an essentially planar WC_3 ring lying in the equatorial plane. The substituent carbon atoms (C(2), C(8), C(9)) and Cl(3) also lie in the equatorial plane. The W–C_α bond lengths are *equal* and slightly shorter than the W=C_α double bond distance of 1.942 (9) Å found in W(C-*t*-Bu)(CH-*t*-Bu)(CH_2-*t*-Bu)(dmpe).[12] Carbon–carbon distances within the four-membered ring are intermediate between those expected for pure double and pure single bonds but are slightly closer to the latter. The three most surprising features are the large C_α–C_β–C_α angle (118.9 (8)°), the short W–C_β distance (far shorter than the W–C_α single bond length of 2.258 (8) Å in W(C-*t*-Bu)(CH-*t*-Bu)(CH_2-*t*-Bu)(dmpe)[12]), and the large W–C(1)–C(2) and W–C(7)–C(8) angles (149.9 (7) and 156.6 (7)°, respectively). These results contrast sharply with those for $Rh(C_3Ph_3)Cl_2(PMe_2Ph)_2$[13] and $[Ir(C_3Ph_3)(CO)(Cl)(PMe_3)_2]^{+}$[14] in which little, if any, multiple metal–carbon bond character is present, and the metallacyclic unit is *compressed* along the C_α–$C_{\alpha'}$ direction. (The C_α–$C_{\alpha'}$ distance in W[C-*t*-BuCMeCMe]Cl_3 is 2.525 (12) Å but in $RhCl_2(PMe_2Ph)_2(C_3Ph_3)$[13] it is only 2.156 (6) Å.)

W[C-*t*-BuCMeCMe]Cl_3 reacts with 1 equiv of *tert*-Butyl alcohol in the presence of NEt_3 to give W[C-*t*-BuCMeCMe](O-*t*-Bu)Cl_2.[15] Addition of a second equivalent of LiO-*t*-Bu to W[C-*t*-BuCMeCMe](O-*t*-Bu)Cl_2 produces only half an equivalent of W(CR)(O-*t*-Bu)$_3$ where R is *t*-Bu or Me. Surprisingly, therefore, W[C-*t*-BuCMeCMe](O-*t*-Bu)($OCMe_2CMe_2O$) can be prepared[16] and is stable toward cleavage of the WC_3 ring or formation of the *β-tert*-butyl-substituted isomer. Furthermore, addition of 1 equiv of pinacol to a mixture of W(CEt)(O-*t*-Bu)$_3$ and 3-hexyne yields an analogous complex, $W(C_3Et_3)$(O-*t*-Bu)($OCMe_2CMe_2O$)[17] (Scheme I). The pinacolate complexes will not metathesize 3-heptyne. At least one of the reasons is that $W(C_3Et_3)$(O-*t*-Bu)($OCMe_2CMe_2O$) reacts with an excess of 3-hexyne to give colorless W(η^5-C_5Et_5)(O-*t*-Bu)O_2[18] and tetramethylethylene quantitatively, possibly via intermediate, unstable W(η^5-C_5Et_5)($OCMe_2CMe_2O$)(O-*t*-Bu).[19]

The question that remained was why alkyne metathesis using W(CR)(O-*t*-Bu)$_3$ catalysts eventually ceases? We know that W_2(O-*t*-Bu)$_6$ cannot be formed since it reacts with dialkylacetylenes to give W(CR)(O-*t*-Bu)$_3$.[21] A simpler "active" system consisting of a mixture of W(CEt)(O-*t*-Bu)$_3$ and excess 3-hexyne was allowed to "decompose" to give an as yet unidentified diamagnetic red complex with the empirical composition W(CEt)$_5$(O-*t*-Bu)$_3$ (by ^{1}H and ^{13}C NMR). This red species slowly (days) also decomposed to give colorless W(η^5-C_5Et_5)(O-*t*-Bu)O_2, the only significant diamagnetic product.

We conclude from these results that tungstenacyclobutadiene complexes are the intermediates in the alkyne metathesis reaction and that they can react with additional alkyne to yield cyclopentadienyl complexes. We can also now expect that cleavage of a tungstenacyclobutadiene ring or further reaction to give (ultimately) cyclopentadienyl complexes will likely prove to be very sensitive to the structure of the complex and (especially) the steric and electronic properties of the ligands.

