

Polyene Oxidation. Part II. Mono-alcohols from the Squalene-singlet Oxygen Reaction

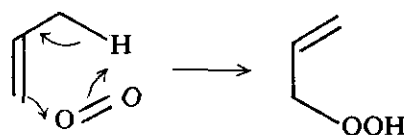
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The structures of two major mono-alcohols obtained by reduction of the hydroperoxides formed during singlet oxygen reaction with squalene (1) have been established as the allylic alcohols (2) and (3) from their chemical behaviour and their spectroscopic properties.

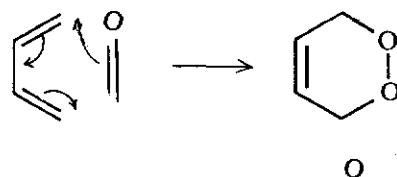
Squalene is a valid model for studies on the behaviour of chemical reagents with polyene systems related to natural rubber. Indeed, current ideas on the mechanisms of rubber auto-oxidation were first formulated on the basis of investigations on the auto-oxidation of squalene (1) itself¹. In the first part of our study of the reaction between singlet oxygen generated by a dye-sensitised photo-reaction and squalene, we have shown that the initial reactivity constant, $\beta = 0.02$, is consistent with the range expected for a trisubstituted double bond². However no information about the nature of the reaction, particularly of its regioselectivity, if any, were available from these rate studies. An earlier report indicated that a hydroperoxide mixture was obtained from this reaction between squalene and singlet oxygen³. We now report on a continuation of our own work to establish the nature of this reaction.

Of the three known modes of reactions of singlet oxygen shown below (Scheme 1)⁴, hydroperoxide formation is the most relevant here. Cycloaddition is unlikely because of the absence of the necessary 1,3-diene system unless a prior isomerisation of the 1,5-diene systems in squalene (1) had occurred; such an event is unprecedented under the mild conditions envisaged for the reaction. Dioxetane formation was also considered unlikely because only activated (that is, electron-rich) double bonds which ultimately cleave into carbonyl products

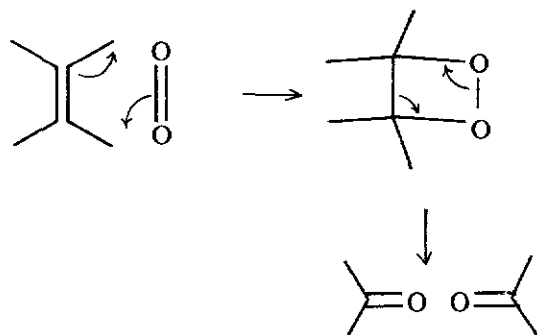
Hydroperoxide formation



Cycloaddition



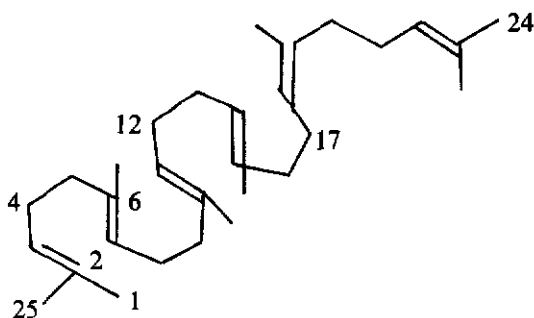
Dioxetane formation



Scheme 1

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can undergo this mode of reaction. Squalene (1) shown below does not satisfy this requirement.



Squalene (1)

The most likely mode of reaction between squalene (1) and singlet oxygen is allylic hydroperoxide formation. The allylic hydroperoxides may subsequently be reduced to allylic alcohols without any rearrangements. The alcohols so obtained are thus good indicators of the initial course of the singlet oxygen reaction.

Regioselectivity is not unknown in reactions of squalene (1). For example, the addition reactions (e.g. with N-bromosuccinimide to produce bromohydrins) of this compound can occur largely at the terminal double bond⁵. This is predictable on steric grounds in that these terminal doubles are less hindered and, in most coiled conformations in polar solutions, more exposed. Therefore, regardless of whichever of the two prevailing mechanisms, peroxide or ene, by which hydroperoxides may be considered to form, reaction should be favoured at the more accessible terminal double bonds in polar solvents. There is however one attendant difficulty. Compounds containing more than one double bond with allylic hydrogens may continue to react at other sites following the initial reaction^{3,6}. It is therefore important with such compounds to arrest the reaction at very early stages in order to have significant amounts of the first formed hydroperoxides otherwise the order of precedence of attack at the double bonds cannot be reliably established.

