111. *The Primary Thermal Oxidation Product of Squalene.*

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The product formed in the early stages of the thermal oxidation of squalene has been examined and found to consist substantially of a diperoxide in which four atoms of oxygen are combined with one squalene molecule. Two oxygen atoms are contained in a hydroperoxide group and two form an intramolecular peroxide ring; two oxygen atoms (one from each group) are attached to adjacent carbon atoms. The probable structure of this compound is suggested after consideration of certain kinetic data on the oxidation of polyisoprenes and related mono-olefins.

THE reaction of olefins with molecular oxygen forms primary products the nature of which depends upon the structure of the olefin. Mono-olefins and 1 : 4-dienes form α-methylenic hydroperoxides, whereas the products from conjugated dienes are preponderantly disubstituted cyclic or polymeric peroxides. No similar characterisation has been made of the oxidation products of a further important type of unsaturated grouping, the 1 : 5-dienes, despite their obvious bearing on the problem of the oxidative ageing of natural rubber. We now report an examination of the product formed in the early stages of the oxidation of a representative of this class of olefin, namely the hexaisoprene, squalene.

The choice of this olefin was dictated partly by a desire to minimise any abnormal effects arising from the proximity of double bonds to the ends of the molecule (a possible objection to the use of the di-isoprene, dihydromyrcene) and partly because kinetic evidence (Bolland and ten Have, *Trans. Faraday Soc.*, 1949, **45**, 93) makes it clear that in the thermal oxidation of this olefin the chain length exceeds 100. The product would thus contain almost 99% of the total combined oxygen in the form of the products from the chain propagation reactions (*i.e.*, the primary product).

In view of the polymeric nature of the hydrocarbon, it is obvious that, even if the oxygen reacts uniquely with the unsaturated centres, the oxidation product itself, and any of its derivatives, must contain a sufficient variety of species to preclude their separate isolation and identification by normally used experimental techniques. The present work has therefore of necessity been limited to the application of analytical methods which give quantitative estimates of chemical groupings in the oxidation product and certain of its derivatives. Certain finer details of the molecular structure of oxidised squalene cannot be established by this approach; it is, however, possible to demonstrate that the primary oxidation product combines certain of the structural characteristics of products derived from conjugated and mono-olefins in a manner which might have been anticipated from the particular spacing of double bonds in 1 : 5-polyenes.

Chemical Examination of Oxidised Squalene.—Earlier work on the oxidation of polyisoprenes (Farmer and Sutton, *J.*, 1942, 139) suggested that, although the oxygen combined in the initial stages of the reaction was converted quantitatively into hydroperoxide groups, yet the yield of hydroperoxide diminished as oxidation progressed. These observations were accounted for in terms of an α-hydroperoxidic primary product which was so thermally unstable as to reduce appreciably the overall yield in which it was obtained. This view requires modification, since careful measurement has now shown that the nature of the oxidised squalene is unchanged over a substantial range of oxidation.

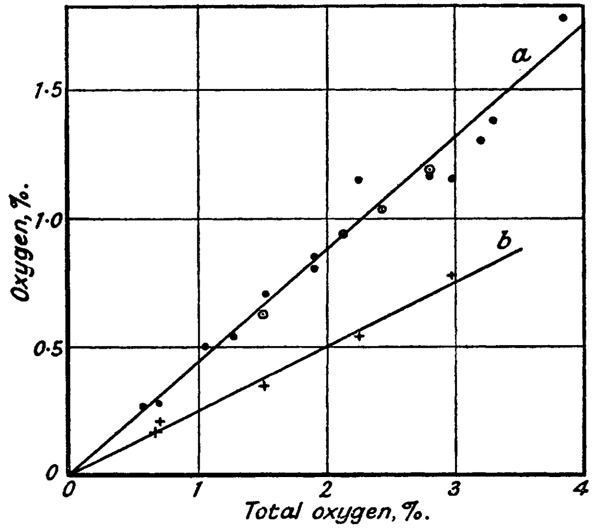
This point has been established by following three analytical characteristics over a series of oxidised squalene samples containing up to 3% of combined oxygen (0·4 mole per mole of squalene) : (*a*) peroxide content, (*b*) active-hydrogen content, and (*c*) average total oxygen content of the fraction of squalene which has been oxidised. Peroxide contents, measured iodometrically and with ferrous thiocyanate reagent, and active-hydrogen contents are presented in the figure. Throughout the introduction of the first 3% of oxygen, each molecule of oxygen incorporated in the squalene introduces 0·90 atom of peroxidic oxygen (reactive towards the above two analytical reagents) and 0·50 atom of active hydrogen. The obvious implication is that one hydroperoxidic group is present in each oxidised molecule. A simple form of chromatographic technique achieves quantitative separation of the oxidised material from the unoxidised squalene. Determination of the amount of unattacked olefin recoverable from a known weight of oxidised squalene of known oxygen content gives at once the average oxygen content of the oxidised squalene (cf. Bolland and Koch, *J.*, 1945, 445). In this way it was found that throughout the total oxygen content range of 0·58 to 2·98%, each molecule of oxidised *product* contained just over four atoms of oxygen :

