necessary linker group required of a hapten for antibody production, and this aspect of the work will be reported separately.

Acknowledgment. We thank the U.S. Army Medical Research and Development Command for support of the work under Contract No. DAMD17-88-C-8021. Valuable discussions with Dr. David Lenz, Biochemical Pharmacology Branch of the USAMRICD, and Stuart Levy of this department.

Supplementary Material Available: Experimental details for the preparation of **1a**, **2**, **3**, and **5** and spectral data for these compounds and **1b-f** as well as details of a stability test for **1b** (9 pages). Ordering information is given on any current masthead page.

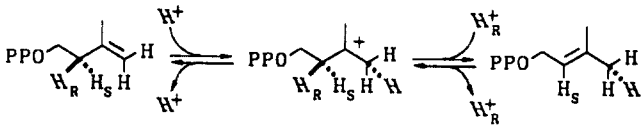
Hydrogen Exchange during the Enzyme-Catalyzed Isomerization of Isopentenyl Diphosphate and Dimethylallyl Diphosphate

Ian P. Street, Dale J. Christensen, and C. Dale Poulter*

Department of Chemistry, University of Utah
Salt Lake City, Utah 84112

Received July 9, 1990

Isopentenyl diphosphate:dimethylallyl diphosphate isomerase (EC 5.3.3.2) catalyzes the interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) by allylic rearrangement of a hydrogen.[1,2] This is an essential activation step in isoprene biosynthesis which provides the electrophilic allylic diphosphate needed as a primer for all of the subsequent prenyl transfer reactions in the pathway.[3] Studies with electronically deactivated alternate substrates and transition-state/reactive-intermediate analogues indicate that the reaction proceeds by addition and elimination of a proton through a carbocationic intermediate or a transition state with considerable carbocationic character.[4,5]



In an elegant series of experiments, Cornforth and co-workers[6-8] determined that isomerization of IPP to DMAPP is antarafacial, with a proton added to the *re* face of the C(3)-C(4) double bond and the *pro-R* (H_R) hydrogen removed from C(2). Thus, the new methyl in DMAPP is in the *E* position. As a consequence of the isomerization, the hydrogens of the (*E*)-methyl group in DMAPP and those at C(4) and the *pro-R* locus of C(2) in IPP exchange with protons from water.[9] However, there are several examples in the literature where label from C(2) of mevalonate is not stereospecifically incorporated into the (*E*)-methyl of the dimethylallyl unit in isoprenoids.[10-22] Croteau and Loomis[19] and

