Table II. Isotope Analysis by Mass Spectrometry

		content, %				
			m/e, [M - 3β-AcOH]+		m/e, [M - 3β, 16α-diAcOH]+	
	steroid analyzed	328 ($^{16}O^{16}O$)	330 ($^{16}O^{18}O$)	332 ($^{18}O^{18}O$)	268 (^{16}O)	270 (^{18}O)
I[a]	3-Ac_2 from **1** treatment	0.5	18	81.5	17	83
	3-Ac_2 from **3** treatment	20	80	0	22	78
		348, 350 ($^{16}O^{79}Br$, ^{81}Br)		350, 352 ($^{18}O^{79}Br$, ^{81}Br)		
	1-Ac and 2-Ac recovered	83		17		
					m/e, [M - 3β, 17β-diAcOH]+	
		328 ($^{16}O^{16}O$)	330 ($^{16}O^{18}O$)	332 ($^{18}O^{18}O$)	268 (^{16}O)	270 (^{18}O)
II[b]	8-Ac_2 from **3** treatment	3	30	67	26	74

[a] Bromo ketone **1** or ketol **3** (0.11 mmol) in 0.75 mL of pyridine was treated with NaOH-$H_2{}^{18}O$ [5 mg (0.125 mmol) of NaOH in 0.25 mL (13.89 mmol) of 99.5 atom % [^{18}O]water; theoretical ^{18}O content of the solution 98.6 atom %] for 8 h at room temperature (condition G). The products were washed with water, acetylated, separated by TLC, and subjected to MS as solid. The recovered bromo ketones **1** and **2** were analyzed as a mixture (16α-Br/16β-Br = 1:1.25). [b] Ketol **3** (0.082 mmol) in 3.5 mL of MeOH was treated with NaOH-$H_2{}^{18}O$ (7 mg of NaOH in 0.19 mL of 99.5 atom % [^{18}O]water) for 3 days at room temperature. The product was isolated and analyzed in the same manner described for **3**-acetate.

3β,17β-Dihydroxy-5-androsten-16-one (**8**) obtained by ketol rearrangement of **3** in CH_3OH-NaOH-$H_2{}^{18}O$ showed 74% ^{18}O labeling at the 16-carbonyl and 91% incorporation into the 17β-hydroxyl group.

The results demonstrate that the formation of 16α-hydroxy 17-ketone by alkaline hydrolysis of 16α- or 16β-bromo ketone is by the direct S_N2 displacement of the 16β-bromine (mechanism B, Scheme II) and not by the putative epoxide mechanism (mechanism A, Scheme I). The quantitative incorporation (98% observed compared to 98.6% theoretical) at the 16α position which was not affected by the equilibrium at the 17-carbonyl (17% in the bromo ketone and 79–83% in the ketol) eliminates the epoxide mechanism which requires the transfer of the carbonyl oxygen to the 16α position. The results also show that epimerization of 16-bromo 17-ketone through the enol does not involve an incorporation of hydroxide ion but that ketol rearrangement through the enediol involves participation of hydroxide ion and results in the ^{18}O exchange of both functions. The reaction mechanisms are formulated in Schemes II and III. 16α-Bromo 17-ketone (**1**) and 16β-bromo 17-ketone (**2**) undergo a rapid equilibrium slightly in favor of 16β.[13] The initial product of alkaline hydrolysis should primarily be [16α-^{18}O]**3**, but with further contact with [^{18}O]-hydroxide ion the 17-carbonyl group exchanges its oxygen through an intermediate **6** and gives rise to doubly labeled **3**. The 16-carbonyl of the ketol **8** also undergoes ^{18}O exchange to give doubly labeled **8**. Under a drastic ketol rearrangement condition (Scheme III), the initial ^{18}O labeling at C-17 would occur by formation of the hydrate **6**. The hydrate may be dehydrated to form [17-^{18}O]enediol **7** and rearrange to give the 16-ketone **8** or, alternatively, may be equilibrated back to form 17-^{18}O-labeled 17-ketone **3**. The labeled **3** may be enolized under the condition to **3a** and give rise to the [17-^{18}O]enediol **7** by protonation. After the rearrangement to 17-^{18}O-labeled 16-ketone **8**, similar ^{18}O-exchange reactions are assumed to occur through an intermediate **9** to give the final product labeled at both the 16 and 17 positions.

