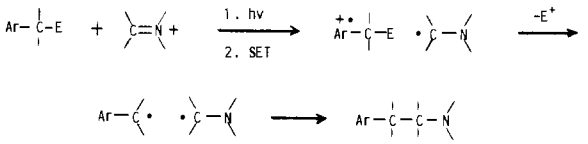
Scheme I



on the type of electrofugal group present at the arene benzylic positions. For example, irradiation ($\lambda \geq 280$ nm) of **12** in MeOH followed by basic workup and chromatography leads to formation of the benzoindolizidine **17**[8] (18%) and the phenylpyrroline **19** (15%). In contrast, the cyclization product **17** is produced *exclusively* (>40%) from photolysis (MeOH or MeCN) of the analogous trimethylsilyl-substituted pyrrolinium salt **13**. Mechanistic information for the transformation **13** → **17** is found in the observation that **13**-d_2, dideutereated at the N–C benzylic position, undergoes photocyclization to form **17**-d_2 with the two deuteriums located at the N–C benzylic position.

Another example of how trimethylsilyl substitution affects the nature of arene–iminium salt photochemistry is revealed in the photochemistry of pyrrolinium perchlorates **14** and **15**. Upon irradiation ($\lambda > 240$ nm) in MeCN followed by basic workup, **14** is converted to the dimethylbenzopyrrolizidine **20** (90%).[11] This novel pyrrolizidine ring-forming process is followed by the simple *N*-benzyl salt **16**, which undergoes efficient photocyclization (MeCN, 90%) to produce **21**. In order to gain evidence to rule out pathways involving the intermediacy of vinylazomethine ylides **23** (Scheme II) in this reaction, **14**-d_2 dideuterated at the N–C benzylic position was prepared and irradiated. The pyrrolizidine **12**-d_2 produced in this case contains both deuteriums at the N–C position, and, thus, is not produced via electrocyclization of **23**. In comparison, irradiation of the silicon-containing salt **15** (MeCN) leads to exclusive production (70%) of the benzoindolizidine **18**. Analysis of the crude photolysate revealed the absence of a Me_3Si analogue of **20** as a photoproduct.

Several aspects of the photocyclization reactions of pyrrolinium salts **12–16** deserve comment. The changes occurring upon replacement of hydrogen by the Me_3Si substituent at benzylic centers in these systems appear to be related to the relative rates of electrofugal group loss converting cation diradicals **22** to neutral diradicals **24** and of other processes open to **22** including C–N bond cleavage and radical coupling (Scheme II). The enhanced efficiency for benzoindolidine formation compared to photofragmentation by cleavage of **22** in the salts **13** is in accord with the greater rate for arene cation radical desilylation vs. deprotonation (Scheme II).[12] Moreover, when the cation diradicals **22** possess the more highly reactive methyl-rather than phenyl-substituted α-pyrrolidinyl radical center and a slow electrofugal group loss pathway (R = H), radical coupling occurs to generate the cation precursor **25** of the pyrrolizidine **20**. However, fast desilylation diverts reaction to indolizidine formation via diradical **24** (R = Me).[13]

(10) The *N*-xylylpyrollinium salts **12–16** were prepared by a sequence involving alkylation of the appropriate pyrrolines with either *o*-$MePhCH_2I$ or *o*-$Me_3SiCH_2PhCH_2I$ followed by perchlorate ion exchange.

(11) The ^1H NMR spectrum of pyrrolizidine **20** contains an AB quartet (3.86 and 4.44 ppm) for NCH_2 and a methyl singlet at 2.24 ppm, and its ^{13}C NMR spectrum indicates the presence of three quaternary aromatic carbons (132.7, 137.0, 147.8 ppm). In comparison, the ^1H NMR and ^{13}C NMR spectra of **18** resemble that of a indolizidine **17** and contain resonances for both sets of diastereotopic benzylic protons and only two quaternary aromatic carbons.

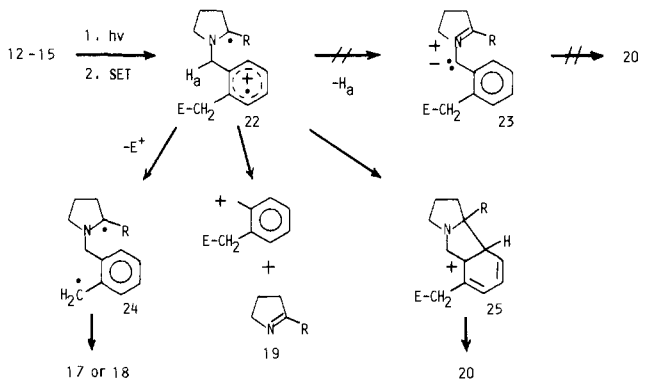
(12) An estimate of the relative rates of arene cation radical desilylation vs. deprotonation has been made through kinetic analysis[1b] of pyrrolinium salt **1** additions to **4** and **5**. This exceptionally inaccurate method suggests that desilylation is ca. 10 times faster than deprotonation.

