Communications to the Editor

Antibody Catalyzed Terpenoid Cyclization

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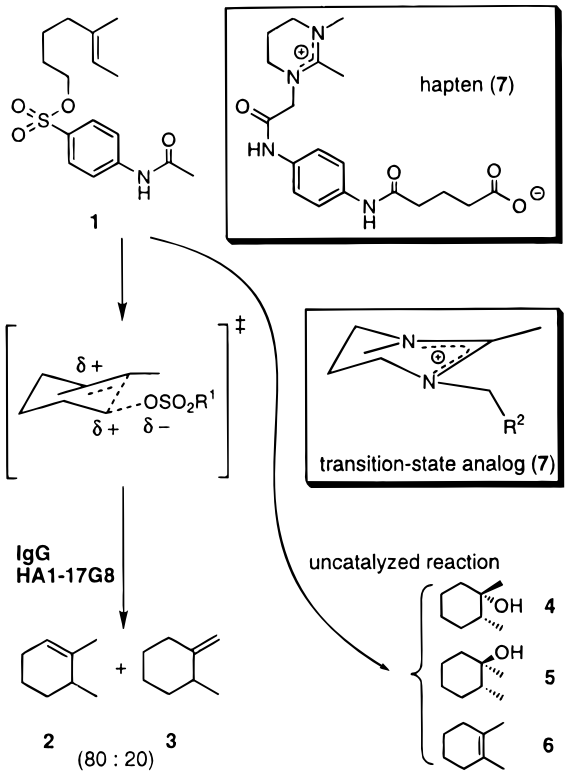
Received December 29, 1995

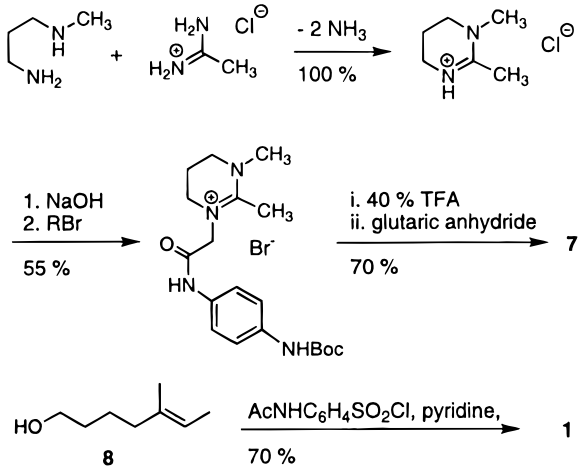
We report the isolation and characterization of an antibody that catalyzes a terpene-like electrophilic cyclization under neutral conditions.[1,2] The reaction mechanism for this cationic cyclization proceeds via the transition state (Scheme 1) in which a chair conformation of the substrate organizes the electron orbital approach of the interacting atoms. The HOMO of the π-bond donates into the incipient carbocation yielding cyclic species. On the basis of these mechanistic principles,[3] hapten **7** was designed as a transition state analog. A notable feature of **7** is a pseudo-halfchair conformation where the positive charge is delocalized over three atoms, thereby closely mimicking the electronic situation at the transition state.

Scheme 2 shows the synthesis of hapten **7**[4] as well as of substrate **1**.[5] The hydrocarbon unit of **1** mirrors the first two isoprene portions of 2,3-oxidosqualene (**9**) except that two methyl substituents at the site of the initial cationic center were omitted to exclude potential elimination reactions (Scheme 3). The arylsulfonate leaving group[6] mimics the role of the opening of the protonated epoxide oxygen of **9** starting the cyclization cascade seen in the lanosterol biosynthesis. Twenty-four monoclonal antibodies against **7** were obtained by standard methods,[7] three of which (1F8, 12E8, 17G8) showed rate enhancement over the uncatalyzed rate ($k_{uncat} = 6 \times 10^{-6}$ s^{-1}) in an initial screen for sulfonic acid release from substrate **1** under biphasic conditions.[8] These same conditions[9] were also employed to study the most active antibody (17G8) for cyclic hydrocarbon formation. With IgG 17G8, only the 6-membered carbocyclic products **2** and **3** were formed.[10,11] Interestingly, for the uncatalyzed reaction again only cyclic material was

Scheme 1. Antibody-Catalyzed Monoterpenoid Formation and Comparison of Spacial Views of the Presumed Transition State and Hapten/Transition State Analog **7** (R^1 = $C_6H_4NHCOCH_3$; R^2 = $CONHC_6H_4NHCO(CH_2)_3COO^-$)



Scheme 2. Synthesis of Hapten **7** and Substrate **1** (RBr = $(H_3C)_3CCONHC_6H_4NHCOCH_2Br$)



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(1) It should be emphasized that *mono*terpene biosynthesis generally follows a different cyclization mechanism than the following, generating an allylic cation from geranyl pyrophosphate (**9**) subsequently rearranging to the linalyl cation which adds to the second double bond in a Markovnikoff fashion (Scheme 3).