By this discovery of the controlled condition of hydrolysis, one of our long-time goals to synthesize sodium 3β,16α-dihydroxy-17-oxo-5-androsten-3-yl sulfate (**4**), the major human fetal 19-carbon steroid found in the umbilical cord blood and hitherto unavailable in crystalline salt form,[14] was achieved in one step in 85% yield from readily available bromo ketone **1** by sulfation with pyridine–chlorosulfonic acid complex followed by addition of NaOH solution.[15]

Acknowledgments. This research was supported in part by USPHS Research Grants HD04945 from the National Institute of Child Health and Human Development and RR05716 from the Division of Research Resources. Assistance by Carol Yarborough is greatly appreciated.

(13) Discrepancy between the 16β assignment for substitution by other nucleophiles[3,4] and the 16α configuration for hydroxide substitution presented here should be noted. Irrespective of which of the bromo ketones (**1** or **2**) is used, the product is seemingly in only one configuration in all cases. When **1** and **2** were separately subjected to the reaction with morpholine under the same condition reported previously,[3] the same equilibrium between **1** and **2** as reported in this paper was observed by NMR analysis. Determination of the total structure of the 16-morpholino derivative by X-ray crystallography is under way to further investigate the stereochemistry of nucleophilic substitution.

(14) Triethylamine and ammonia salts of the sulfate **4** were previously synthesized in a relatively low yield by involving five steps from 16α,17α-epoxy-3β-hydroxy-5-pregn-20-one (Wynne, K. N.; Renwick, A. G. C. *Biochem. J.* **1976**, *156*, 419) and three steps from the 16α-bromo ketone **1** (Numazawa, M.; Osawa, Y. *Steroids* **1978**, *32*, 519).

(15) The 16α-bromo 17-ketone **1** (2 g, 4.88 mmol) in 10 mL of dry pyridine was added to 1.5 equiv of pyridine–chlorosulfonic acid complex in 20 mL of pyridine with stirring under ice cooling. After 20 min, the reaction mixture was poured into a chilled 0.1 N NaOH solution (1 L) and allowed to stand at 0 °C for 3 h. The solution was passed through a column of Amberlite XAD-2 (4 × 100 cm). After the solution was washed with H_2O, the absorbed steroid was eluted with MeOH. The eluate was condensed to 20 mL and allowed to stand at 4 °C for 24 h. The solid (1.95 g) precipitated was collected by filtration and recrystallized from MeOH–Et_2O to give **4** (1.63 g, 4.21 mmol) as colorless needles: mp >280 °C; IR (KBr) ν_{max} 3440 (OH), 1738 (C=O), 1235 (SO_4); ^1H NMR [Py-d_5–CD_3OD (1:3)] δ 0.92 (3 H, s, 18-CH_3), 0.97 (3 H, s, 19-CH_3), 4.19–4.70 (2 H, m, 3α-H and 16β-H). Anal. Calcd for $C_{19}H_{27}O_6SNa \cdot H_2O$: C, 53.76; H, 6.89; S, 7.55. Found: C, 53.54; H, 6.83; S, 7.42.

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Contribution of Orbital Alignment to Organic and Enzymatic Reactivity

Sir:

Storm and Koshland[1] have proposed that a 10° misalignment of reactant groups relative to an ideal orientation can cause a massive decrease in rate. If this is true, then enzymes could achieve much of their catalytic ability by optimizing orientational relationships at the active sites ("orbital steering").[1] The evidence supporting the significance of orbital alignment in catalysis rests mainly on the widely differing lactonization rates found among structurally similar hydroxy acids.[2]

(1) D. R. Storm and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **94**, 5815 (1972).

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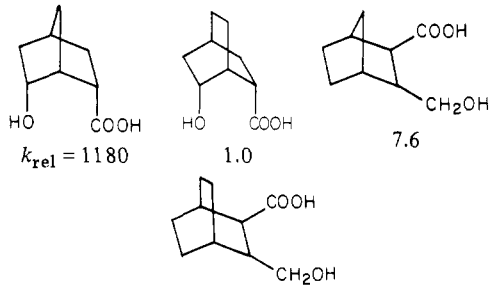