(13) Perhaps another manifestation of the more rapid rate of arene cation radical desilylation vs. deprotonation might be found in the product spectra and yields from reaction of **4** and **5** with **1**. Thus, the much higher yield of benzylpyrrolidine **6** from **5** vs. **4** could reflect the faster rate of electrofugal group loss vs. cage collapse of the initially formed radical cation pair. This would lead to higher yields of the in-cage coupling product vs. materials generated by out-of-cage processes. However, this same trend is not seen for additions to the phenylpyrrolinium salts **2** and **3**.

Table I. Photoaddition Product Yields from Irradiation of Arene–Iminium Salt Systems in MeOH

pyrrolinium perchlorate	arene	photoproducts (yields)
1	**4**	**6** (2%) + **7** (23%) + **8** (35%) + **11** (1%)
1	**5**	**6** (40%) + **7** (26%) + **8** (10%) + **11** (16%)
2	**4**	**9** (24%) + **11** (15%)
2	**5**	**9** (22%) + **11** (20%)
3	**4**	**10** (20%) + **11** (21%)
3	**5**	**10** (24%) + **11** (24%)

Scheme II



The results summarized above demonstrate that the electron-transfer photochemistry of arene–iminium salt systems can be induced by irradiation of either the donor or acceptor component. This feature along with the control offered by the nature of benzylic-disposed electrofugal group on the type of the heterocyclic products formed suggests that photocyclization reactions of these systems will be synthetically significant.

Acknowledgment. We are pleased to acknowledge the support provided by NIH (GM-27251) and NSF (CHE-08240) for these studied. The technical assistance of Lori Klingler in accumulating a portion of the fluorescence quenching data is acknowledged.

Registry No. 1, 2730-96-3; **2**, 69105-60-8; **3**, 2826-88-2; **4**, 108-88-3; **5**, 770-09-2; **12**, 92014-43-2; **13**, 92014-45-4; **14**, 92014-47-6; **15**, 92014-49-8; **16**, 56519-58-5.

Catalytic Versatility of Angiotensin Converting Enzyme: Catalysis of an α,β-Elimination Reaction

Thomas E. Spratt and E. T. Kaiser*

Laboratory of Bioorganic Chemistry and Biochemistry, Rockefeller University New York, New York 10021

Received April 27, 1984

We wish to report that angiotensin converting enzyme (ACE) catalyzes the α,β-elimination of *p*-nitrothiophenol from *N*-(3-benzoyl-2-((*p*-nitrophenyl)thio)propanoyl)-L-phenylalanine (**1**), a ketone substrate with a leaving group β to the ketone function. This substrate was employed in an effort to determine whether ACE can catalyze proton abstraction from an activated methylene group in a suitably designed ketone substrate. In earlier studies, carboxypeptidase (CPA), an exopeptidase containing an active site proposed to resemble that of ACE,[1] had been shown to catalyze stereospecifically proton incorporation into (*R*)-3,3-dideuterio-2-benzyl-3-(*p*-methoxybenzoyl)propionic acid (*R*-**2**-d_2),[2-4]

(1) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Rubin, B.; Ondetti, M. A. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1979**, *38*, 2778.
(2) Sugimoto, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1978**, *100*, 7750.
(3) Sugimoto, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1979**, *101*, 3946.