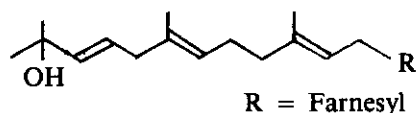
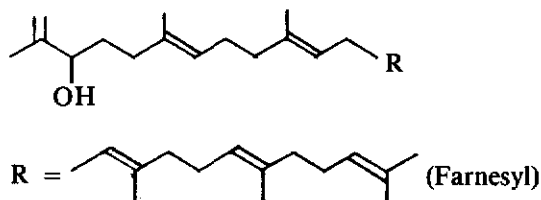
MATERIALS AND METHOD

Proton nuclear magnetic resonance (n.m.r.) spectra were recorded on the Perkin-Elmer R12B instrument operating at 60 MHz. C-13 spectra were obtained on the Varian CFT-20 machine at 20 MHz. High resolution electron impact mass spectra (EIMS) were recorded on the University of Malaya AEI MS3074. Petroleum ether refers to the boiling point 60°C–80°C fraction. Merck Silica Gel 60 (230–400 mesh) was used for the flash chromatography.

Methylene Blue Sensitised Oxidation of Squalene (1)

A solution of 5 g squalene (1) in 100 ml 1:4 methanol-benzene was placed in the apparatus used previously². A few milligrammes of methylene blue was added and a steady stream of oxygen was then passed into the blue solution through the sintered glass bottom. A General Electric Company 400 watts Solarcolor lamp placed 8 cm away was switched on. The solution was kept cool by running water through the cold finger in the apparatus. The progress of the reaction was followed by both thin layer chromatography and gas chromatography². After 45 min, the reaction was stopped. The reaction mixture was transferred to a 250 ml round-bottom flask and cooled to 0°C while 5 g triphenylphosphine was added in batches to reduce the first formed hydroperoxides. This reaction was allowed to proceed overnight after reaching ambient temperature. The solvent was then evaporated under reduced pressure and the bulk of precipitated triphenylphosphine oxide was filtered off. The precipitate was washed with petroleum ether and the washing was combined with the filtrate obtained earlier. A viscous oil was obtained and this was placed in a column containing Merck silica gel (230–400 mesh), 50 mm in diameter to a height of 16 cm; 10% ethyl acetate in petroleum ether was passed through the column under nitrogen pressure such that the rate of fall of the solvent level was approximately 5 cm per minute. Fractions of 50 ml were collected. The fractions containing mono-ols were detected by thin layer chromatography, combined and evaporated

under reduced pressure to give the mono-ol fraction (1.58 g). This fraction was placed on a second column of diameter 30 mm with 16 cm of silica gel and the elution under nitrogen pressure at the same rate of fall of solvent level was repeated with collection of fractions in 25 ml volumes. The fractions were checked by thin layer chromatography. When combined, a mono-ol mixture rich in 1.2 g secondary alcohol (2), and 0.14 g tertiary alcohol (3) was obtained.

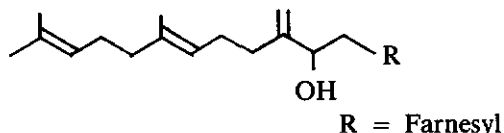


A repetition of the flash chromatography with fresh silica gel in the 30 mm column gave 0.580 g pure secondary alcohol (2) and a mixed fraction (0.40 g). Both (2) and (3) were viscous and colourless oils which on standing became more viscous and coloured, an indication that polymerisation had occurred. Proton, C-13, mass and infra-red spectra of the two compounds were recorded.

Sarrett Oxidation of Secondary Alcohol (2)

The Sarrett reagent was prepared by adding in portions, 400 mg anhydrous chromium trioxide to 4 ml anhydrous pyridine at 15°C. A slurry of the yellow complex in pyridine was obtained. The alcohol (200 mg) in 2 ml dry pyridine was added in one portion to the slurry. The mixture was stirred and left overnight at ambient temperature before being poured into 30 ml ether; the precipitated chromium salts were filtered off. The filtrate was washed

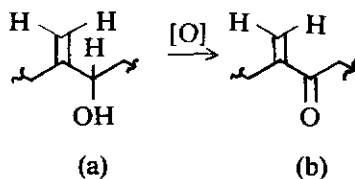
several times with water to remove pyridine, dried over sodium sulphate and evaporated to give a viscous yellow oil characterised only from its proton n.m.r. and infra-red spectra as the ketone (4).