Acknowledgment. We thank the National Science Foundation for support (Grants CHE 80-23448 to M.R.C. and 82-21282 to R.R.S.) and the Dow Central Research Department for a fellowship to S.F.P.

Registry No. [NEt_4][W(C-*t*-Bu)Cl_4], 78251-20-4; W(C-*t*-Bu)(dme)Cl_3, 83542-12-5; W(η^5-C_5Et_4-*t*-Bu)(EtC≡CEt)Cl_2, 83511-01-7; W(η^5-C_5Me_4-*t*-Bu)(Me≡CMe)Cl_2, 83511-02-8; [W(η^5-C_5Et_4-*t*-Bu)Cl_4]$_2$, 83511-03-9; [W(η^5-C_5Me_4-*t*-Bu)Cl_4]$_2$, 83511-04-0; W(C-*t*-BuCEtCEt)Cl_3, 83487-36-9; W(C-*t*-BuCMeCMe)Cl_3, 83487-37-0; W[C-*t*-BuCMeCMe](O-*t*-Bu)Cl_2, 83487-38-1; W[C-*t*-BuCMeCMe](O-*t*-Bu)($OCMe_2CMe_2O$), 83487-39-2; W(CEt)(O-*t*-Bu)$_3$, 82228-88-4; W(C_3Et_3)(O-*t*-Bu)($OCMe_2CMe_2O$), 83487-40-5; W(η^5-C_5Et_5)(O-*t*-Bu)O_2, 83511-05-1; 3-hexyne, 928-49-4; 2-butyne, 503-17-3; *tert*-butyl alcohol, 75-65-0; pinacol, 76-09-5; tetramethylethylene, 563-79-1.

Supplementary Material Available: Listings of positional parameters and observed and calculated structure factors (14 pages). Ordering information is given on any current masthead page.

(12) Churchill, M. R.; Youngs, W. J. *Inorg. Chem.* **1979**, *18*, 2454.
(13) Frisch, P. D.; Khare, G. P. *Inorg. Chem.* **1979**, *18*, 781.
(14) Tuggle, R. M.; Weaver, D. L. *J. Am. Chem. Soc.* **1970**, *92*, 5523.
(15) The *tert*-butoxide ligand is believed to be in the equatorial position in W[C-*t*-BuCMeCMe](O-*t*-Bu)Cl_2. Anal. Calcd for $WC_{13}H_{24}Cl_2O$: C, 34.61; H, 5.36. Found, C, 34.56; H, 5.41. ^{1}H NMR (C_6D_6) δ 3.08 (s, 3, CMe), 2.16 (s, 3, CMe), 1.75 (s, 9, O-*t*-Bu), 1.39 (s, 9, C-*t*-Bu); ^{13}C{^{1}H} NMR (C_6D_6) δ 265.6 and 259.1 (J_{CW} = 93, 116 Hz, C_α), 134.2 (C_β), 87.9 ($OCMe_3$), 42.7 ($CCMe_3$), 31.1 and 29.6 ($OCMe_3$ and $CCMe_3$), 24.3 and 12.4 (CMe).
(16) ^{13}C{^{1}H} NMR (C_6D_6) δ 232.1 and 225.2 (J_{CW} = 122, 134 Hz, C_α), 128.9 (C_β), 88.3 ($O_2C_2Me_4$), 75.9 ($OCMe_3$), 40.4 ($CCMe_3$), 31.9, 31.7 and 27.6 ($OCMe_3$, $CCMe_3$ and $O_2C_2Me_4$, not assignable), 22.0 and 13.0 (CMe). Molecular ion found at 496 in mass spectrum.
(17) ^{13}C{^{1}H} NMR (C_6D_6) δ 226.5 ($C_\alpha CH_2CH_3$), 132.9 ($C_\beta CH_2CH_3$), 75.6 ($OCMe_3$), 31.8 ($OCMe_3$), 29.3 (CCH_2CH_3), 27.6 ($O_2C_2Me_4$), 23.2 (CCH_2CH_3), 16.0 and 12.9 (CCH_2CH_3). Molecular ion found at 496 in mass spectrum.
(18) W(η^5-C_5Et_5)(O-*t*-Bu)O_2. Anal. Calcd for $WC_{19}H_{34}O_3$: C, 46.17; H, 6.93. Found: C, 45.78; H, 6.80. Mass spectrum molecular ion at 494. ^{13}C{^{1}H} NMR (C_6D_6) δ 123.6 (η^5-C_5Et_5), 79.7 ($OCMe_3$), 30.3 ($OCMe_3$), 19.3 (CH_2CH_3), 15.7 (CH_2CH_3).
(19) This type of decomposition of glycolates was proposed as the way in which tungsten(IV) halide complexes converted glycols into olefins.[20]
(20) (a) Sharpless, K. B.; Flood, T. C. *J. Chem. Soc., Chem. Comm.* **1972**, 370. (b) Sharpless, K. B.; Umbreit, M. A.; Nick, M. T.; Flood, T. C. *J. Am. Chem. Soc.* **1972**, *94*, 6538.
(21) Schrock, R. R.; Listemann, M. L.; Sturgeoff, L. G. *J. Am. Chem. Soc.* **1982**, *104*, 4291.

