Tetrahedron Letters No. 25, pp. 1207-1211, 1962. Pergamon Press Ltd.
Printed in Great Britain.

FREE RADICAL ADDITIONS TO SQUALENE[1]

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(Received 27 August 1962)

SQUALENE is cyclized to a number of other triterpenes, including the precursor of lanosterol and thus of cholesterol, by oxidative hydroxylation of a terminal double bond.[2] It is usually suggested that this is a two-electron process, involving "OH+", but it has seemed to us that many of the requirements of the reaction would be better served by a free radical process. According to this view, the cyclization would be initiated by the addition of a species such as OH· to the double bond; transfer of the second electron would occur only after cyclization, e.g. by hydroxylation of the product radical. Free radical cyclization of suitable dienes is well known, most commonly in the so-called "cyclopolymerization" reactions,[3] although a simple addition-cyclization of bromotrichloromethane and diallyl ether has also been reported.[4]

Squalene has been shown to incorporate two moles of thioacetic acid in a radical addition, but the product was not characterized.[5]

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[1] Support of this work by the National Institute of Health is gratefully acknowledged.
[2] Cf. D. Goodman, J. Biol. Chem. 236, 2429 (1961).
[3] G. Butler and R. Angelo, J. Am. Chem. Soc. 79, 3128 (1957), and later work.
[4] W. Friedlander, 133rd Meeting American Chemical Society, 1958.
[5] J. Cunneen, J. Chem. Soc. 134 (1947).

We have reinvestigated this reaction in order to determine whether radical addition proceeds with cyclization, or whether the previous results only reflected inertness of the four remaining double bonds of a simple adduct. Using n.m.r. to characterize the total product, we have added 0.23, 0.98, and 1.71 moles of thioacetic acid to squalene with irradiation, and find in all three cases that the ration of vinyl hydrogen to the methyl hydrogen of the thioacetyl group is that expected if no cyclization has occurred. These results are confirmed by titration of the double bonds in the product. Since addition across a terminal double bond produces two methyls on saturated carbon, while internal addition produces only one, the ratio of methyl hydrogens on saturated carbon (9.10 τ) to thioacetyl methyl groups (7.73 τ) was used to determine the relative reactivities of the two types of double bonds. From the three reactions the ratios of terminal to internal rate constants are respectively 13.04, 12.34, and 12.89 (statistically corrected). This high selectivity for attack on the terminal bonds is similar to van Tamelen's observations on epoxidation in polar solvents,[6] although our system is non-polar.

The results with squalene are consistent with data on the addition of thioacetic acid to geranyl acetate, a model for the first diene system of squalene, and to diallyl ether. In both these cases addition went cleanly without cyclization, as was demonstrated by analysis, double bond titration, and n.m.r. spectra, and in the case of geranyl acetate it was again found that addition occurred only to the double bond bearing the gem-dimethyl group. These results suggest that in the

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[6] E. van Tamelen and T. Curphey, *Tetrahedron Letters* No. 3, 121 (1962)

free radical cyclizations which are known a stepwise process is involved, formation of a simple radical with subsequent attack on the nearby double bond. This is consistent with our finding in competitive rate studies that diallyl ether is slightly less reactive in the bromotrichloromethane addition than is ethyl allyl ether (on statistical correction); the second double bond does not assist in the addition to the first one, even though the overall product is cyclized.

It was hoped that a free radical hydroxylation of squalene would not suffer from the rapid chain transfer observed with thioacetic acid. Volman has reported that hydroxyl radical, produced by irradiation of hydrogen peroxide, will add to allyl alcohol to afford mainly dimeric products.[7] Under similar conditions we find that OH· will add to 3,3-dimethylallyl alcohol, again affording chiefly dimeric products.[8] This suggested that with this system hydroxyl radical addition to squalene would not be followed by rapid chain transfer or termination, so even a non-concerted cyclization should be possible. However, addition without cyclization was the only observed reaction. In a typical run 20 ml. of squalene, 30 ml. of 30% H_2O_2, and 270 ml. of t-butanol was irradiated for 72 hours with a 450 watt high pressure mercury lamp in a Vycor well; the solution was maintained at 25° under a nitrogen atmosphere. The excess H_2O_2 was decomposed with PbO and after filtration the solvent was removed in vacuo(In an identical control reaction without irradiation the squalene was recovered unchanged). Chromatography on alumina afforded

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[7] D. Volman and J. Chen, J. Am. Chem. Soc. 81, 4141 (1959).

[8] E. Barrett, Ph.D. thesis, Columbia University, 1962.

23% recovered squalene, eluted with hexane, and the remainder was then eluted with chloroform-methanol. By sublimation at 80°/0.1 mm., 20% by weight of 2,5-dihydroxy-2,5-dimethylhexane could be isolated; 80% of the remaining material distilled at 220°/ 0.03 mm., and this was further characterized.

By titration the molecule showed an average of four double bonds and 1.5 glycol units. Only 0.6-0.9 moles of acetone are formed on periodate cleavage, however, suggesting an almost random scattering of glycol units through squalene, in contrast to the thioacetic acid results. That no appreciable amount of cyclization has occurred is confirmed by the results of n.m.r. spectra. Although the methyl groups in squalene are found near 8.4 τ, addition to the double bond shifts them upfield. If the bond has been saturated by glycol formation the methyl groups will now be near 8.88 τ, as we find them in a sample of squalene polyglycol prepared with peracetic acid, but with cyclization the effect of nearby hydroxyl will no longer be present and the methyls will be near 9.10 τ.[9] In our product the methyl groups are found at 8.43 τ (70%) and 8.78-8.88 τ (30%). There is no absorption above 9.0 τ, so no detectable cyclization (less than 5%) has occurred. The n.m.r. spectrum of the crude reaction product, before any fractionation whatever, similarly shows methyl groups only below 9.0 τ.

The formation of dihydroxydimethylhexane from t-butanol is expected if hydroxyl radical abstracts hydrogen and the resulting radical dimerizes, but interestingly no 1,1-dimethylglycol could be detected in the products(no formaldehyde was formed with HIO_4).

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[9] L. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, New York, 1959, chap. 4.

Accordingly it seems that the rate at which radicals are hydroxylated in this system is not abnormally high, and the result with squalene that the first-formed radical hydroxylates more rapidly than it cyclizes thus reflects slowness of cyclization, rather than speed of the competing reaction. Of course, if the molecule were rigidly held in the proper configuration on an enzyme all this might change.