RESULTS AND DISCUSSION

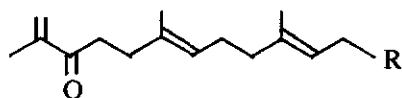
In the event, brief reaction of squalene in the polar solvent, 1:4 methanol-benzene, with singlet oxygen produced by methylene blue sensitised irradiation of oxygen was followed by reduction of any hydroperoxides formed with triphenylphosphine. The mono-ol fraction was resolved into two major components by careful flash chromatography. Flash chromatography is a remarkable speedy method for chromatographic separation⁷ and was crucial in our case because the compounds isolated proved to be unstable on standing.

The less polar alcohol (2) had molecular formula $C_{30}H_{50}O$ (high resolution EIMS requires M^+ 426.3862, observed 426.3831). Loss of water giving rise to a weak m-equiv. peak at 408 in the mass spectrum suggested an alcohol. This was confirmed by a broad infra-red band at 3400 cm^{-1} . An infra-red band at 900 cm^{-1} pointed to the presence of a vinylic methylene group. Based on the allylic hydroperoxide mode of singlet reaction with olefins, the part structure (a) was considered for this alcohol (2).



Evidence for the secondary nature of the alcohol group came when the alcohol could

be oxidised with Sarrett's reagent to the α, β -unsaturated ketone (5) which was characterised by the typical carbonyl band at 1670 cm^{-1} in the infra-red spectrum.



R = Farnesyl

Ketone (5)

Further evidence for the part structure (a) and its conversion by oxidation to part structure (b) was provided by the proton n.m.r. spectra

of both the alcohol (2) (Figure 1) and the derived ketone (5). The methine proton in (a) appeared as a triplet ($J = 6\text{ Hz}$) at 4.00 p.p.m. whereas the vinylic methylene protons appeared as one-proton singlets with fine structures at 4.82 p.p.m. and 5.00 p.p.m. (partially obscured by other olefinic protons). In the derived ketone (5), this pair of methylene protons was shifted downfield to appear clearly as one-proton broad singlets at 5.65 p.p.m. and 5.92 p.p.m., a result only consistent with proximity to a carbonyl group.

The spectral evidence so far could not distinguish between three possible structures arising out of singlet oxygen attack at each of the three individually different pairs of double bonds in the squalene molecule to give a vinylic

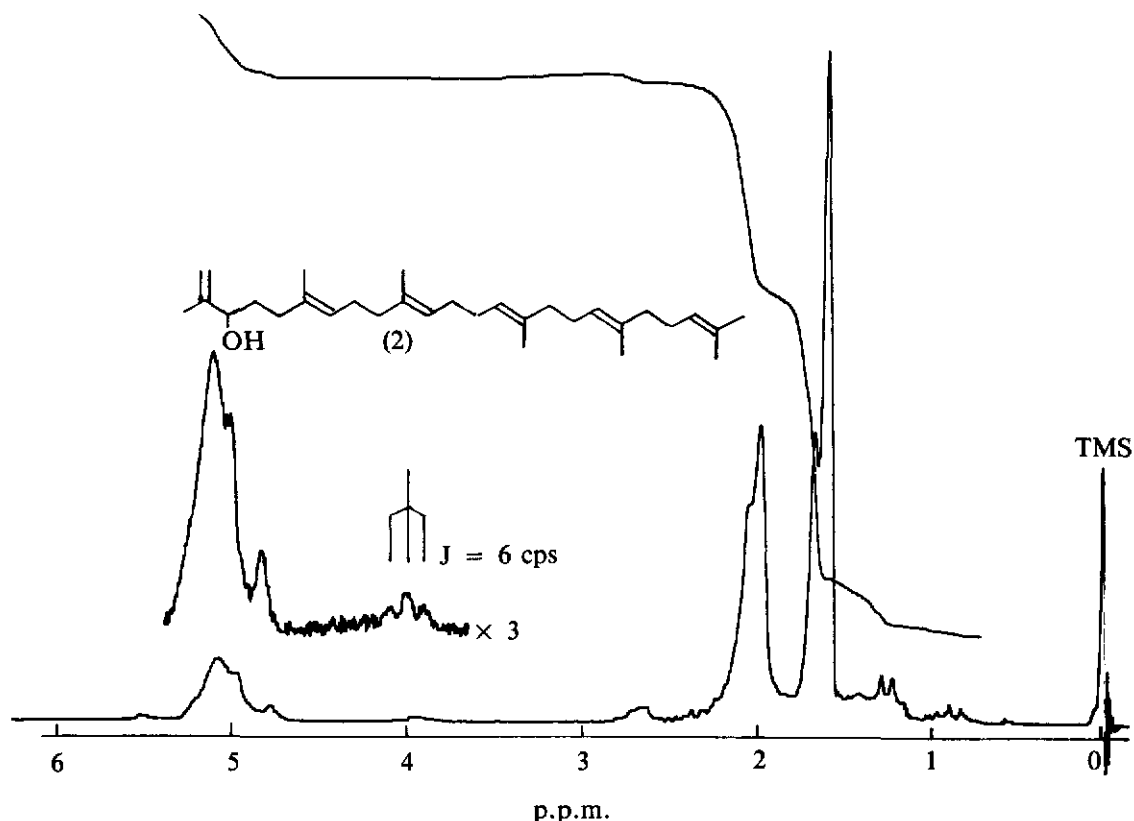
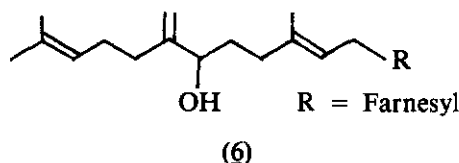
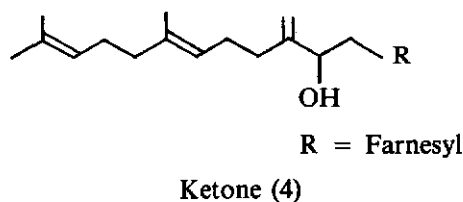
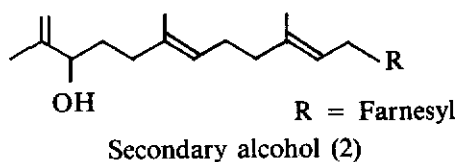
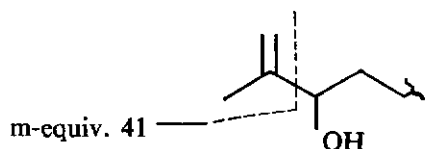