Protiodesilylation Reactions of Simple β-Hydroxysilanes (and α-Hydroxysilanes). Homo-Brook Rearrangements[1]

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β-Hydroxysilanes have been of considerable interest as precursors to geometrically defined olefins and heteroatom-substituted olefins because of their stereospecific olefin-forming β-elimination reactions, and therefore a number of methods to prepare diastereomerically pure β-hydroxysilanes have been developed.[2] We have recently become interested in the possibility that the R_3Si group in a β-hydroxysilane could be replaced by H (protiodesilylation) or by another substituent, thus enabling β-hydroxysilanes to serve as precursors to saturated organic systems. Here we report that simple unactivated β-hydroxysilanes can undergo protiodesilylation when treated with base in aqueous dimethyl sulfoxide (Me_2SO), that unactivated α-hydroxysilanes also undergo protiodesilylation (essentially a Brook rearrangement followed by hydrolysis) under these conditions, and that both reactions take place with complete retention of stereochemistry at carbon.

Cleavage of unactivated carbon–silicon bonds is ordinarily quite difficult. Our earlier work with α,β-dihydroxysilanes[3] suggested to us that base-induced protiodesilylation reactions should be facilitated by the presence of a β hydroxyl as shown in the mechanistic rationale in Scheme I. Simple (unactivated) β-hydroxysilanes normally undergo facile β-elimination reactions when treated with base under aprotic conditions (e.g., KH/THF). (The reaction is considerably accelerated by the presence of anion-

(1) A portion of this work was presented at the 15th Middle Atlantic Regional Meeting of the American Chemical Society, Washington, DC, Jan 1981; Abstr 306.
(2) (a) Hudrlik, P. F.; Peterson, D. *J. Am. Chem. Soc.* **1975**, *97*, 1464–1468. (b) Hudrlik, P. F.; Peterson, D.; Rona, R. J. *J. Org. Chem.* **1975**, *40*, 2263–2264. (c) Utimoto, K.; Obayashi, M.; Nozaki, H. *Ibid.* **1976**, *41*, 2940–2941. (d) Hudrlik, P. F.; Hudrlik, A. M.; Rona, R. J.; Misra, R. N.; Withers, G. P. *J. Am. Chem. Soc.* **1977**, *99*, 1993–1996. (e) Sato, T.; Abe, T.; Kuwajima, I. *Tetrahedron Lett.* **1978**, 259–262. (f) Yamamoto, K.; Tomo, Y.; Suzuki, S. *Ibid.* **1980**, *21*, 2861–2864. (g) Hudrlik, P. F.; Hudrlik, A. M.; Misra, R. N.; Peterson, D.; Withers, G. P.; Kulkarni, A. K. *J. Org. Chem.* **1980**, *45*, 4444–4448. (h) Hudrlik, P. F.; Hudrlik, A. M.; Nagendrappa, G.; Yimenu, T.; Zellers, E. T.; Chin, E. *J. Am. Chem. Soc.* **1980**, *102*, 6894–6896. (i) Davis, A. P.; Hughes, G. J.; Lowndes, P. R.; Robbins, C. M.; Thomas, E. J.; Whitham, G. H. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1934–1941. (j) Hudrlik, P. F.; Kulkarni, A. K. *J. Am. Chem. Soc.* **1981**, *103*, 6251–6253.
(3) (a) Hudrlik, P. F.; Schwartz, R. H.; Kulkarni, A. K. *Tetrahedron Lett.* **1979**, 2233–2236. (b) Hudrlik, P. F.; Nagendrappa, G.; Kulkarni, A. K.; Hudrlik, A. M. *Ibid.* **1979**, 2237–2240.