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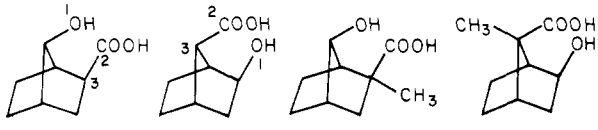
Opposition to the orbital steering concept was voiced on theoretical grounds by several people, particularly Bruice,[3] Jencks,[4] and Lipscomb.[5] Bruice, for example, concluded that orbital steering violates certain molecular force-field and reaction-surface considerations. On the other hand, calculations by Hoare[6] incorporating solvent cage effects suggest that appreciable rate enhancements could indeed arise from orbital steering at active sites. Moreover, X-ray work of Dunitz[7] on compounds with intramolecular N···C=O association points to a "strongly preferred orientation for nucleophilic attack, as has been postulated from analysis of [Koshland's] kinetic data". The fact of the matter is that orbital steering has never been proved or disproved. There is thus a need to test the concept rigorously, and this has motivated the experiments described below.

In order to determine how lactonization rates depend on alignment, one needs a pair of hydroxy acids with the following properties: (1) The two compounds must have measurable but differing angular relationships between their hydroxy and carboxy groups. Since molecular flexibility would lead to uncertainty in this regard, a rigid carbon framework must support the functionalities. (2) Despite the angular differences, the initial OH/COOH distances must be the same. (Koshland's compounds are seen to disobey this stipulation.) (3) The hydroxy groups must possess identical inherent reactivities (e.g., one hydroxy should not be primary and the other tertiary). The same holds true for the carboxy groups. (4) The lactone products must have identical strain energies. Obviously, these constitute a nonrealizable ideal. The ideal can be approximated, however, by a norbornyl system bearing a hydroxy and carboxy group on *nonequivalent* carbons as in I; interchanging the two groups to give II modifies the



I II III IV

alignment while keeping the other parameters relatively constant. Thus, force-field calculations[8] on I and II show that the compounds have similar energies (within 1 kcal/mol) and similar O_1C_2 distances (2.83 and 2.81 Å) but contrasting $O_1C_2C_3$ angles (70° and 80°).[9] This 10° alignment variation should, if orbital steering theory is correct, produce a 10^4 difference in lactonization rates.[10] A comparable rate effect would be predicted for III and IV, whose O_1C_2 distances are both 2.69 Å while their $O_1C_2C_3$ angles are 76° and 85°, respectively.

Table I. Effect of Structure on the IR and Saponification Rates of Lactones and on the Rates of Acid-Catalyzed Lactonization of Hydroxy Acids at 25.0 °C

compd	angle,[a] deg	IR, cm^{-1} (lactone)[b]	k_{OH^-}, M^{-1} min^{-1} (lactone)	k_{H^+}, M^{-1} min^{-1} (hydroxy acid)	k_{H^+} (rel)[c]
I	70	1778	45	0.0083	1
II	80	1771	7	0.01	1.2
III	76	1780	13	0.30	36
IV	85	1768	1	0.18	22

[a] Represents the angle between the hydroxy oxygen, carbonyl carbon, and α-carbon as determined by force-field calculations. [b] IR carbonyl stretching frequency of lactones in $CHCl_3$. [c] Relative rate of acid-catalyzed lactonization of hydroxy acids on the base of the column directly to the left.

Compound I and its lactone were synthesized by dechlorinating the adduct of 1,1-dimethoxy-2,3,4,5-tetrachlorocyclopentadiene and acrylic acid with Na/THF to give 7,7-dimethoxybicyclo-[2.2.1]hept-5-ene-*endo*-2-carboxylic acid.[11] After hydrolysis of the ketal in 5% H_2SO_4, the ketone and double bond were reduced with $NaBH_4/CH_3OH$ and H_2/5% Pd–C, respectively, thereby forming *syn*-7-hydroxybicyclo[2.2.1]heptane-2-*endo*-carboxylic acid.[12] Esterification with CH_2N_2, epimerization in $NaOCH_3/CH_3OH$, and hydrolysis of the ester gave compound I which was lactonized with TsOH/benzene. Hydroxy acid II was prepared by converting 3-bromotricyclene into the corresponding carboxylic acid via the Grignard and then rearranging the acid in CH_2Cl_2 with 75% H_2SO_4.[13,14] Compound III was secured by methylating 7,7-dimethoxybicyclo[2.2.1]hept-5-ene-*endo*-2-carboxylic acid with LDA and CH_3I.[15] Hydrolysis of the product mixture in 5% H_2SO_4 gave the ketone which was reduced with $NaBH_4$, lactonized with acid, and finally subjected to H_2/5% Pd–C. Compound IV was obtained by rearranging the Diels–Alder adduct of cyclopentadiene and methacrylic acid[14,16] in 75% H_2SO_4 at 25 °C. Multiple column chromatographies and/or sublimations of the four lactones gave high-purity materials (although in low overall yields). Mass spectrometry, ^1H and ^{13}C NMR and IR spectroscopy, elemental analysis, and known chemistry supplied the structure proof.

