the pseudobase 4a-FlC_2H_5OH be produced in an excited state. However, the latter is nonfluorescent in solution[18] and, furthermore, a Baeyer-Villiger-type rearrangement is not likely with 4a-$FlC_2H_5OOCH_2C_6H_5$ (the hydroxyl group of 4a-FlROOCH(*OH*)R′ is essential for this mechanism also). We put forth the mechanism of eq 4 for



(4)

consideration. The six-center concerted decomposition of 4a-$FlROOCHR_1R_2$ finds precedence in the self-reaction of secondary peroxy radicals via the Russell mechanism,[19] and, particularly, thermal decompositions of hydroxyalkyl peroxides and hydroperoxides,[20] as well as the proposed route of decay of *O*-benzyl-*N*-benzylamino radicals following their coupling,[21] and others.[22] These reactions are characterized by (k^H/k^D) isotope effects comparable[19a,20c] with those seen in this study. From bond energies, it can be calculated that the cyclic mechanism of eq 4 is exothermic by as much as 90 kcal/mol.[23] This, plus the activation enthalpy, should be quite sufficient to populate the first excited singlet (or triplet) level of the flavin fragment.[24] The mechanism of eq 4 is in accord with the fact that oxidized flavin is not the emitter in the luciferase-catalyzed reaction.[25] In the enzymatic reaction, ring closure to re-form flavin would be required as previously proposed by Hamilton for his oxene hydroxylation mechanism.[26]

The results reported herein, along with the results of McCapra and Leeson[27] with somewhat similar systems,[28] indicate the presence of a general class of efficient chemiluminescent reactions which yield an electronically excited product via fragmentation of a mixed peroxide formed by addition of R_1R_2CHOOH to the >C=N⁺< moiety of a suitable nitrogen heterocycle. This mechanism would not appear to involve the intermediacy of a 1,2-dioxetane.

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(18) The chemiluminescence emission (λ_{max} 530 nm) does not correspond to the fluorescence of $Fl^+{}_{ox}C_2H_5$ (λ_{max} >650 nm). The latter is the only fluorescent chromophore originally in solution. Therefore, the possibility of energy transfer from electronically excited 4a-FlC_2H_5OH to a fluorescer can be ruled out. In other words, the excited product of the reaction is not 4a-FlC_2H_5OH.
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(24) The reasonable assumption is made that the nonflavin carbonyl fragment (which would be an aliphatic acid in the reaction of $Fl^+_{ox}R$ + $CH_3(CH_2)_8CH(OH)OOH$ and benzaldehyde in the reaction of $Fl^+_{ox}R$ + $C_6H_5CH_2OOH$) will not be generated in an excited state since the energy difference between its lowest excited state and ground state would be expected to be considerably higher than that for the flavin fragment.
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(28) These chemiluminescent reactions result on addition of hydroperoxides to 1,3,10-trimethylisoalloxazinium perchlorate (A) at the 10a position. The 10a-flavin peroxides cannot be alternative models for the luciferase bound oxygenated FMN intermediate as claimed, because the spectral characteristics of the latter are distinctly different from that of 10a adducts of A.
(29) This study will be submitted by C.K. in partial fulfillment of the requirements for the Ph.D. in Chemistry.

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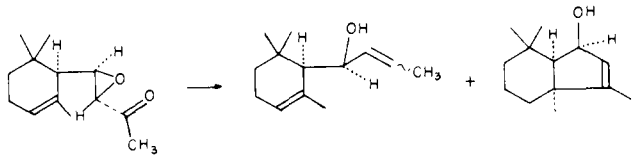
Five- and Six-Membered-Ring Formation from Olefinic α,β-Epoxy Ketones and Hydrazine

Sir:

A few years ago, Ohloff reported[1] that treatment of the conjugated epoxide **1** with hydrazine in methanol gave the expected allylic alcohols **2** from the Wharton reaction,[2] together with an equal amount of an unexpected product, the cyclopentenol **3.**



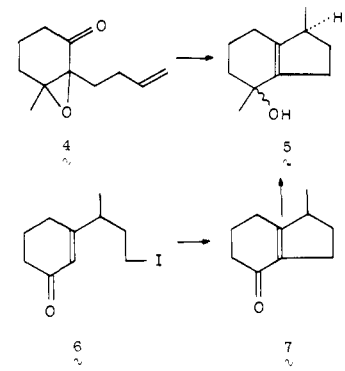
1 2 3

We now report studies which were initiated because the mechanism which was suggested[1] for the formation of **3** seemed very unlikely to us, and because we hoped to develop Ohloff's observation into a cyclization method of some generality.