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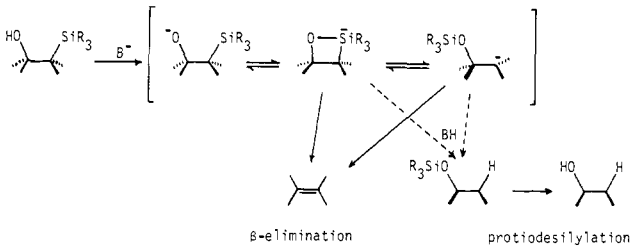
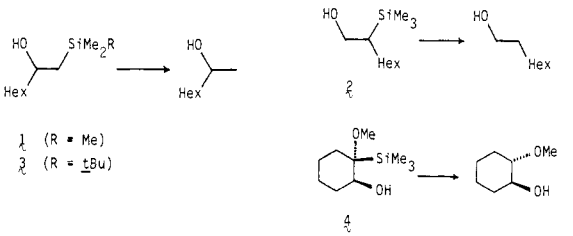
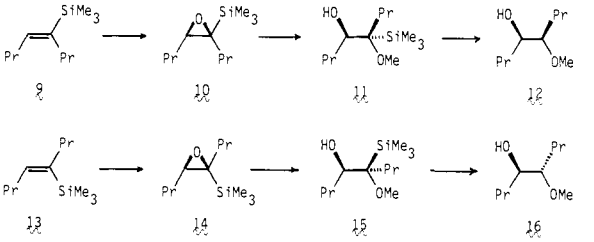
Scheme I



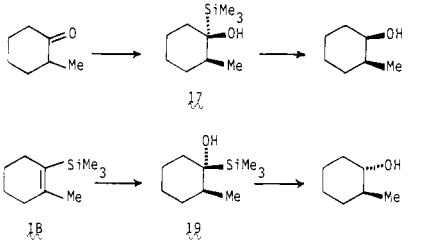
Scheme II



Scheme III



Scheme IV

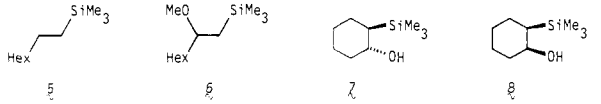


stabilizing groups on the carbon bearing the silicon.) If a proton source were present, the postulated four-membered ring species[4] (or incipient carbanion) might undergo protonation (resulting in protiodesilylation), the facility of the reaction aided by ring strain.

We have prepared compounds **1–4** as substrates to test this concept. When β-hydroxysilane **1**[5] was subjected to the normal β-elimination conditions (KH in THF), 1-octene was formed as expected. No 2-octanol (which would have resulted from protiodesilylation) could be detected. Not surprisingly, a similar experiment in aqueous THF resulted in no reaction. However, treatment of **1** with KO-*t*-Bu in Me_2SO[6] resulted in mixtures of β-elimination and protiodesilylation products in a very fast reaction. Addition of *t*-BuOH or other proton donors to the reaction mixture retarded the reaction and favored formation of the protiodesilylation product. The best conditions we found were with water as the proton source.

Thus, treatment of **1** with a 5% solution of KO-*t*-Bu in 19:1 Me_2SO:H_2O (room temperature, 16 h) yielded only 2-octanol (89% yield), with no observable 1-octene (Scheme II). When β-hydroxysilane **2**[7,8] was similarly treated with KO-*t*-Bu in aqueous Me_2SO, reaction was slower (complete in 2–3 days), presumably because the incipient carbanion is at a secondary center. However, the protiodesilylation product, 1-octanol, was the only product formed (71% yield) with no observable 1-octene. A similar reaction of compound **3**,[2j] with a more hindered silyl group, required 4.5 days at room temperature, but the protiodesilylation product, 2-octanol, was formed cleanly. When the α-methoxy-β-hydroxysilane **4**[3b] was treated with KO-*t*-Bu in aqueous Me_2SO under the conditions used for β-hydroxysilane **1**, *trans*-2-methoxycyclohexanol[9] (75% yield) was formed with no observable 1-methoxycyclohexene. The reaction was shown to be complete after only 1 h, indicating an activating influence of the α-methoxy group.