Figure 1. The 60 MHz proton nuclear magnetic resonance spectrum of (2) recorded in CDCl_3 with tetramethylsilane (TMS) as internal standard.

methylene alcohol. These possibilities are (2), (4) and (6).



The mass spectrum of the alcohol shares the same base peak at *m*-equiv. 69 ($C_3H_5^+$) as squalene (1). However, the second most intense peak at *m*-equiv. 41 (73%) shown by high resolution to be due to $C_3H_5^+$ provided some evidence to support (2) as the structure for this alcohol. This peak is comparatively weak in the parent squalene (23%) and is enhanced in the spectrum of the alcohol possibly as a result of the expected α -cleavage shown below:

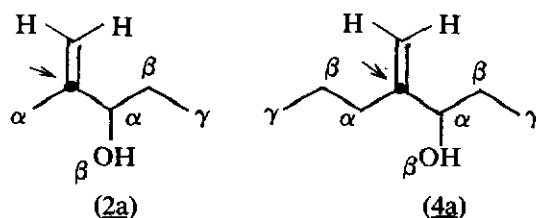


No feature in the structures (4) and (6) could be expected to contribute to such an enhancement of this fragment.

We had originally intended to establish the gross structure of the alcohol by chemical degradation. The instability of this compound in our hands however made such attempts unfruitful.

In particular, we failed to obtain crystalline derivatives (for example, the 3,5-dinitrobenzoate or 3-nitrophthalate) from it or from the oxidation product (2,4-dinitrophenylhydrazone and semicarbazone). An attempt to dehydrate the alcohol to a 1,3-diene and then to trap the diene with a Diels-Alder dienophile (maleic anhydride) was also unsuccessful.

Previously, Roberts *et al.* had demonstrated that C-13 n.m.r. shifts could be predicted accurately using empirical increments supported by correction factors for *geminal* alkyl substituents⁸. Considering substitution to the γ -positions, the three structures could be simplified to (2a) for (2) and (4a) for both (4) and (6).



$$C-C-C-C = C-C-C-C$$

$$\gamma' \quad \beta' \quad \alpha' \quad k' \quad k \quad \alpha \quad \beta \quad \gamma$$

$$\delta_c(k) = 123.5 + \sum Z_{ik} + \sum S_i$$

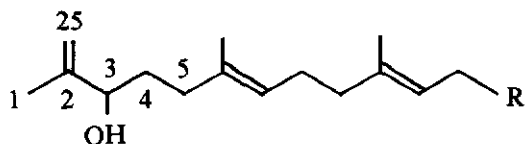
(2a)