Before discussing orientational effects on cyclization rates of I–IV, we must first assess the ring strain in the corresponding lactones. Table I lists their IR carbonyl stretching frequencies[17] (a parameter which ranges from 1740 cm^{-1} for δ-lactones to 1840 cm^{-1} for β-lactones).[18] The IR frequencies of lactones I and II are seen to differ by 7 cm^{-1} and those of III and IV by 12 cm^{-1}. These differences are regarded as small and certainly not indicative of appreciable ring-strain variations. Saponification rates, another strain-sensitive parameter, are also listed in Table I.[19] Lactone II, with a hydrolysis rate sixfold less than that of I, probably has the more hindered carbonyl. But the effect is trivial relative to the 10^5 rate changes found among diverse aliphatic lactones.[20]

(2) As pointed out by R. M. Moriarty and T. Adams, *J. Am. Chem. Soc.*, **95**, 4070 (1973), three of the hydroxy acids studied by ref 1 had incorrect structures. This problem does not, however, invalidate the arguments in ref 1 because most of the compounds, including the four shown here, were correctly assigned.

(3) T. C. Bruice, A. Brown, and D. O. Harris, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 658 (1971).

(4) M. I. Page and W. P. Jencks, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1678 (1971).

(5) S. Scheiner, W. N. Lipscomb, and D. A. Kleier, *J. Am. Chem. Soc.*, **98**, 4770 (1976).

(6) D. G. Hoare, *Nature (London)*, **236**, 437 (1972).

(7) H. B. Bürgi, J. D. Dunitz, and E. Shefter, *J. Am. Chem. Soc.*, **95**, 5065 (1973).

(8) We thank Professor N. L. Allinger, University of Georgia, for carrying out these calculations. See: N. L. Allinger and S. H. M. Chang, *Tetrahedron*, **33**, 1561 (1977).

(9) In accordance with Storm and Koshland,[1] we selected the angle between the hydroxy oxygen, carbonyl carbon, and α-carbon as an arbitrary measure of alignment. This is not necessarily the angle of reaction in the transition state.

(10) D. R. Storm and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **94**, 5805 (1972).

(11) P. G. Gassman and P. G. Pape, *J. Org. Chem.*, **29**, 160 (1964).

(12) H. C. Brown and J. Muzzio, *J. Am. Chem. Soc.*, **88**, 2811 (1966).

(13) J. D. Roberts, E. R. Trumbull, Jr., W. Bennett, and R. Armstrong, *J. Am. Chem. Soc.*, **72**, 3116 (1950).

(14) S. Beckmann and H. Geiger, *Chem. Ber.*, **94**, 48 (1961).

(15) P. L. Creger, *J. Am. Chem. Soc.*, **89**, 2500 (1967).

(16) J. S. Meek and W. B. Trapp, *J. Am. Chem. Soc.*, **79**, 3909 (1957).

(17) Carbonyl frequencies of the lactones in $CHCl_3$ were secured by interpolating the carbonyl peak between two known polystyrene bands with a Perkin-Elmer 467 spectrophotometer set at a scan mode of 5x.

(18) L. J. Bellamy, "The Intra-red Spectra of Complex Molecules", Wiley, New York, 1958.

(19) Saponification rates (±10%) were determined in 10% dioxane–90% water (v/v) at 25.0 °C by using either an Acta II spectrophotometer (λ 230 nm) or a Radiometer pH stat.

(20) E. T. Kaiser and F. J. Kézdy, *Prog. Bioorg. Chem.*, **4**, 239 (1976).

Thus, we reach the reasonable conclusion that interchanging the hydroxy and carboxy groups of I/II or III/IV does not create within the lactone pairs significant energy differences which could complicate comparisons of the hydroxy acids.