To establish the role of the β hydroxyl, we prepared[10] compounds **5–8** and treated them with 5% KO-*t*-BU in 19:1 Me_2SO:H_2O. Compounds **5–7** were completely unreactive (4 days at room temperature), while compound **8** was slowly converted to cyclohexanol (30% after 3 days at room temperature). These results demonstrate the importance of the β hydroxyl and support the steric requirement implied by the pathway in Scheme I (i.e., syn alignment of the OH and SiR_3).



The reaction of **4** to form *trans*-2-methoxycyclohexanol suggested that the protiodesilylation reactions take place with retention of configuration. In order to establish the stereochemistry of the reactions conclusively, we prepared the α-methoxy-β-hydroxysilanes **11**[8] and **15**[8] from the corresponding epoxysilanes[13] by treatment with methanol in the presence of CF_3CO_2H (Scheme III). The isomeric purities of epoxides **10**[8] (>99%) and **14**[8] (98%) were determined by VPC, and those of **11** and **15** were assumed to be the same, on the basis of the known stereospecific acid-catalyzed ring openings of α,β-epoxysilanes[2d,i] and the β-elimination reactions of **11** and **15**.

When β-hydroxysilanes **11** and **15** were treated with 5% KO-*t*-Bu in 19:1 Me_2SO:H_2O, the conditions used above, approximately equal amounts of β-elimination and protiodesilylation products were formed in a fast reaction. When the corresponding reactions were carried out in 4:1 Me_2SO:H_2O (24 h), the β-elimination products (4-methoxy-4-octenes) comprised only ~25% of the product mixture, and the protiodesilylation products were easily purified by fractional bulb-to-bulb distillation. From **11** was obtained methoxy alcohol **12**[8] (51% yield) in >99% isomeric purity; from **15** was obtained methoxy alcohol **16**[8] (58% yield) in 97.5% isomeric purity. These results indicate that these protiodesilylation reactions take place with complete stereospecific retention of configuration at carbon.

A number of fluoride-induced protiodesilylations of β-hydroxy*alkenyl*silanes[14] and of some β-hydroxy-α-alkoxysilanes[15] have been reported. The former reactions have been shown to proceed with retention of configuration,[14b–d] and a pathway involving the well-known affinity of fluoride for silicon was sug-

(4) Trindle, C.; Hwang, J.-T.; Carey, F. A. *Ibid.* **1973**, *38*, 2664–2669.
(5) Carey, F. A.; Toler, J. R. *J. Org. Chem.* **1976**, *41*, 1966–1971.
(6) Tetramethylsilane is cleaved by KO-*t*-Bu in Me_2SO: Price, C. C.; Sowa, J. R. *J. Org. Chem.* **1967**, *32*, 4126–4127.
(7) Compound **2** was prepared from vinyltrimethylsilane by treatment with pentyllithium followed by paraformaldehyde (followed by reduction with $LiAlH_4$; 68% yield).
(8) (a) Satisfactory IR and NMR spectra were obtained. (b) A satisfactory high-resolution mass spectrum was obtained.
(9) Winstein, S.; Henderson, R. B. *J. Am. Chem. Soc.* **1943**, *65*, 2196–2200.
(10) **5**[11] was prepared from 1-octene by hydrosilylation; **6**[8] was prepared from **1** by treatment with MeLi followed by MeI; **7**[2i,12] and **8**[2i,12] were prepared as described in ref 12b.
(11) Nozakura, S. *Bull. Chem. Soc. Jpn.* **1956**, *29*, 784–789; *Chem. Abstr.* **1957**, *51*, 8086e.
(12) (a) Musker, W. K.; Larson, G. L. *Tetrahedron Lett.* **1968**, 3481–3483. (b) Lambert, J. B.; Finzel, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 2020–2022.
(13) The precursor vinylsilane **9** was prepared from 4-octyne by hydrosilylation (Yamamoto, K.; Nunokawa, O.; Tsuji, J. *Synthesis* **1977**, 721–722); vinylsilane **13** was prepared from the corresponding vinyl halide by treatment with Na and Me_3SiCl (Hudrlik, P. F.; Kulkarni, A. K.; Jain, S.; Hudrlik, A. M. *Tetrahedron*, in press).
(14) (a) Chan, T. H.; Mychajlowskij, W. *Tetrahedron Lett.* **1974**, 3479–3482. (b) Snider, B. B.; Karras, M.; Conn, R. S. E. *J. Am. Chem. Soc.* **1978**, *100*, 4624–4626. (c) Snider, B. B.; Conn, R. S. E.; Karras, M. *Tetrahedron Lett.* **1979**, 1679–1682. (d) Fristad, W. E.; Bailey, T. R.; Paquette, L. A. *J. Org. Chem.* **1980**, *45*, 3028–3037.
(15) Magnus, P.; Roy, G. *J. Chem. Soc., Chem. Commun.* **1979**, 822–823; *Organometallics* **1982**, *1*, 553–559.