Total oxygen (%).	Atoms of oxygen/mole oxidised squalene :	
	Found.	Calc.
0·58	4·4	4·07
1·65	4·4	4·18
1·9	4·1	4·21
2·3	4·1	4·25
2·95	4·4	4·33
2·98	4·3	4·33

The calculated oxygen content of the oxidised squalene is derived on the suppositions that each time a squalene molecule is oxidised, four oxygen atoms are introduced, and that oxidation proceeds with equal facility at oxidised and unoxidised molecules.

Examination of the Separated Oxidation Product.—Analyses carried out on the oxidised fraction separated chromatographically agreed well with the indications obtained from analyses of the unfractionated oxidised samples as quoted above. The total oxygen content corresponded fairly well to the presence of four oxygen atoms per molecule, while peroxide and active hydrogen analyses indicated the presence of two peroxidic oxygen atoms and one active hydrogen atom per molecule. Cryoscopic determinations of the molecular weight of the oxidised material gave a mean value of 490. Since the molecular weight of the squalene molecule oxidised to contain four oxygen atoms is 474, there is little evidence of intermolecular bridging by oxygen. Nevertheless, traces of gel were on occasion observed adhering to the reaction vessel at the end of experiments. This product which might well owe its insolubility to polymeric peroxidation was ignored in all the analyses quoted here.

Nature of Unreactive Oxygen.—Examination of the infra-red spectrum of oxidised squalene at the Department of Colloid Science, Cambridge, has revealed the presence of only trivial amounts of carbonyl oxygen. The two oxygen atoms per oxidised squalene molecule not reactive toward peroxide reagents do not therefore reside in ketonic, aldehydic, acidic, or ester groupings. Application of the method of Swern, Findley, Billen, and Scanlan (*Ind. Eng. Chem. Anal.*, 1947, **19**, 414) for the estimation of epoxide oxygen revealed the presence of only a fractional amount of this grouping (0·06 epoxide group per oxidised molecule). The remaining possible groups—ether or cyclic peroxide—are sufficiently unreactive to analytical reagents to render their direct quantitative determination a difficult if not insoluble problem. Examination of the completely hydrogenated product does, however, provide a method with diagnostic possibilities.

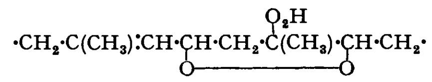


Relationship between total oxygen content and (a) *peroxidic and* (b) *hydroxylic oxygen contents in autoxidised squalene. Peroxide contents measured iodometrically and with ferrous thiocyanate reagent are indicated by* ○ *and* ● *respectively. The curves correspond to yields of* (a) 0·90 *atom of peroxidic oxygen and* (b) 0·50 *atom of hydroxylic oxygen per molecule of oxygen incorporated in the squalene.*

Hydrogenation of Oxidised Squalene.—Samples of both oxidised squalene containing 2—3% of oxygen and the chromatographically separated oxidation product were completely hydrogenated in presence of Raney nickel. Measurements of total oxygen and active hydrogen contents and titrations with lead tetra-acetate were carried out on such hydrogenation products. The analytical results can be most satisfactorily interpreted if the overall effect of hydrogenation is to convert the hydroperoxide group in each oxidised molecule (thus accounting for the two peroxidic oxygen atoms and one active hydrogen atom per oxidised molecule shown to be present) into a hydroxyl group, with the simultaneous conversion of the two hitherto unaccounted-for oxygen atoms into two hydroxyl groups. The reactivity towards lead tetra-acetate requires further that two of these hydroxyl groups should be adjacent.