0002-7863/84/1506-6440$01.50/0

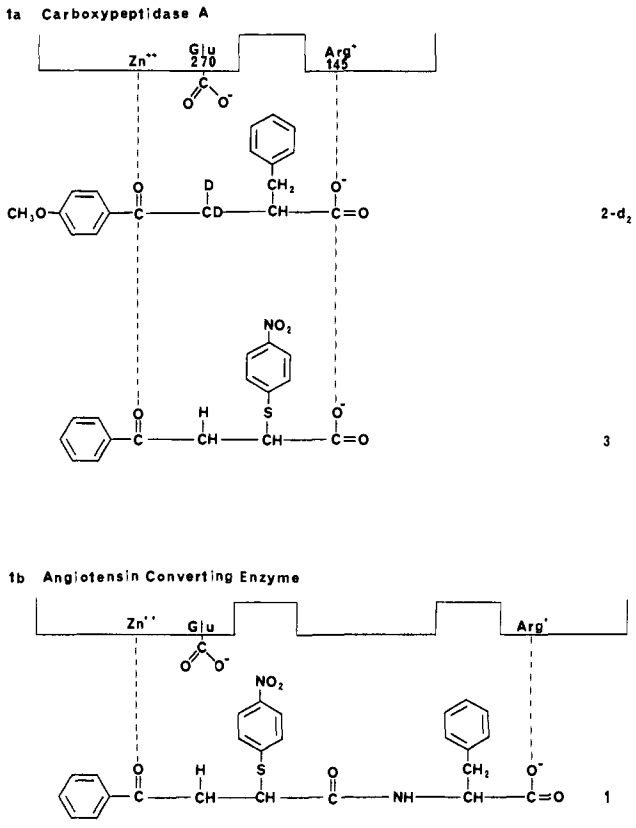


Figure 1. Schematic representations of (a) the active site of CPA and (b) the proposed active site of ACE bound to substrates discussed in the text.

a ketone that has been shown to bind to the active site of CPA in a mode analogous to that of peptide substrates[5-7] and the α,β-elimination of *p*-nitrothiophenol from (+)-3-benzoyl-2-((*p*-nitrophenyl)thio)propionic acid ((+)-**3**).[8]

We studied **1** as a substrate for ACE because the carboxyl-terminal extension of **3** by a phenylalanyl residue would place the ketone and leaving group moieties in positions in the active site of ACE comparable to their positions in the active site of CPA when **3** is used as the CPA substrate (see Figure 1a,b). The substrate **1** was obtained through the following synthetic scheme. (*E*)-3-Benzoylpropenoic acid[9] was treated with DCC and *N*-hydroxysuccinimide at −20 °C in THF for 12 h. The resultant active ester was coupled to L-phenylalanine in dioxane/water (1/1, v/v) containing 2 equiv of Na_2CO_3. Michael addition of *p*-nitrothiophenol to *N*-((*E*)-3-benzoylpropenoyl)-L-phenylalanine in THF at room temperature gave **1**. The two diastereomers of **1** were purified by HPLC on silica gel.[10]

ACE[11] was found to catalyze the elimination of *p*-nitrothiophenol from one diastereomer of **1**, r-**1**, but not from the other, u-**1**. The reaction was followed spectrophotometrically at 416 nm, under anaerobic conditions, in 1.0 mM Veronal buffer, 0.30 M KCl, and 0.10 mM $ZnCl_2$, pH 7.0, with 3% *p*-dioxane at 25.0 °C. Under conditions of $[S]_0 >> [E]_0$, the reaction exhibited simple Michaelis–Menten kinetics, yielding the following kinetic parameters: $k_{cat} = (1.01 \pm 0.07) \times 10^{-2}$ s^{-1}; $K_M = (1.34 \pm 0.12) \times 10^{-4}$ M. The reaction was completely inhibited in the presence of 3×10^{-4} M *N*-[3-(N-benzoyl-L-phenylalanyl)propanoyl]-L-phenylalanine, a potent inhibitor of ACE (IC_{50} = 28 nM).[12] Both r-**1** and u-**1** were found to be competitive inhibitors of the ACE-catalyzed hydrolysis of *N*-(2-furanacroyl)-L-phenylalanylglycylglycine[13] in 0.010 M MOPS, 0.30 M KCl, and 0.10 mM $ZnCl_2$, pH 7.0, with 6% *p*-dioxane at 25.0 °C. The K_I values for r-**1** and u-**1** were determined to be $(8.91 \pm 1.04) \times 10^{-5}$ M and $(8.30 \pm 0.52) \times 10^{-4}$ M, respectively.