gested.[14a,16] Simple β-hydroxy*alkyl*silanes were found to be unreactive to these conditions.[14a,17] The work described here suggests that the fluoride ion induced reactions may proceed according to Scheme I, with fluoride ion acting as a base to generate alkoxide.

According to the mechanistic rationale of Scheme I, the base-induced protiodesilylation of a β-hydroxysilane might be viewed as a homo-Brook rearrangement (followed by hydrolysis of the resulting silyl ether). The Brook rearrangement,[18] the conversion of an α-hydroxysilane to a silyl ether with a catalytic amount of base (typically Na/K alloy or an amine), is normally very slow unless the carbon bearing the silicon is substituted with an anion-stabilizing group (e.g., phenyl).[19] We were therefore interested in determining whether protiodesilylations of simple unactivated α-hydroxysilanes could be accomplished under our conditions.

α-Hydroxysilane **17**[8] was prepared by addition of Me_3SiLi to 2-methylcyclohexanone (54% yield) (Scheme IV). The stereochemistry was initially assigned by assuming predominant attack of the silyl reagent trans to the methyl group. When **17** was treated with 5% KO-*t*-Bu in 19:1 Me_2SO:H_2O at room temperature, reaction was complete in 1 h, giving the protiodesilylation product, 2-methylcyclohexanol, in 72% yield. The stereochemistry of the product (97% cis) suggested that the protiodesilylation took place with predominant or complete retention of configuration. The Brook rearrangements of *α-phenyl-α*-hydroxysilanes under quite different conditions (Na/K alloy in ether, or with amines in various solvents) have been shown to take place with *inversion* of configuration at carbon.[18] Therefore an additional experiment was undertaken to confirm the stereochemistry in our reaction.

The isomeric α-hydroxysilane **19**[8a] was prepared from vinylsilane **18**[20] by treatment with BH_3·THF followed by H_2O_2/NaOH[21] (89% crude yield). When **19** was treated with 5% KO-*t*-Bu in 19:1 Me_2SO:H_2O (1 h), *trans*-2-methylcyclohexanol (>99% trans) was formed in 69% yield. These results indicate that these protiodesilylation reactions of α-hydroxysilanes, like those of the β-hydroxysilanes discussed above, take place with stereospecific retention of configuration at carbon.[22]

Acknowledgment. We thank the National Science Foundation (Grant No. CHE-7926181) for support of this work.

Registry No. 1, 58541-11-0; **2**, 83511-14-2; **3**, 79705-13-8; **4**, 61580-73-2; **5**, 3429-76-3; **6**, 83511-15-3; **7**, 20584-41-2; **8**, 20584-43-4; **9**, 64997-08-6; **10**, 83511-16-4; **11**, 83511-17-5; **12**, 83511-18-6; **13**, 83511-19-7; **14**, 83511-20-0; **15**, 83511-21-1; **16**, 83511-22-2; **17**, 83511-23-3; **18**, 55860-92-9; **19**, 83511-24-4; Me_3SiLi, 18000-27-6; 1-octanol, 111-87-5; *trans*-2-methoxycyclohexanol, 7429-40-5; cyclohexanol, 108-93-0; 2-methylcyclohexanone, 583-60-8; *cis*-2-methylcyclohexanol, 7443-70-1; *trans*-2-methylcyclohexanol, 7443-52-9; 2-octanol, 123-96-6.

(16) A few examples of base-induced protiodesilylations (*without* fluoride ion) of β-hydroxyalkenylsilanes (Ruden, R. A., personal communication, and ref 14d) and β-hydroxy-α-alkoxysilanes (ref 3b, and footnote 18 therein) were known. Fluoride-induced protiodesilylations of epoxysilanes (Chan, T. H.; Lau, P. W. K.; Li, M. P. *Tetrahedron Lett.* **1976**, 2667–2670) and base-induced protiodesilylations of α-silyl esters having a β-OH group[2f] are known and have been found to take place with retention of stereochemistry at carbon; for these reactions, the β-hydroxyl group is presumably not necessary.