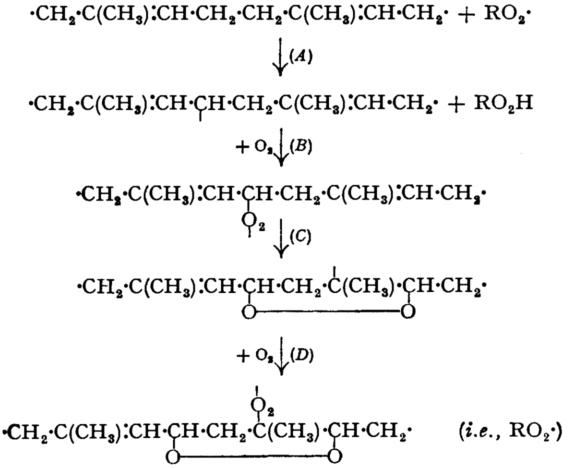
The only oxygenated groups which would fulfil these requirements are disubstituted peroxides of the cyclic or polymeric types : the former must predominate on the evidence of the molecular weight of the separated oxidation product (see above). Since four-membered ring peroxides may safely be ruled out, the inference from the lead tetra-acetate titrations is that in each oxidised squalene molecule the hydroperoxidic oxygen atoms and one oxygen atom in the cyclic peroxide are attached to two neighbouring carbon atoms.

Discussion.—It is not possible to formulate unequivocally the structure of the primary oxidation product from group analyses alone. It may be safely deduced, however, that the predominant oxidation product is a squalene molecule containing four oxygen atoms, two of which are contained in a hydroperoxide group and two in a cyclic peroxide group, with one atom from each grouping attached to adjacent carbon atoms. In advancing (I) as the probable



$$\cdot CH_2\cdot C(CH_3){:}CH\cdot\underset{\underset{O\text{———————}}{|}}{C}H\cdot CH_2\cdot\overset{O_2H}{\overset{|}{C}}(CH_3)\cdot\underset{\underset{\text{———————}O}{|}}{C}H\cdot CH_2\cdot \qquad (I.)$$

structure, consideration has been given to kinetic evidence (*Trans. Faraday Soc., loc. cit.*) which provides a detailed knowledge of the mechanism by which squalene and oxygen interact. The cycle of reactions by which (I) would be formed during the propagation of the oxidation chain is as follows :



$$\cdot CH_2\cdot C(CH_3){:}CH\cdot CH_2\cdot CH_2\cdot C(CH_3){:}CH\cdot CH_2\cdot + RO_2\cdot$$
$$\downarrow (A)$$
$$\cdot CH_2\cdot C(CH_3){:}CH\cdot\underset{|}{C}H\cdot CH_2\cdot C(CH_3){:}CH\cdot CH_2\cdot + RO_2H$$
$$+O_2 \downarrow (B)$$
$$\cdot CH_2\cdot C(CH_3){:}CH\cdot\underset{\underset{O_2}{|}}{C}H\cdot CH_2\cdot C(CH_3){:}CH\cdot CH_2\cdot$$
$$\downarrow (C)$$
$$\cdot CH_2\cdot C(CH_3){:}CH\cdot\underset{\underset{O\text{———————}}{|}}{C}H\cdot CH_2\cdot\overset{|}{C}(CH_3)\cdot\underset{\underset{\text{———————}O}{|}}{C}H\cdot CH_2\cdot$$
$$+O_2 \downarrow (D)$$
$$\cdot CH_2\cdot C(CH_3){:}CH\cdot\underset{\underset{O\text{———————}}{|}}{C}H\cdot CH_2\cdot\overset{\overset{|}{O_2}}{\overset{|}{C}}(CH_3)\cdot\underset{\underset{\text{———————}O}{|}}{C}H\cdot CH_2\cdot \qquad (\textit{i.e.}, RO_2\cdot)$$