Acid-catalyzed lactonizations of hydroxy acids I, II, III, and IV have relative rates of 1, 1.2, 36, and 22, respectively (Table I).[21] The faster rates found for III and IV probably originate from steric acceleration of the type detected by Bunnett[22] in the lactonization of 3-substituted 2-(hydroxymethyl)benzoic acids. The crucial observation, however, is that I and II lactonize at nearly identical rates despite the 10° difference in alignment between the hydroxy and carboxy groups.[23] Similarly, III and IV display virtually no rate dependence on orientation within the confines of a 9° variation. We have thus demonstrated experimentally for the first time that *an angular displacement of a few degrees is not kinetically significant*.[24] Orbital steering theory, predicated upon a sensitive relationship between rate and small angle changes, seems indefensible in light of our results.

Acknowledgments. This work was supported by the National Science Foundation and the National Institutes of Health.

(21) Second-order rate constants for lactonization, determined spectrophotometrically at 25.0 °C in 0.2–10 N HCl, were extrapolated to zero ionic strength. The resulting uncertainty in the rate constants (perhaps ±50%) has no bearing on the main conclusions of this paper.

(22) J. F. Bunnett and C. F. Hauser, *J. Am. Chem. Soc.*, **87**, 2214 (1965).

(23) It is likely but unproven that hydroxy addition to the carbonyl is the rate-determining step in the lactonizations. Obviously, the observed rate constant is a function of the addition rate whether formation *or* collapse of the tetrahedral intermediate is rate determining.

(24) Alternatively, one might conclude that the angles of 70° and 80° for I and II in Table I bracket an optimal lactonization angle near 75°. This seems unlikely since it would require a *different* optimal angle for III and IV (one near 81°). Furthermore, 75° is smaller than the optimal angle suggested by recent X-ray work: W. B. Schweizer, G. Procter, M. Kaftory, and J. D. Dunitz, *Helv. Chim. Acta*, **61**, 2783 (1978). Unfortunately, it is experimentally impossible to secure more than a two-point plot of k_{obsd} vs. angle while still obeying the stipulations listed in the third paragraph of this article.

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Extended Unsaturated Carbenes.[1] Generation and Trapping of an Alkatetraenylidenecarabene, $(R)_2C{=}C{=}C{=}C{=}C{=}C{:}$[2]

Sir:

To date, the first three members of the homologous unsaturated carbenes (**1**), namely, alkylidenecarbenes[3] (**2**), alkenylidenecarbenes[3] (**3**), and alkadienylidenecarbenes[1] (**4**) have been observed

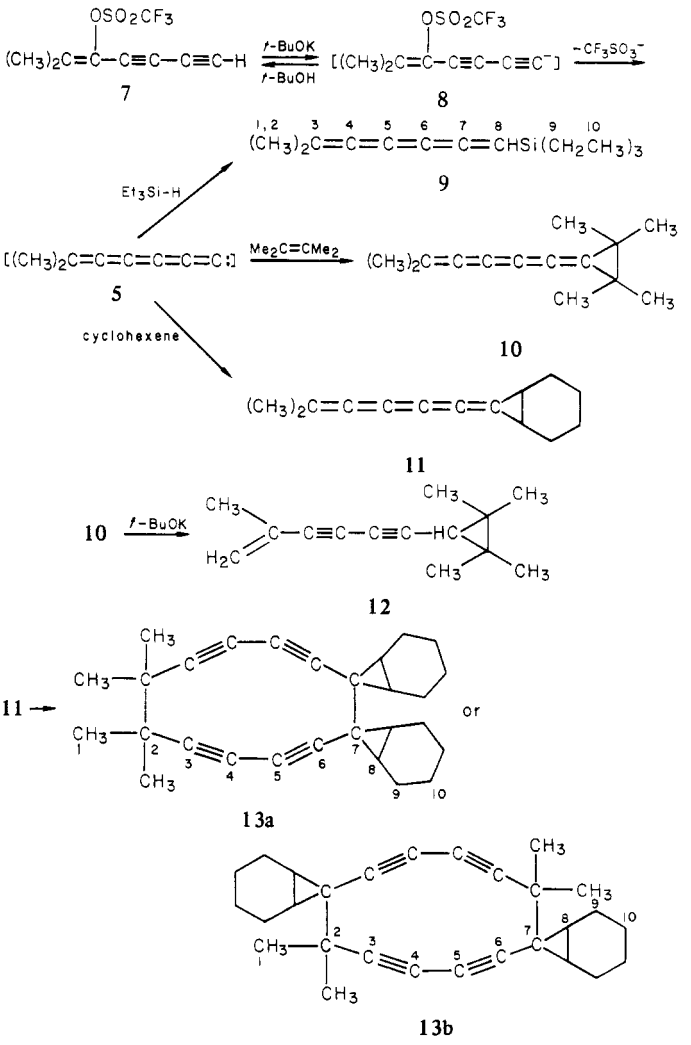
$R_2C{=}(C{=})_nC{:}$ **1**, $n = 0$–∞ $\quad R_2C{=}C{:}$ **2** $\quad R_2C{=}C{=}C{:}$ **3** $\quad R_2C{=}C{=}C{=}C{:}$ **4**