(2) For an entry to monoterpene cyclases isolated from plants, see: (a) Rajaonarivony, J.; Gershenzon, J.; Croteau, R. *Arch. Biochem. Biophys.* **1992**, *296*, 49−57. For a review on terpene cyclase isolation, see: (b) Croteau, R.; Cane, D. E. In *Methods in Enzymology*; Law, J. H., Rilling, H. C., Eds.; Academic Press: New York, 1985; pp 383−405.

(3) (a) Ruzicka, L.; Eschenmoser, A.; Heusser, H. *Experientia* **1953**, *9*, 357−367. (b) Stork, G.; Burgstahler, A. W. *J. Am. Chem. Soc.* **1955**, *77*, 5068−5077. (c) Eschenmoser, A.; Ruzicka, L.; Jeger, O.; Arigoni, D. *Helv. Chim. Acta* **1955**, *38*, 1890−1905.

(4) (a) Brown, D. J.; Evans, R. F. *J. Chem. Soc.* **1962**, 527−533. (b) *Ibid*. **1962**, 4039−4045.

(5) Julia, M.; Descoins, C.; Baillarge, M.; Jacquet, B.; Uguen, D.; Groeger, F. A. *Tetrahedron* **1975**, *31*, 1737−1744.

(6) (a) Li, T.; Janda, K. D.; Ashley, J. A.; Lerner, R. A. *Science* **1994**, *264*, 1289−1293. (b) Li, T.; Hilton, S.; Janda, K. D. *J. Am. Chem. Soc.* **1995**, *117*, 3308−3309. (c) Johnson, W. S.; Bailey, D. M.; Owyang, R.; Bell, R. A.; Jaques, B.; Crandall, J. K. *J. Am. Chem. Soc.* **1964**, *86*, 1959−1966.

(7) Köhler, G.; Milstein, C. *Nature* **1975**, *256*, 495−497.

(8) Hexane/chloroform/phosphate buffer (83:2:15%), 50 mM, pH 7.1; shaken in a sealed Teflon tube for defined time units.

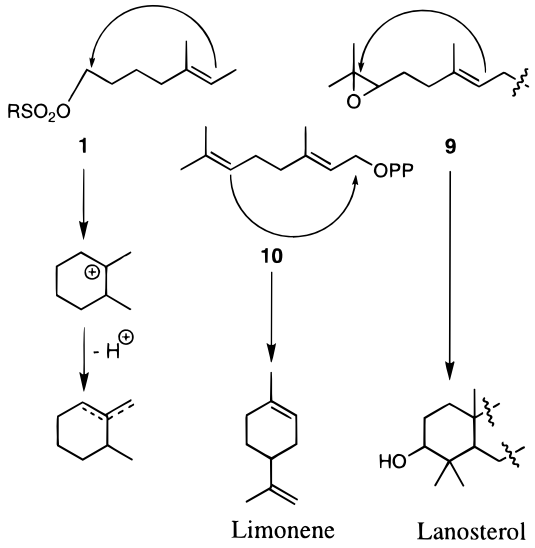
(9) See also: Ashley, J. A.; Janda, K. D. *J. Org. Chem.* **1992**, *57*, 6691−6693.

(10) Carbocycles **2** and **3** were formed with an ee of 24 and 37%, respectively. Without crystallographic data for IgG 17G8, it is difficult to speculate on the origins of this result. However, hapten **7** displays a planar geometry in the region where the stereocenter is being generated, and thus, we would not expect appreciable optical induction to be enforced in this region of the antibody's combining site.

(11) It should be noted that natural terpene cyclase reactions also display multiple product formation (see: ref 2a, pp 52 and 56).

S0002-7863(95)04316-2 CCC: $12.00

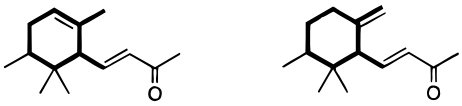
Scheme 3. Comparison of Antibody-Catalyzed Reaction with 2,3-Oxidosqualene Cyclization to Lanosterol and Geranyl Pyrophosphate Cyclization to Limonene



observed;[12] however, now the two diastereomeric tertiary alcohols **4** and **5** and a small amount of 1,2-dimethylcyclohexene (**6**) were formed. These findings suggest that the antibody binding pocket is more than a simple hydrophobic and/or proton cleft, since product distribution between catalyzed and uncatalyzed reactions share no congruency except for being cyclic. Thus, antibody 17G8 rerouted the naturally occuring cationic cyclization reaction.