where RO_2H represents the completed oxidation product (I). The chain carrier $RO_2\cdot$ (like the other three types of radical concerned in the cycle) is reproduced after four reactions. This contrasts with the chain-propagation sequence in the case of many mono-olefins and 1 : 4-dienes where the cycle consists of only two reactions :

$$RH + RO_2\cdot \longrightarrow RO_2H + R\cdot \qquad R\cdot + O_2 \longrightarrow RO_2\cdot$$

where the chain carrier $R\cdot$ is derived from the olefin RH by removal of an α-methylene hydrogen and $RO_2\cdot$ by addition of an oxygen molecule to $R\cdot$ in an α-methylenic position and the main primary oxidation product is an α-methylenic hydroperoxide.

The critical point at which these two types of oxidation-chain cycle diverge lies in reaction (*C*) : in the case of squalene the peroxido-radical cyclises by interaction with the neighbouring double bond more efficiently than it abstracts a hydrogen atom from another squalene molecule. The nature of the elementary reaction ($RO_2\cdot$ + olefinic centre) undoubtedly determines the type of oxidation product which any olefin forms. Since, in general, the interaction of a peroxide radical with either a double bond or an α-methylene group appears to be of comparable efficiency, the balance in favour of one or the other mode of reaction may be altered by differences in the type of olefinic system. Thus the formation of α-methylenic hydroperoxides from mono-olefins and 1 : 4-dienes on the one hand and mainly polymeric peroxides from conjugated olefins on the other can be correlated with variations in the exothermicity of the reactions of the respective peroxide radicals with either the appropriate α-methylenic groups or double bonds (Bolland and Gee, *Trans. Faraday Soc.*, 1946, **42**, 244). In the present case the determining factor appears to be predominantly a steric one : the particular spacing of the double bonds must be sufficiently favourable for cyclisation of the peroxide radical to replace the normal hydrogen atom extraction process of peroxide radicals disposed next to a trisubstituted ethylenic bond—as exemplified by 1-methyl*cyclo*hexene (Farmer and Sundralingam, *J.*, 1942, 121). It is significant, however, that the chain propagation steps (*A*) and (*B*), in which the influence of the neighbouring double bonds should be small, may be shown to occur at almost precisely the same rates as the corresponding reactions in the oxidation of a methyl-substituted mono-olefin like 4-methylhept-3-ene (Bolland and ten Have, *loc. cit.*).

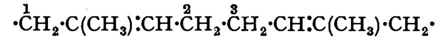
Owing to the unsymmetrical nature of the unsaturated centres in squalene, the three propagation steps (*A*)—(*C*) may each be formulated in two ways, which differ only in the side of the double bond involved in the reactions. The alternatives given above were chosen as the more probable on the following grounds.

(*A*) The relative efficiencies of the analogous reaction $RO_2\cdot + RH \longrightarrow RO_2H + R\cdot$ for a series of olefins (Bolland, *Trans. Faraday Soc.*, in the press) lead to the conclusion that in the unsaturated system $\cdot\overset{1}{C}H_2\cdot C(CH_3){:}CH\cdot\overset{2}{C}H_2\cdot$ hydrogen abstraction from the α-methylene group (2) occurs some three times more readily than from α-methylene group (1).

(*B*) The attachment of the oxygen molecule in this reaction to the resonating hydrocarbon radical so that the double bond retains its original position is preferred on infra-red spectral evidence (obtained at Cambridge): oxidised squalene did not apparently contain the symmetrically disubstituted type of double bond which would necessarily result from oxygen addition at the tertiary carbon atom. It is, however, unlikely that differentiation between the two possibilities is clear-cut, since ample evidence of the insensitivity of this type of addition reaction to the structure of the hydrocarbon radical has been obtained (*idem, ibid.*).