and their properties and chemistry explored. In this communication, we report the generation and trapping of the six-carbon homologue (**1**, $n = 4$), specifically 5-methyl-1,2,3,4-hexatetraenylidenecarbene (**5**), a member of the alkatetraenylidenecarbene (**6**) family.

$(CH_3)_2C{=}C{=}C{=}C{=}C{=}C{:}$ **5** $\quad (R)_2C{=}C{=}C{=}C{=}C{=}C{:}$ **6**

In analogy to the generation[1] of **4** via a γ elimination from 1-ethynylvinyl triflate, carbene **5** was obtained from 1-butadiynylvinyl triflate (**7**) prepared in 70% overall yield in a three-step process from $(CH_3)_2CHCOCl$ and $Me_3SiC{\equiv}C{-}C{\equiv}CSiMe_3$.[4]

Scheme I



Reaction of 2.4 mmol of butadiynylvinyl triflate (**7**) and 3.0 mmol of freshly sublimed *t*-BuOK in a mixture of excess trap (36 mmol) and 1,2-dimethoxyethane at 0 °C over an argon atmosphere proceeded as shown in Scheme I. Rapid deprotonation and equilibrium formation of anion **8** were indicated by reisolation of deuterium-incorporated triflate **7** in deuterated media. Subsequent slow loss of the triflate ion results in carbene **5** that may be trapped by silane or olefins. "Insertion" of carbene **5** into triethylsilane results, after column chromatography on silica gel, in 10% isolated yield of the novel substituted cumulene[5] **9**. Addition to tetramethylethylene gave 41% of enediyne **12**, presumably via base-catalyzed isomerization of the initially formed adduct **10**.[6] Trapping with cyclohexene results in a 31% isolated yield of the cyclyne **13**, the result of either a (symmetry forbidden) cycloaddition or some kind of a free-radical dimerization of initial adduct **11**. Compounds **9**, **12**, and **13** were characterized and identified by spectral means as summarized in Table I. These

(1) Paper 12 in a series on unsaturated carbenes. Paper 11: Stang, P. J.; Fisk, T. E. *J. Am. Chem. Soc.* **1979**, *101*, 4772–4773.

(2) Presented at the 180th National Meeting of the American Chemical Society, San Francisco, August 1980.

(3) Stang, P. J. *Chem. Rev.* **1978**, *78*, 383–405. Hartzler, H. D. In "Carbenes"; Moss, R. A.; Jones, M., Eds.; Wiley-Interscience: New York, 1975; Vol. II, Chapter 2, pp 43–100.

(4) Stang, P. J; Ladika, M. *Synthesis*, in press.

(5) For reviews on cumulenes, see: Fischer, H. In "The Chemistry of Alkenes"; Patai, S., Ed.; Wiley-Interscience: London, 1964; Chapter 13, pp 1025–1160. Murray, M. In "Methoden der Organischen Chemie", Müller, E., Ed.; Georg Thieme Verlag: Stuttgart, 1977; Vol. V/2a, pp 963–1076.

(6) Direct evidence for adduct **10** comes from the observation of a small close second spot on the TLC of product **12** and an additional band at 2050 cm^{-1} in the IR strongly characteristic of cumulenes.[1,5]

(7) Karich, G.; Jochims, J. C. *Chem. Ber.* **1977**, *110*, 2680–2694. Bertsch, K.; Karich, G.; Jochims, J. C. *Ibid.* **1977**, *110*, 3304–3313. Hartzler, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 4527–4531.

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