To establish the structure of the olefinic product of the elimination reaction of r-**1**, the reaction was allowed to proceed nearly to completion under aerobic conditions. After the precipitated bis(*p*-nitrophenyl) disulfide was removed by filtration, the enzyme was separated from the product by gel filtration through Sephadex G-15. The product fractions were combined, acidified with dilute HCl, and extensively extracted with ethyl acetate. The product, which was separated from the buffer, starting material, and *p*-nitrothiophenol by HPLC on silica gel, was identified as the (*E*)-*N*-(3-benzoylpropenoyl)-L-phenylalanine through its 360-MHz ^{1}H NMR spectrum.[8,14]

To determine the configurations of the two diastereomers of **1**, the r (reactive) and the u (unreactive) isomers were reacted with CPA in 0.010 M MOPS and 0.50 M NaCl, pH 7.5, at 25.0 °C. We expected that CPA would first cleave the amide bond to yield L-phenylalanine and **3** then subsequently catalyze the α,β-elimination of *p*-nitrothiophenol from the (+) isomer but not from the (−) isomer of **3**.[8] We found that CPA catalyzed the formation of *p*-nitrothiophenol from r-**1**, but not from u-**1**, with the concomitant production of (*E*)-3-benzoylpropenoic acid as determined by ^{1}H NMR.[8,14] This shows that the ketone portion of r-**1** must have the same absolute configuration as (+)-**3**. Since the absolute configuration of (+)-**3** has been proposed to be *R*,[8] we conclude that the configuration of r-**1** is *R*,*S* and that of u-**1** is *S*,*S*.[15]

In summary, ACE has been demonstrated to catalyze an elimination reaction with a suitably designed ketone substrate containing a leaving group β to the ketone. The k_{cat}/K_M value of 75.4 ± 4.5 M^{-1} s^{-1} for this catalytic action is comparable to the second-order rate constant of the hydroxide ion catalyzed elimination of *p*-nitrothiophenoxide from r-**1** (k_{OH^-} = 24.7 ± 0.8 M^{-1} s^{-1} in 0.10 M NaCl at 25.0 °C), approximately 6000 times greater than the second-order rate constant of the acetate ion catalyzed elimination reaction (k_{OAc^-} = 0.0114 ± 0.0004 M^{-1} s^{-1} in 0.10 M NaCl at 25.0 °C) and at least 9–10 orders of magnitude greater than the second-order rate constant estimated from the pseudo-first-order rate constant of the water (≃55 M) catalyzed elimination reaction ($k_w < 10^{-6}$ s^{-1} in 0.10 M NaCl at 25.0 °C). The ACE-catalyzed elimination reaction is directly analogous to an α,β-elimination process catalyzed by CPA. The products of the reactions for both enzymes are the respective trans olefins and the rate constants are similar; the k_{cat}/K_M for the ACE-catalyzed elimination reaction is 75.4 ± 4.5 M^{-1} s^{-1}, while that for the CPA-catalyzed reaction is 4.97 ± 0.34 M^{-1} s^{-1}.[8] The difference in the rate constants may be due to the greater lability of **1**. The second-order rate constant for the hydroxide ion catalyzed elimination of *p*-nitrothiophenoxide from **1** is 24.7 ± 0.8 M^{-1} s^{-1} while that for **3** is 4.7 ± 0.1 M^{-1} s^{-1} [8] at 25 °C in 0.10 M NaCl. These results provide strong support for the hypothesis that the catalytic sites of ACE and CPA are very similar[1] and illustrate the catalytic versatility of ACE.

(4) Spratt, T. E.; Sugimoto, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1983**, *105*, 3679.
(5) Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3875.
(6) Lipscomb, W. N.; Hartsuck, J. A.; Reeke, G. N.; Quiocho, F. A.; Bethge, P. A.; Ludwig, M. L.; Steitz, T. A.; Muirhead, H.; Coppola, J. C. *Brookhaven Symp. Biol.* **1968**, *21*, 24.
(7) Rees, D. C.; Hozatko, R. B.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3288.
(8) Nashed, N. T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1981**, *103*, 3611.
(9) Becker, E. I.; Miesse, C. "Organic Syntheses", Wiley: New York, 1955; Collect. Vol. III, p 109.
(10) r-**1**, mp 142–144 °C dec, $[\alpha]^{25}_D$ +62.4° (*c* 16, ethyl acetate); u-**1**, mp 109–112 °C dec, $[\alpha]^{25}_D$ +2.7° (*c* 15, ethyl acetate). The ^{1}H NMR spectra are consistent with the assigned structures.
(11) El-Dorry, H. A.; Bull, H. G.; Iwata, K.; Thornberry, N. A.; Cordes, E. H.; Soffer, R. L. *J. Biol. Chem.* **1982**, *257*, 14128.
(12) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *J. Med. Chem.* **1981**, *24*, 964.
(13) Bunning, P.; Holmquist, B.; Riordan, J. F. *Biochemistry* **1983**, *22*, 103.
(14) Sugiyama, N.; Gasha, T.; Kataoka, H.; Kashima, C. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 971.
(15) The stereochemistry of the reactive diastereomer, r-**1**, corresponds to that of an L,L dipeptide, while the stereochemistry of the unreactive diastereomer, u-**1**, corresponds to that of a D,L dipeptide.