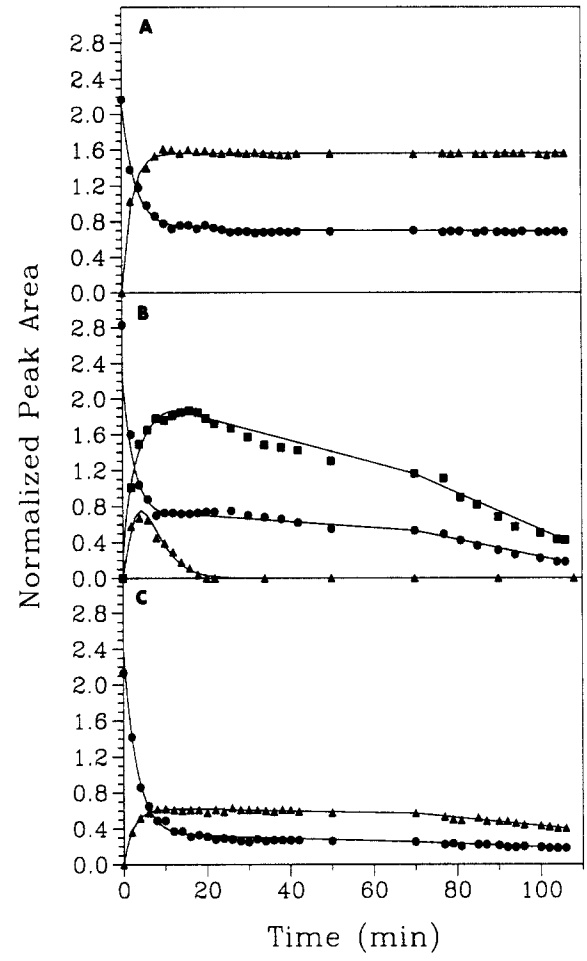


Figure 1. Exchange of hydrogens in IPP and DMAPP with D_2O. Spectra were recorded at 2-min intervals (16 transients/spectrum at 500 MHz) in 0.5 mL of buffer containing 50 mM K_2HPO_4, 200 mM KCl, 10 mM $MgCl_2$, and 0.5 mM dithiothreitol, pH 7.0. Samples were equilibrated at 24 °C before addition of enzyme, and the probe temperature was maintained at 24 °C. Chemical shifts were referenced to internal *tert*-butyl alcohol (1 mM), and integrals for each resonance were normalized to the *tert*-butyl alcohol standard. Part A: hydrogens at C(1) in IPP (●) and DMAPP (▲). Part B: methyl hydrogens in IPP (●), (*E*)-DMAPP (▲), and (*Z*)-DMAPP (■). Part C: hydrogens at C(2) in IPP (●) and DMAPP (▲).

Shibuya et al.[22] suggested that isomerization of IPP to DMAPP was a likely step for scrambling to occur in the systems studied. Koyama et al.[23,24] discovered that there was a substantial loss of stereoselectivity for pig liver isomerase when ethyl and ethylidene derivatives of IPP and DMAPP were used as alternate substrates. With an ample supply of IPP isomerase available from our efforts

* Author to whom correspondence should be addressed.

(1) Satterwhite, D. M. *Methods Enzymol.* **1985**, *110*, 92-99.
(2) Shah, D. H.; Cleland, W. W.; Porter, J. W. *J. Biol. Chem.* **1965**, *240*, 1946-1951.
(3) Poulter, C. D.; Rilling, H. C. *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; pp 209-219.
(4) Reardon, J. E.; Abeles, R. H. *Biochemistry* **1986**, *25*, 5609-5616.
(5) Muehlbacher, M.; Poulter, C. D. *Biochemistry* **1988**, *27*, 7315-7328.
(6) Cornforth, J. W.; Popjak, G.; Yengoyan, L. *J. Biol. Chem.* **1966**, *241*, 277-281.
(7) Cornforth, J. W.; Popjak, G. *Methods Enzymol.* **1969**, *15*, 359-371.
(8) Clifford, K.; Cornforth, J. W.; Mallaby, R.; Phillips, G. T. *J. Chem. Soc., Chem. Commun.* **1971**, 1599-1600.
(9) Poulter, C. D.; Rilling, H. C. *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; pp 162-209.
(10) Yeowell, D. A.; Schmidt, H. *Experimentia* **1964**, *29*, 250-252.
(11) McCapra, F.; Money, A.; Scott, A. I.; Wright, I. G. *Chem. Commun.* **1965**, 537-538.
(12) Goggel, H.; Arigoni, D. *Chem. Commun.* **1965**, 538-539.
(13) Battersby, A. R.; Brown, R. T.; Kapil, I. S.; Plunkett, A. O.; Taylor, J. B. *Chem. Commun.* **1966**, 46-47.
(14) Hüni, J. E. S.; Hiltebrand, H.; Schmidt, H.; Gröger, D.; Johne, S.; Mothes, K. *Experientia* **1966**, *22*, 656.
(15) Coscia, C. J.; Guarnaccia, R. *J. Am. Chem. Soc.* **1967**, *89*, 1280-1281.
(16) Inouye, H.; Veda, S.; Nakamura, Y. *Tetrahedron Lett.* **1967**, 3221-3226.
(17) Biollaz, M.; Arigoni, D. *Chem. Commun.* **1969**, 633-634.
(18) Corbella, A.; Gariboldi, P.; Jommi, G.; Scolastico, C. *J. Chem. Soc. D* **1969**, 634-635.
(19) Croteau, R.; Loomis, W. D. *Phytochemistry* **1972**, *11*, 1055-1066.
(20) Floss, H. G. *Tetrahedron* **1976**, *32*, 873-897.
(21) Foss, H. G.; Anderson, J. A. *The Biosynthesis of Mycotoxins*; Steyn, P. S., Ed.; Academic Press: New York, 1980; pp 17-67.
(22) Shibuya, M.; Chou, H.-M.; Fountoulakis, M.; Hassam, S.; Kim, S.-U.; Kobyashi, K.; Otsuka, H.; Rogalska, E.; Cassady, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1990**, *112*, 297-304.
(23) Koyama, T.; Ogura, K.; Seto, S. *J. Biol. Chem.* **1973**, *248*, 8043-8051.
(24) Koyama, T.; Katsuki, Y.; Ogura, K. *Bioorg. Chem.* **1983**, *12*, 58-70.

