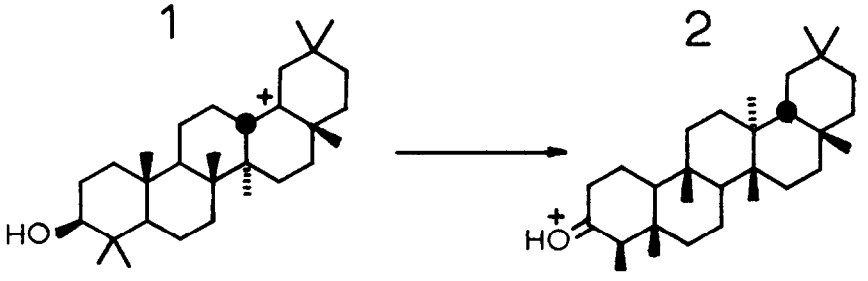
Tetrahedron Letters No.7, pp. 821-825, 1968. Pergamon Press. Printed in Great Britain.

THE ENZYMATIC REQUIREMENT FOR "CONCERTED" BACKBONE REARRANGEMENT

H. W. Whitlock, Jr. and M. C. Smith
Department of Chemistry, University of Wisconsin
Madison, Wisconsin

(Received in USA 25 October 1967)

One of the important features of the biogenetic isoprene rule as formulated by Eschenmoser *et al*[1] is the appearance of backbone rearrangements in the post-cyclization structural modification of squalene, e.g. 1 → 2. The correctness of this postulate has been demonstrated in a number of cases of



biosynthesis of pentacyclic triterpenes[2] and tetracyclic triterpenes and steroids,[2,3,4,5,6] and it now seems safe to assume that in general this type of rearrangement does in fact involve a sequential series of 1,2 shifts of hydrogens and methyl groups. The recent appearance of reports[7,8] describing enzyme preparations capable of the cyclization and subsequent rearrangement of 2,3-oxidosqualene and its congeners raises the following question. What necessary role must one ascribe to an enzyme associated with backbone rearrangements of the type 1 → 2 such that there are no detectable olefinic intermediates between initial carbonium ion and final olefin? Studies of model reactions reported here suggest a simple answer to this question.

As models we chose the acid catalyzed conversion of glutin-5(10)-ene (3) and friedel-3-ene (4) to olean-12-ene (5).[9,10] The mass spectral cracking pattern[11] of 5 enables one to partially dissect the deuterium content of 5-d_x into that present in rings A and B (6) and that in rings C, D and E (7·). Our interpretation of the interconversions of 3, 4 and 5, as summarized in figure 1, is based on the following observations.

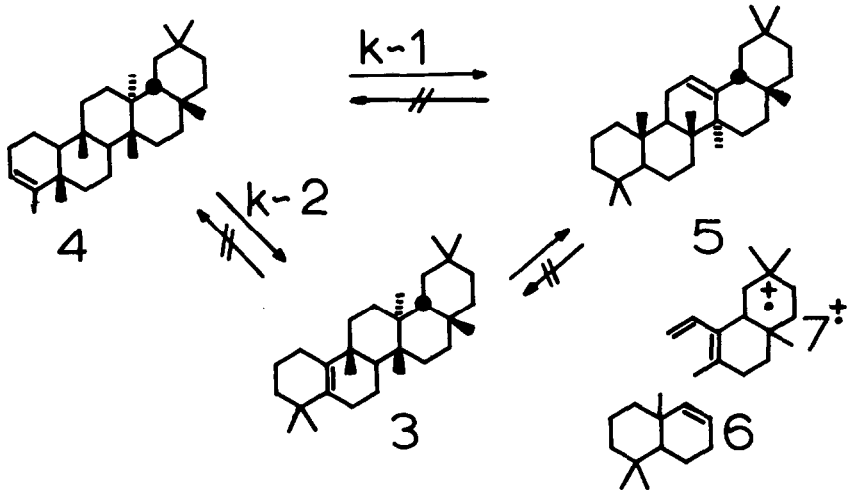


figure 1

1) A solution of 1.91 g of 3 in a mixture of 44 ml trifluoroacetic acid-d_1 and 1120 ml of dry chloroform was allowed to stand at 25° for 7 min. Chromatography of the product mixture on silver nitrate-impregnated silica gel afforded 1.87 g (97.8% recovery) of 3 and 5.7 mg of pure 5.[12] Deuterium analyses, table 1, indicate that, even ignoring $^1H/^2H$ exchange in the starting material, the reaction 3 → 5 must proceed with <u>initial</u> formation of at least[13] 92% 5-d_1.

Table 1[a]

ION	$3^{+\cdot}$	$5^{+\cdot}$	$7^{+\cdot}$
% d_0	96.5	--	91.4
d_1	3.5	84.5	8.6
d_2	--	14.0	--
d_3	--	1.5	--
d_4	--	--	--

a) The deuterium content of 3 and 5 was derived from low voltage spectra; that of $7^{+\cdot}$ from 70 e.v. The percentages given are estimated to be ± 2%.