(17) In accord with these observations, we found that β-hydroxysilane **1** was inert to CsF in acetonitrile at 80 °C and that **1** and **4** were inert to CsF in Me_2SO at room temperature, reacting very slowly at 80 °C to give mixtures of products resulting from elimination and protiodesilylation.

(18) Brook, A. G. *Acc. Chem. Res.* **1974**, 7, 77–84, and references cited therein.

(19) A few examples are known where no anion-stabilizing groups are present: Brook, A. G.; Warner, C. M.; McGriskin, M. E. *J. Am. Chem. Soc.* **1959**, *81*, 981–983. Brook, A. G.; Iachia, B. *Ibid.* **1961**, *83*, 827–831. See also: Manuel, G.; Mazerolles, P.; Gril, J. *J. Organomet. Chem.* **1976**, *122*, 335–343.

(20) Eaborn, C.; Jackson, R. A.; Pearce, R. *J. Chem. Soc., Perkin Trans. 1* **1975**, 470–474.

(21) de Jesus, M.; Rosario, O.; Larson, G. L. *J. Organomet. Chem.* **1977**, *132*, 301–320.

(22) While this manuscript was in preparation, we learned that Wilson has carried out an aliphatic Brook rearrangement (by using KH in HMPA) which occurred with retention of configuration at carbon: Wilson, S. R.; Hague, M. S.; Misra, R. N. *J. Org. Chem.* **1982**, *47*, 747–748.

Cryoenzymology of Proteases: NMR Detection of a Productive Thioacyl Derivative of Papain at Subzero Temperature†

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It is generally accepted[3] that the hydrolysis of peptides and amides catalyzed by the thiol protease papain can be represented by a minimal three-step pathway[4] as in Scheme I. The reactions are controlled by a thiolate ion (cys-25) at the active site of papain in a sequence involving binding, acylation, and deacylation. Structural evidence for the thioacyl intermediate **1** is limited to electronic absorption data in which acylation of papain by *N*-cinnamoylimidazole gave rise to a UV spectrum red shifted by 20 nm relative to the model, (*S*)-*trans*-cinnamoylcysteine.[5] More direct evidence bearing on this point comes from the observation[6] of a species assigned to a dithioester structure with λ_{max} 313 nm (cf. dithioacetate, λ_{max} 305 nm) in the papain-catalyzed hydrolysis of methyl thionohippurate. As a result of the development in our laboratory of reliable protocols for the observation of covalently bound intermediates of enzymes and their substrates by ^{13}C NMR spectroscopy at subzero temperatures, we can now report on the direct observation of a productive thioacyl intermediate prepared from papain and [^{13}C=O]-*N*-benzoylimidazole by adapting the techniques of cryoenzymology[7] to a ^{13}C NMR experiment. To monitor the extent of benzoylation of papain and the rate of deacylation, we used the high reactivity of 2,2′-dipyridyl disulfide[8] toward the thiolate ion of cys-25 in papain at pH 3.8 to titrate free thiolate in aliquots of incubation mixtures corresponding to the time course NMR experiment, using 1–2 mM solutions of papain and a large excess (~20 mM) of substrate in formate buffer. After many trials the following conditions gave completely reproducible results in which a suitable concentration (~1 mM) and $t_{1/2}$ (>30 min) of the intermediate were achieved. Papain (1.7 mM) in formate buffer (0.1 M, pH 4.1) was mixed with 90% enriched [^{13}C=O]-*N*-benzoylimidazole[9,10] (23.6 mM) in 25% Me_2SO-d_6 at 0 °C then rapidly cooled to −6 °C. An aliquot of this solution was kept at −6 °C and active site thiol concentration measured throughout the NMR time course.

At 0 °C papain was 96% acylated (thiolate assay) while at −6 °C the half-life of deacylation is 96 min. The time course of the CMR experiment is shown in Figure 1 a–f. The broad (25 ± 5 Hz) resonance at 196.0 ppm is assigned to the thiobenzoate (**2**, Scheme II) of papain labeled at ^{13}C=O (cf. phenylthiobenzoate, δ 189.1;[11] *n*-butyl thioacetate, 194.1[12]). The rate of disappearance of the signal at 196.0 ppm (allowing for experimental error due

† Dedicated to the memory of the late Professor F. Šorm.

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