Steady state kinetics were performed by following the release of the sulfonic acid which partitioned into the aqueous phase. The antibody exhibited Michaelis−Menten saturation behavior and a Lineweaver−Burk plot of velocity versus substrate concentration gave $k_{cat} = 4.2 \times 10^{-4}$ s^{-1}, $K_M = 35$ μM, and $k_{cat}/K_M = 12$ mol^{-1} s^{-1}.[13] Addition of 2 equiv of **7** to the antibody completely inhibited the catalytic activity,[14] strongly suggesting that the two combining sites of the immunoglobulin are responsible for the catalytic activity. The IC_{50} value of the inhibitor was approximately 1.2 μM. Exhaustive dialysis of the 17G8-**7** complex did not restore the catalytic activity of 17G8, suggesting that **7** was either covalently bound or that tight-binding inhibition[15] occurred.

Antibody 17G8 shows remarkable product specificity in view of the possible product diversity of cationic cyclization and no loss of activity even when being recharged with fresh substrate.[16] In addition, IgG 17G8 appears to completely exclude water from its catalytic site thereby producing only olefinic products. In light of the exclusive formation of structures **2** and **3**, it is tempting to speculate that an antibody amino acid residue is located on the "upper part" of the substrate that abstracts a proton from the evolving terminal carbocation. Interestingly, products **2** and **3** match the core structures of α- and γ-irone, respectively,[17] that are natural terpene constituents of violet-derived perfumes. The action of antibody 17G8 and the irone-



α-irone **γ-irone**

forming plant enzyme on their substrates is comparable in respect to the deprotonation of the terminal carbocation of the cyclization sequence yielding only two of the three possible regioisomers. Structural and mechanistic studies of antibody 17G8 may illuminate the heretofore hidden protein−substrate interactions[18] involved in the enzyme-controlled terpene cyclizations of nature.

Acknowledgment. This Research was supported by a stipend from the Scientific Committee of the NATO through the Deutscher Akademischer Austauschdienst (J.H.), NIH Grant GM-43858 (K.D.J.), and the Skaggs Institute for Chemical Biology. We thank D. M. Kubitz and M. Wolfe for technical assistance with the hybridoma work.

Supporting Information Available: A listing of the preparation of KLH- and BSA-immunoconjugates, the preparation of monoclonal antibodies, the gas capillary chromatography conditions, the initial screening diagram, a dose response plot for the IC_{50} determination, and experimental details/characterization data for the preparation of **1** and **7** (17 pages). See any current masthead page for ordering and Internet access instructions.

JA954316W

(12) The complete absence of any linear product like **8**, arising from simple hydrolysis of substrate **1** may be attributed to our biphasic assay conditions as well as the strength of the anchimeric effect of the double bond found in **1** (see: ref 6c).

(13) Most natural monoterpene, sesquiterpene, and diterpene cyclases exhibit k_{cat} values in the range of 0.01−0.35 s^{-1} and K_M values of 0.5−3.0 μM for their respective pyrophosphate substrates (ref 2a, p 53).

(14) Since a fully functional IgG molecule was used, this corresponds to a 1:1 ratio of inhibitor to binding site. The same finding was also observed with antibodies 1F8 and 12E8.

(15) Williams, J. W.; Morrison, J. F. In *Methods in Enzymology*; Purich, D. L., Ed.; Academic Press: New York, 1979; pp 437−467.

(16) A 24 h assay consisting of 16 μM IgG 17G8 and 600 μM **1** resulted in 95% solvolysis of **1**. No activity loss was observed after replacing the hexane phase and recharging the system with fresh substrate.

(17) Compounds **2**, **3**, and **6** are analogous in their double-bond regiochemistry to α-, γ- (the fragrance principle of violets), and β-irones, respectively. See: Jaenicke, L.; Marner, F.-J. *Pure Appl. Chem.* **1990**, *62*, 1365−1368 and research on biosynthesis cyclization mechanisms therein.

(18) See this article and references therein: Corey, E. J.; Virgil, S. C.; Cheng, H.; Baker, C. H.; Matsuda, S. P.; Singh, V.; Sarshar, S. *J. Am. Chem. Soc.* **1995**, *117*, 11819−11820.