2) Allowing the above reaction to proceed to 40% completion gives a mixture of 3-d and 5-d whose isotopic composition can be satisfactorily explained by the pseudo-first order kinetic scheme with $k_e/k_i = 8$.

$$\begin{array}{ccc} 3\text{-}d_0 & \xrightarrow{k_e} & 3\text{-}d_1 \\ {\scriptstyle k_i}\downarrow & & \downarrow{\scriptstyle k_i} \\ 5\text{-}d_1 & & 5\text{-}d_2 \end{array}$$

3) The reaction of friedel-3-ene (4) under the above conditions was interrupted at the state of 91% recovered 4, 2.2% 3-d_x and 1.2% of 5. Mass spectrometry, with corrections applied as above, showed initially formed 5 to be 99% d_1. The isolated 3 was 94% d_1 and 6% d_2.

4) Olean-12-ene (5) is not indefinitely stable under these conditions.[14] Those products that still contain the $\Delta^{12,13}$ unsaturation, although substantially deuterated, appear to carry little of the deuterium in rings A and B however.

From the deuterium content of the products at early time it follows that no other olefins are important contributors to the rearrangement of 3 to 5, but that 3 is an intermediate 2/3 of the time for 4 → 5.

In answer to the initially posed question, then, one does not have to envisage any special role for the enzymes associated with the naturally occurring reaction other than an ability to hold the reacting system in a relatively non-basic environment. The biosynthesis of any particular carbon skeleton is then determined by the relative rates at which the members of the set of rapidly interconverting carbonium ions[15] are deprotonated.

Acknowledgment. Financial support by The Eli Lilly Company and The National Institutes of Health is acknowledged. In particular we wish to thank the Armstrong Cork Company for generous supplies of cork wax.

References

(1) A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, Helv. Chim. Acta, 38, 1890 (1955).

(2) H. H. Rees, E. I. Mercer and T. W. Goodman, Biochem. J., 99, 726 (1966).

(3) J. W. Cornforth, R. H. Cornforth, G. Donninger, G. Popjak, Y. Shimizu, S. Ichii, E. Forchielli and E. Caspi, J. Am. Chem. Soc., 87, 3224 (1965).

(4) J. W. Cornforth, R. H. Cornforth, A. Pelter, M. G. Horning and G. Popjak, Tetrahedron, 5, 311 (1959).

(5) R. K. Maudgal, T. T. Tchen and K. Bloch, J. Am. Chem. Soc., 80, 2589 (1958).

(6) T. T. Tchen and K. Bloch, J. Biol. Chem., 226, 931 (1957).

(7) E. E. van Tamelen, K. B. Sharpless, J. D. Willet, R. B. Clayton and A. L. Burlingame, J. Am. Chem. Soc., 89, 3921 (1967).

(8) P. D. G. Dean, P. R. Ortiz de Montellano, K. Bloch and E. J. Corey, J. Biol. Chem., 242, 3014 (1967).

(9) J. L. Courtney, R. M. Gascoigne and A. Z. Szumer, J. Chem. Soc., 881 (1958).

(10) E. J. Corey and J. J. Ursprung, J. Am. Chem. Soc., 78, 5041 (1956).

(11) J. Karliner and C. Djerassi, J. Org. Chem., 31, 1945 (1966).

(12) Identification of the olefins was by means of melting points, mass spectra and optical rotation. No evidence was found for the formation of trifluoroacetates, isotopic purity (96% d_1) of recovered acid was established, and the results reported are demonstrably repeatable. Mass spectra were determined on a modified CEC-103C spectrometer (250° glass inlet) and agree with controls run on an MS-902 instrument (direct inlet).

(13) This is arrived at by consideration of the manner of fragmentation of $\underset{\sim}{5}^{+\cdot}$-$d_x$. Representing $\underset{\sim}{5}$ as 6/7, $\underset{\sim}{5}$-d_2 may be comprised of $\underset{\sim}{6}$-d_1/$\underset{\sim}{7}$-d_1 or $\underset{\sim}{6}$-d_2/$\underset{\sim}{7}$-d_0, the latter representing "dirty" rearrangement. $\underset{\sim}{5}$-d_3 may be $\underset{\sim}{6}$-d_3/$\underset{\sim}{7}$-d_0 or $\underset{\sim}{6}$-d_2/$\underset{\sim}{7}$-d_1. The presence of only 8.6% of $\underset{\sim}{7}$-d_1 places an upper limit of (1.5 + 7.1) $\underset{\sim}{7}$-d_1 or 6.9% $\underset{\sim}{6}$-d_2/$\underset{\sim}{7}$-d_0. Therefore a minimum estimate of 92% $\underset{\sim}{6}$-d_1/$\underset{\sim}{7}$-d_x for $\underset{\sim}{5}$ is arrived at.

(14) G. Brownlie, M. B. E. Fayez, F. S. Spring, R. Stevenson and W. S. Strachan, J. Chem. Soc., 1377 (1956).

(15) Or their biological equivalent such as sulfonium ions.