0002-7863/90/1512-8577$02.50/0

to overproduce the enzyme,[25] we reinvestigated the stereochemistry of reaction for the normal substrates and have determined the complete time course of hydrogen exchange for IPP and DMAPP with yeast isomerase.

Exchange of hydrogens in IPP and DMAPP with water was detected by following the loss of signal in ^{1}H NMR spectra upon incubation of IPP with isomerase in D_2O. For the experiment summarized in Figure 1, IPP (10 mM) was incubated with 0.1 mg (sp act. 19 μmol min^{-1} mg^{-1}) of recombinant *Saccharomyces cerevisiae* IPP isomerase. Upon addition of enzyme, a rapid conversion of IPP to DMAPP was seen as evidenced by the appearance of resonances for DMAPP[26] at δ 5.44 (C(2)), 4.45 (C(1)), 1.75 ((*E*)-methyl), and 1.71 ((*Z*)-methyl) ppm and a concomitant decrease in the intensity of resonances for IPP[27] at δ 4.86 (C(4)), 4.06 (C(1)), 2.40 (C(2)), and 1.77 (methyl) ppm.

Figure 1A shows the time course of the reaction for the C(1) methylene protons. A rapid change in the concentrations of IPP and DMAPP was observed upon addition of enzyme. Equilibrium was reached within 10 min, and the intensities of the peaks remained constant thereafter. The C(1) hydrogens did not exchange with D_2O during the course of the experiment, and $K_{eq}^{24°} = 2.2$ was calculated from the relative intensities of the C(1) resonances. The time course for signals in the methyl region is shown in Figure 1B. During the first few minutes, the intensity of the resonance for the methyl group in IPP decreased rapidly as signals for the (*E*)-methyl and (*Z*)-methyl groups in DMAPP increased. However, rapid exchange of the (*E*)-methyl protons in DMAPP with D_2O quickly reduced the intensity of that signal until, after 20 min, it was no longer detected. The intensity of the resonance for the (*Z*)-methyl group of DMAPP reached a maximal value after 10 min. However, in contrast to the C(1) methylene protons, signals for the methyl group in IPP and the (*Z*)-methyl in DMAPP slowly decreased after equilibrium was reached, indicating exchange of these protons with D_2O. Addition of more enzyme after 70 min increased the rate of the slow exchange reaction by approximately 4-fold. Similar behavior was observed for the protons attached to C(2) of IPP and DMAPP, as shown in Figure 1C. In this case, the C(2) signal in IPP decreased rapidly, with a concomitant increase for C(2) in DMAPP, as equilibrium was obtained. Between 20 and 70 min, the intensities remained essentially constant. However, upon addition of more IPP isomerase at 70 min, the C(2) signals in both compounds decreased as well! After continued incubation for 19 h, only the two resonances at δ 4.06 and 4.45 ppm for the C(1) methylene protons of IPP and DMAPP, respectively, remained in the ^{1}H NMR spectrum. Both signals were simple doublets with a 6.9-Hz coupling to phosphorus. A ^{2}H spectrum of IPP and DMAPP from this sample gave resonances for all positions, except for C(1), with intensities consistent with a 1:2.2 mixture of isomers.

Rates for the three exchange processes seen in Figure 1 were estimated from initial slopes. The first exchange resulted in the rapid loss of intensity[28] for the *pro-R* C(2) and C(4) hydrogens of IPP and the rapid rise and fall of the (*Z*)-methyl signal in DMAPP. These exchanges are coincident with establishment of the equilibrium. The second exchange resulted in loss of intensity for the methyl group in IPP and the (*E*)-methyl in DMAPP at a rate of approximately 2% that of the first process. The slowest exchange (0.5% of the fast process) resulted in loss of the *pro-S* and olefinic signals at C(2) in IPP and DMAPP, respectively.

For an enzyme, yeast IPP isomerase catalyzes interconversion of IPP and DMAPP with a rather low degree of stereochemical fidelity. At least three kinetically distinguishable exchange processes were detected. The most facile was consistent with the accepted stereochemistry for isomerization, an antarafacial protonation of the *re* face of the double bond in IPP and elimination of the H_R proton at C(2). If one assumes that the catalytic residues are optimally positioned for addition/elimination by the preferred stereochemistry, the slower exchanges most likely arise from other conformers of the enzyme–substrate complex. It is interesting to note that elimination of *either* C(2) proton will give DMAPP or of *any* methyl proton will give IPP from the tertiary carbocationic species. Thus, there is no stereochemical imperative for the reaction catalyzed by IPP isomerase and there are no consequences in vivo for low stereoselectivity beyond adding to the consternation of chemists by scrambling isotope in artificially labeled precursors.