Acknowledgment. We thank Dr. H. Bull of Merck Sharp and Dohme Laboratories for a sample of ACE used in the early phases of this research and for the gift of *N*-(3-phenyl-1-carboxylpropyl)-L-lysyl-L-proline. The support of this research by NIH Grant AM 32539 is gratefully acknowledged.

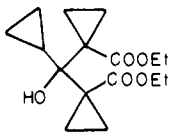
A Method for the Generation of Electronegatively Substituted Cyclopropyl Anions under Preparatively Useful Conditions. Aldol Condensation with Carbonyl Partners[1]

Leo A. Paquette,* Craig Blankenship, and Gregory J. Wells

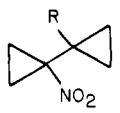
Evans Laboratories, The Ohio State University
Columbus, Ohio 43210

Received June 29, 1984

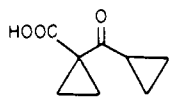
Despite the intrinsically greater acidity of protons attached to three-membered rings,[2] cyclopropanes carrying carbonyl, nitro, and sulfonyl groups exhibit markedly decreased equilibrium acidities relative to suitable acyclic congeners.[3,4] This is because proton abstraction is accompanied by formation of an exocyclic π-bond in order to maximize charge delocalization. The increase in p character with resultant development of planarity leads to a sharp enhancement of ring strain[5] and heightened chemical reactivity. The consequences are dramatic. For example, all attempts to deprotonate ethyl cyclopropanecarboxylate have led to formation of the trimeric self-condensation product **1**.[6,7]



1



2a, R = NO_2
b, R = NO



3

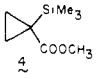
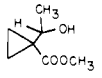
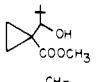
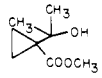
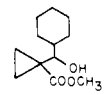
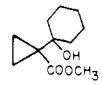
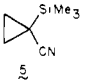
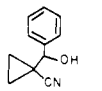
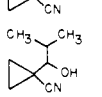
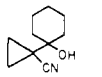
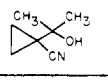
Nitrocyclopropane leads spontaneously in the presence of strong base to **2a** and **2b**.[7,8] The dianion of cyclopropanecarboxylic acid is capable of reaction with select reactive electrophiles.[9-11] Near 50 °C, however, dimerization occurs rapidly to deliver **3**.[11]

As a consequence, simple electronegatively substituted cyclopropanes have not been available for use as basic building blocks in organic synthesis. Although bulky substituents appended to the three-membered ring[12,13] or bonded to the carbonyl group[14] are known to retard the proclivity for dimerization, steric inhibition complicates the question of synthetic utility. We can now report that fluoride ion induced desilylation of simple α-(trimethylsilyl)-substituted cyclopropane derivatives combines a convenient method of carbanion generation with a means for efficient electrophile capture. Since aldol condensations of cyclopropyl anions have not been previously reported,[15] this utilitarian C-C bond-forming process is highlighted here.

Slow addition (2-3 h) of methyl 1-(trimethylsilyl)cyclopropanecarboxylate (**4**)[9] to cold (0 °C) tetrahydrofuran solutions containing dry tetra-*n*-butylammonium fluoride (TBAF, 1.5-2.5 equiv) and the selected aldehyde or ketone led to rapid reaction. After 30 min at 0 °C, workup afforded the respective β-hydroxy esters **6-10**, which were readily purified by chromatography (column or gas phase) without evidence of retroaldol fragmentation. Comparable handling of nitrile **5**[16] proved equally satisfactory (Table I). All new compounds were characterized spectroscopically and by combustion analysis.