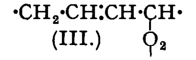
(*C*) The accepted mechanisms of abnormal (peroxide initiated) addition to ethylenic bonds (Mayo and Walling, *Chem. Reviews*, 1940, **27**, 351) and formation of head-to-tail polymers (*e.g.*, Flory, *J. Polymer Sci.*, 1947, **2**, 36) require that radical addition should be more facile at the less highly substituted carbon atom in the double bond.

It is to be emphasised that the structure (I) represents only the most frequently occurring diperoxide structure: the alternative ways in which reactions (*A*) and (*B*) can proceed are sufficiently efficient to assure the presence of an appreciable proportion of other species in the oxidation product. Provided, however, that formation of six-membered peroxide rings by step (*C*) can proceed at either side of a trisubstituted double bond more efficiently than the competing intermolecular reactions involving a double bond or α-methylene group of another squalene molecule, the reaction product will be a diperoxide of the same type as (I). This situation arises with special force in the case of the central $\Delta^{1:5}$-system in the squalene molecule (II). In reactions (*A*) and (*B*) the attachment of a peroxide radical group at carbon atom 2 or 3 will be favoured. Addition of this radical at the mono-substituted end of either of the neighbouring double bonds is unlikely, on the grounds that ring-closure of the radical (III) in the oxidation of mono-olefins (to give four- or five-membered rings) is not observed. The condition of diperoxide formation is thus again that addition of the peroxide radical at even the less favoured side of the neighbouring double bond should be more efficient than reaction with other olefin molecules.



$$\cdot\overset{1}{C}H_2\cdot C(CH_3){:}CH\cdot\overset{2}{C}H_2\cdot\overset{3}{C}H_2\cdot CH{:}C(CH_3)\cdot CH_2\cdot$$

(II.)



$$\cdot CH_2\cdot CH{:}CH\cdot\underset{\textstyle O_2}{\underset{|}{C}}H\cdot$$

(III.)

EXPERIMENTAL.

Crude squalene obtained from basking shark liver oil was freed from saponifiable material and purified by repeated molecular distillation. Before use samples, in the form of a 2% solution in light petroleum (b. p. 40—60°), were passed through a 12-cm. alumina column.

Thermal oxidation of squalene was carried out in a constant-pressure apparatus of the type already described (Bolland, *Proc. Roy. Soc.*, 1946, *A*, **186**, 218; Bolland and ten Have, *Trans. Faraday Soc.*, 1947, **43**, 201). The temperature employed was 55° and the oxygen pressure *ca.* 760 mm. Hg.

Separation of Oxidised Product.—The oxidised material was quantitatively removed from known weights of oxidised squalene by passing the latter in the form of a 2% solution in light petroleum (b. p. 40—60°) through a 12-cm. alumina column. From the weight of squalene recovered from the solution, the oxygen content of the oxidised portion was calculated: elementary analyses on the recovered squalene demonstrated the absence of oxygenated groups (*e.g.*, C, $87{\cdot}6_5$; H, 12·3. Calc. for $C_{30}H_{50}$: C, 87·75; H, 12·25%). The oxidised *product* was eluted from the column with 150 ml. of acetone. Recovery yields averaged 90%. Evaporation of the acetone solution in a vacuum at room temperature left a slightly yellow liquid of viscosity similar to that of the original squalene. Analyses on different samples gave: (*a*) C, $76{\cdot}7_5$; H, 10·8; O (by diff.), $12{\cdot}4_5$; (*b*) C, $76{\cdot}5_5$; H, $10{\cdot}6_5$; O, 12·8; (*c*) C, 76·0; H, $10{\cdot}6_5$; O, $13{\cdot}3_5$; (*d*) C, 75·5; H, 10·6; O, 13·9%. The molecular weight, determined cryoscopically in benzene, was (*a*) 454, (*b*) 533 ($C_{30}H_{50}O_4$ requires C, 75·9; H, 10·62; O, 13·48%; *M*, 474). The peroxidic oxygen contents of three separated oxidised products measured colorimetrically were 5·75, 6·5, and 5·1%, showing that no marked decomposition had taken place during the chromatographic treatment.