In the search for systems which might cyclize we finally discovered that epoxy ketone **4** was[3] cyclized with hydrazine in methanol to the hydrindenol **5** in 85% yield.

The structure of **5** (mixture of diastereoisomers) was strongly suggested by its NMR: δ 0.98 (2 d, J = 6 Hz, 3 H), 1.19 (2 s, 3 H), 1.21-2.90 (complex m, 10 H). In particular, the absence of vinyl hydrogen absorptions showed that essentially none of the "normal" Wharton product was formed. There also appeared to be no six-membered ring formed in the reaction.[4]

Conclusive evidence for the structure of **5** was obtained by independent synthesis via 3-(4-iodo-2-butyl)-2-cyclohexenone (**6**), which was readily obtained by Birch reduction of 3-(*m*-methoxyphenyl)-1-butanol,[5] followed by tosylation of the resulting dihydroanisole and exchange with iodide (sodium iodide

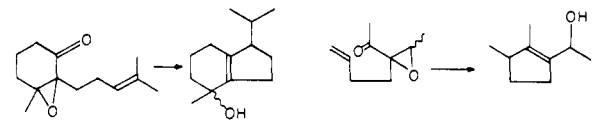


4 5

6 7

in refluxing acetone to form **6** directly). Cyclization (potassium *tert*-butoxide in refluxing *tert*-butyl alcohol), followed by isolation by preparative VPC (20% SE-30), gave the pure indenone **7**:[6] IR 1680, 1640 cm^{-1}; NMR δ 0.83 (d, J = 8 Hz, 3 H); *m/e* 150.1044. Addition of lithium methyl gave a mixture of tertiary alcohols **5,** essentially indistinguishable (NMR, VPC) from the product of the hydrazine cyclization.

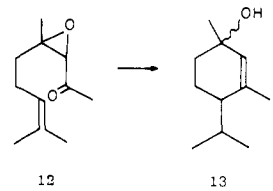
The type of substitution around the side-chain double bond does not appear to be crucial. The dimethyl homologue **8**[7] of **4** was similarly cyclized to **9** (~60% yield after Kugelrohr distillation): NMR δ 0.60 (d, J = 4 Hz), 0.68 (d, J = 4 Hz), 0.85 (s), 0.90 (s), 1.25 (s), 1.4-2.8 (m), 5.2 (br s, OH).



8 9 10 11

The cyclization is also successful with acyclic systems. This was established with the epoxide **10** derived from 3-ethylidene-6-hepten-2-one. The latter was made in excellent overall yield by titanium tetrachloride mediated aldol condensation[8] of acetaldehyde with 2-trimethylsilyloxy-2,6-heptatriene (from the oxy-Cope rearrangement of the trimethylsilyl ether[9] of 3-methyl-1,5-hexadien-3-ol[10]), followed by dehydration (toluenesulfonic acid-benzene, reflux). Cyclization with hydrazine gave the cyclopentenecarbinol **11** in 70% yield as a mixture of the two possible diastereoisomers, identified by direct comparison with authentic material made by reduction (lithium aluminum hydride) of 2,3-dimethyl-1-acetylcyclopentene.[11]

Finally, although there is a clear preference for five- rather than six-membered-ring formation (**4** → **5**), six-membered rings can nevertheless be made. This was demonstrated by cyclization of the epoxide **12** derived from the related conjugated ketone.[12] Hydrazine cyclization gave a 1:1 mixture of the two epimers of **13** in 60% yield. Identification was made by comparison with the mixture of epimers from lithium methyl and 3-methyl-4-isopropyl-2-cyclohexenone.[13] No simple Wharton elimination product (independently synthesized) could be found.[14,15]



12 13

Very subtle (geometric?) factors affect the cyclization reactions. We were, for instance, unable to find any evidence of cyclization with the epoxy ketone **14.**[16] Only the normal Wharton product could be found.[17] This was also the case with