Because of the stringent need for dry TBAF and the recognized difficulties in achieving this end result without partial degradation,[17] the effect of the fluoride ion source was briefly examined. Although the more stable benzyltrimethylammonium fluoride (BTAF)[18] is not as soluble in THF at this temperature, its presence served in several instances to improve substantially the yield of

Table I. Desilylation-Aldol Condensation of 4 and 5

substrate	fluoride source	carbonyl reagent	mol equiv	product		yield, %
	TBAF	$CH_3C(=O)H$	5.0		**6**	90
	TBAF	$(CH_3)_3CC(=O)H$	5.0		**7**	51
	TBAF	$CH_3C(=O)CH_3$	5.0		**8**	68
	TBAF		1.2		**9**	27
	TBAF		2.2			49
	TBAF		1.2		**10**	45
	TBAF		2.0			42
	TBAF		2.4		**11**	47
	BTAF		3.0			83
	CsF		2.6			66[a]
	TBAF	$(CH_3)_2CHC(=O)H$	2.2		**12**	13
	BTAF		2.0			60
	CsF		3.0			47[b]
	TBAF		1.5		**13**	43
	BTAF		2.6			43
	BTAF	$CH_3C(=O)CH_3$	4.3		**14**	55

[a] This composite yield includes 7% of isolated 11 and 59% of its *O*-(trimethylsilyl) derivatives. [b] 23% of 12 and 24% of 12-$OSiMe_3$.

(1) Silicon in Organic Synthesis. 27. For Part 26, see: Paquette, L. A.; Wells, G. J.; Wickham, G. *J. Org. Chem.*, in press.
(2) Cram, D. J. "Fundamentals of Carbanion Chemistry", Academic Press: New York, 1965; Chapter 1.
(3) Bordwell, F. G.; Vanier, N. R.; Matthews, W. S.; Hendrickson, J. B.; Skipper, P. L. *J. Am. Chem. Soc.* **1975**, *97*, 7160.
(4) Kinetic acidities determined by base-catalyzed exchange rates have sometimes given evidence of the reverse ordering (Breslow, R. *Acc. Chem. Res.* **1973**, *6*, 393). The difficulties of judging carbanion stabilities from kinetic acidities have been commented on elsewhere (Bordwell, F. G.; Matthews, W. S.; Vanier, N. R. *J. Am. Chem. Soc.* **1975**, *97*, 442).
(5) (a) Haas, H. B.; Shechter, H. *J. Am. Chem. Soc.* **1953**, *75*, 1382. (b) Zimmerman, H.; Thyagarajan, B. S. *Ibid.* **1960**, *82*, 2505.
(6) Pinnick, H. W.; Chang, Y.-H.; Foster, S. C.; Govindan, M. J. *J. Org. Chem.* **1980**, *45*, 4505.
(7) Kai, Y.; Knochel, P.; Kwiatkowski, S.; Dunitz, J. D.; Oth, J. F. M.; Seebach, D.; Kalinowski, H.-O. *Helv. Chim. Acta* **1982**, *65*, 137.
(8) Wagner, H.-U.; Boche, G. *Helv. Chim. Acta* **1983**, *66*, 842.
(9) Ainsworth, C.; Kuo, Y. N. *J. Organomet. Chem.* **1973**, *46*, 73.
(10) Warner, P. M.; De, D. *J. Org. Chem.* **1982**, *47*, 893.
(11) Jahngen, E. G. E.; Phillips, D.; Kobelski, R. J.; Demko, D. M. *J. Org. Chem.* **1983**, *48*, 2472.
(12) Walborsky, H. M.; Hornyak, F. M. *J. Am. Chem. Soc.* **1955**, *77*, 6026.
(13) (a) Böhm, I.; Hirsch, E.; Reissig, H.-U. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 574. (b) Reissig, H.-U.; Böhm, I. *J. Am. Chem. Soc.* **1982**, *104*, 1735. (c) Reichelt, I.; Reissig, H.-U. *Chem. Ber.* **1983**, *116*, 3895; *Liebigs Ann. Chem.* **1984**, 531.
(14) Wemple, J. *Tetrahedron Lett.* **1975**, 3255.
(15) We have previously described a fluoride ion induced desilylation of a 1-dienyl-1-(trimethylsilyl)cyclopropane and condensation with acetone as a key step in a new α-vetispirene synthesis: Yan, T.-H.; Paquette, L. A. *Tetrahedron Lett.* **1982**, 3227.
(16) Wells, G. J.; Yan, T.-H.; Paquette, L. A. *J. Org. Chem.*, in press.
(17) (a) Kawashima, T.; Ishii, T.; Inamoto, N. *Tetrahedron Lett.* **1983**, 739. (b) Sharma, R. K.; Fry, J. L. *J. Org. Chem.* **1983**, *48*, 2112.
(18) Kuwajima, I.; Nakamura, E.; Shimizu, M. *J. Am. Chem. Soc.* **1982**, *104*, 1025.

0002-7863/84/1506-6442$01.50/0