Peroxide Determination.—Two distinct methods were used: (*a*) Dastur and Lea's iodometric procedure (*Analyst*, 1941, **66**, 90) and (*b*) the oxidation of ferrous to ferric thiocyanate, measured colorimetrically. In the latter case the effect of varying the concentration of the various constituents of the reagent was studied systematically. It was found that the total acid concentration could be varied between 0·1N and 1·1N with no appreciable change in estimated peroxide content, but below certain critical concentrations the apparent peroxide content decreased with decreasing ferrous salt concentration, but increased with decreasing thiocyanate concentration. Increase in concentration of these three reagent constituents above these critical values had no appreciable effect on the colour developed. The reagent finally adopted varied slightly from that of Bolland *et al.* (*Trans. India Rubber Inst.*, 1941, **17**, 29) and consisted of 0·5 g. of ammonium thiocyanate in 100 ml. of 94% methanol/water, 0·5 ml. of 36N-sulphuric acid, and 0·04 g. of ferrous ammonium sulphate (dissolved in *ca.* 0·3 ml. water to ensure complete solubility when mixed with the methanol-rich reagent). The methanol was redistilled over ferric alum before use to remove oxidisable impurities. The reagent was used immediately after being made up.

Active-hydrogen Determinations.—These were carried out by the modified Zerewitinoff method described by Bolland (*ibid.*, 1941, **16**, 267).

Hydrogenations.—These were carried out in the conventional constant-pressure type of apparatus of 200-ml. capacity. Weighed samples were dissolved in 15—20 ml. of redistilled ethanol, and *ca.* 3 g. of Raney nickel added. Hydrogenation was usually complete in 7—10 hours. The catalyst was separated off by filtration through a wad of " Hyflo Super-cel " which was then washed with either petrol or ethanol, depending on whether unoxidised squalene was present in the hydrogenated sample or not. The filtrate, which was shown to be entirely free from hydroperoxide, was made up to known volume. Aliquots were pipetted into the active-hydrogen apparatus, and the solvent evaporated off in a vacuum at room temperature. The remainder was evaporated down and weighed. Losses during the above procedure were thus shown to be less than 2%. The reduced material was dissolved in 46% or 90% methanol–benzene mixture, depending on whether the starting material contained considerable amounts of squalene or not, before treatment with lead tetra-acetate.

Reaction with Lead Tetra-acetate.—This process was carried out according to Criegee's method (*Ber.*, 1931, **64**, 260). Aliquots of the reduced product were left for various periods in contact with 0·1N-lead tetra-acetate in anhydrous acetic acid. Maximum reaction was reached after about 6 hours. Compared with the reduced oxidation product, reduced squalene showed only slight reactivity toward lead tetra-acetate.

The following results were obtained with hydrogenated samples of separated and unseparated oxidation products. In each case the total number of hydroxyl groups and the number contained in 1 : 2-diol groupings per oxidised squalene molecule are calculated.

Total oxygen (%).	Active H (%).	No. of OH groups per oxidised mol.: Total.	As 1 : 2-diol.
	(*a*) Unseparated oxidised squalene.		
2·93	0·113	2·66	1·5
2·98	0·113	2·62	$1{\cdot}5_5$
	(*b*) Separated oxidation product.		
—	0·491	2·31	1·5
—	0·428, 0·433	2·03	$1{\cdot}5_5$
—	0·407, 0·412	1·93	—
—	—	—	1·6

These active-hydrogen values (*b*) should be regarded as minimum values: condensation between hydroxyl groups during the necessary drying treatment of the sample before active-hydrogen determination must inevitably be favoured by the relatively high hydroxyl content of the reduced separated oxidation product.

The total oxygen content of two samples of separated oxidation product after reduction averaged $10{\cdot}4_5$% [C, 76·0; H, 13·6; O (by diff.), 10·4; and C, $76{\cdot}3_5$, 76·4; H, 13·1, 13·1; O (by diff.), 10·5%].

The hydrogenation product of (I) would be $C_{30}H_{62}O_3$ (oxygen content, 10·2%) and would contain three hydroxyl groups (Found, *ca.* 2·6) and one 1 : 2-diol group (Found, 0·78).

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