Acknowledgment. This project was supported by NIH Grant GM 25521.

(25) Street, I. P.; Poulter, C. D. *Biochemistry*, in press.

(26) ^{1}H NMR spectrum (D_2O): δ 1.71 (s, 3 H, (*E*)-methyl), 1.75 (s, 3 H, (*Z*)-methyl), 4.45 (dd, $J_{H,H}$ = 6.9 Hz, $J_{H,P}$ = 6.9 Hz, 2 H, H at C(1)), and 5.44 ppm (t, $J_{H,H}$ = 6.9 Hz, 1 H, H at C(2)).

(27) ^{1}H NMR spectrum (D_2O): δ 1.77 (s, 3 H, methyl), 2.40 (t, $J_{H,H}$ = 6.6 Hz, 2 H, H at C(2)), 4.06 (td, $J_{H,H}$ = 6.6 Hz, $J_{H,P}$ = 3.3 Hz, 2 H, H at C(1)), and 4.86 ppm (s, 2 H, H at C(4)).

(28) k_{cat} = 9 s^{-1} for yeast IPP isomerase.[25]

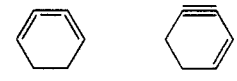
1,2,3-Cyclohexatriene and Cyclohexen-3-yne: Two New Highly Strained C_6H_6 Isomers

William C. Shakespeare and Richard P. Johnson*

Department of Chemistry, University of New Hampshire Durham, New Hampshire 03824

Received July 16, 1990

Enormous effort has been devoted toward the exploration of structural limitations in organic compounds.[1] Cyclic butatrienes[2] and cyclic enynes[3] are two fundamental classes of strained hydrocarbons for which limiting ring sizes are as yet unknown. We describe here evidence for the synthesis and trapping of 1,2,3-cyclohexatriene (**1**) and cyclohexen-3-yne (**2**). These highly strained and reactive substances redefine known limitations for their respective homologous series. Additionally, **1** and **2** are of interest as new benzene isomers.



1 2

Less strained, larger ring homologues of **1** and **2** are known. Szeimies and co-workers have reported the successful trapping of 1,2,3-cycloheptatriene; however, attempts to prepare **1** were unsuccessful.[4] We have described the preparation and isolation of 1,2,3-cyclononatriene.[5] Strained cyclic enynes have been generated by a variety of methods; the smallest previously reported homologue is cyclohepten-3-yne.[3]

A new and presumably general route to cyclic butatrienes is exemplified by the present synthesis of **1** (Scheme I). Kinetic deprotonation of enone **4**[6] with lithium diisopropylamide (LDA), followed by addition of *N*-phenyltrifluoromethanesulfonimide,[7] gave 1,3-diene **5** in 50% yield.[8] Reaction of **5** with CsF in DMSO

(1) For recent reviews on hydrocarbon structural limitations, see: (a) Wiberg, K. *Chem. Rev.* **1989**, *89*, 975. (b) Warner, P. M. *Chem. Rev.* **1989**, *89*, 1067. (c) Borden, W. T. *Chem. Rev.* **1989**, *89*, 1095. (d) Billups, W. E.; Haley, M. M.; Lee, G.-A. *Chem. Rev.* **1989**, *89*, 1147. (e) Johnson, R. P. In *Molecular Structures and Energetics*; Leibman, J. F., Greenberg, A., Ed: VCH Publishers: Deerfield Beach, FL, 1986; Vol. 3, pp 85–140.

(2) Review: Johnson, R. P. *Chem. Rev.* **1989**, *89*, 1111.

(3) Review: Meier, H.; Harold, N.; Molz, T.; Bissinger, H. J.; Kolshorn, H.; Zountsas, J. *Tetrahedron* **1986**, *42*, 1711.

(4) Schülter, A. D.; Belzner, J.; Heywang, U.; Szeimies, G. *Tetrahedron Lett.* **1983**, *24*, 891.

(5) Angus, R. O., Jr.; Johnson, R. P. *J. Org. Chem.* **1984**, *49*, 2880.

(6) Shih, C.; Fritzen, E. L.; Swenton, J. S. *J. Org. Chem.* **1980**, *45*, 4462.

(7) McMurry, J. G.; Scott, W. J. *Tetrahedron Lett.* **1983**, *24*, 979.

0002-7863/90/1512-8578$02.50/0