(4a)

| Reference | 123.5 | Reference | 123.5 |
|---------------------------------------|--------------|---------------------------------------|--------------|
| $2 \times Z_{\alpha} (C)$ | 21.2 | $2 \times Z_{\alpha} (C)$ | 21.2 |
| $1 \times Z_{\beta} (OH)$ | 6.0 | $1 \times Z_{\beta} (OH)$ | 6.0 |
| $1 \times Z_{\beta} (C)$ | 7.2 | $2 \times Z_{\beta} (C)$ | 14.4 |
| $1 \times Z_{\gamma} (C)$ | -1.5 | $2 \times Z_{\gamma} (C)$ | -3.0 |
| $S_{\alpha\alpha} (gem) \text{ corr}$ | -4.8 | $S_{\alpha\alpha} (gem) \text{ corr}$ | -4.8 |
| | <u>151.6</u> | | <u>157.3</u> |

Using values for the various substituents (Z_{ik}) and a correction ($S_{\alpha\alpha}$) for the *geminal* dialkyl substituents^{8,9}, calculated shifts for the indicated carbon for (2a) and (4a) are 151.6 p.p.m. and 157.3 p.p.m. respectively. This difference is sufficiently large to be distinguishable in a spectrum. In the C-13 spectrum of the alcohol (Figure 2), there occurs a peak at 151.93 p.p.m.

and none in the region of 157.3 p.p.m. Thus on the basis of this predicted shift for the carbon terminus, it is concluded that the most likely structure for this alcohol is (2). With this *proviso*, the partial proton and C-13 assignments are:



R = Farnesyl

(2)

Chemical shifts

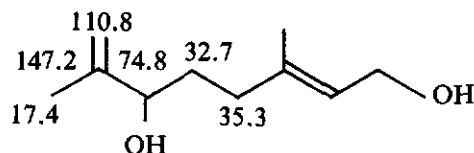
| C-13, decoupled CDCl ₃ , p.p.m. (TMS)* | Proton, CDCl ₃ p.p.m. (TMS) |
|---|---|
| C-1 17.68 | -CH ₃ 1.55-1.70 (21H) |
| C-2 151.93 | -CH ₂ - 1.80-2.90 (20H) |
| C-25 109.57 | =CH- 4.9-5.3 (5H, br. s) |
| C-3 75.28 | 3-H 4.00 (1H, t, J=6 Hz) |
| C-4 31.63 | 25-H 4.82 (1H, br. s) & |
| C-5 35.74 | 5.00 (part hidden) |

*The other signals [and their equivalent in squalene (1)] are 16.10 (16.10), 25.67 (25.68), 26.82 (26.88), 28.32 (28.41), 39.83 (39.93), 124.10 and 124.33 (124.51), 131.25 (131.04) and 134.96 (134.86).

i.r., cm⁻¹: 3400 (-OH) and 900 (>C=CH₂)

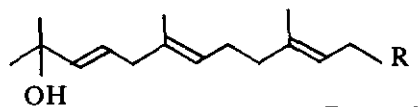
EIMS, m-equiv. (intensity %): 426 (M⁺, 0.4), 149(10), 135(11), 121(10), 109(15), 107(11), 95(20), 93(20), 91(10), 81(44), 79(12), 69(100), 68(13), 67(21), 57(14), 55(26), 53(11), 43(C₃H₇⁺, 19), 43(C₂H₃O⁺, 24), 41(73).

The partial C-13 assignment is based upon the assignments for the monoterpene (7)¹⁰.



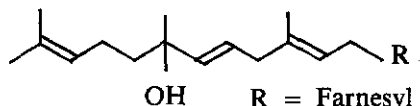
(7) 13-C chemical shifts in p.p.m. (TMS)

The second more polar mono-ol (ν_{\max} 3400 cm⁻¹) had the same molecular formula C₃₀H₅₀O, (observed high resolution EIMS, M⁺ 426.3773). Loss of water to give a M⁺ -H₂O peak at m-equiv. 408 is consistent with the alcohol nature of this compound. However this alcohol could not be oxidised to a ketone. Therefore it was tertiary. This information and the known mode of singlet oxygen attack suggested that the alcohol was one of three possibilities, (3), (8) or (9):



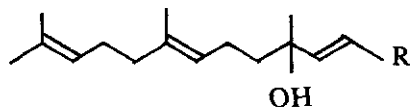
R = Farnesyl

Tertiary alcohol (3)



R = Farnesyl

(8)

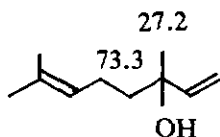


R = Farnesyl

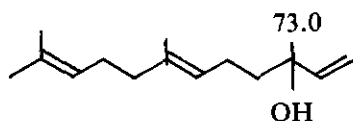
(9)

Firm evidence for (3) was given by the *gem*-dimethyl singlet in the proton n.m.r. spectrum (Figure 3) at 1.2 p.p.m. integrating for six

protons and this is supported by C-13 shift for the hydroxyl bearing carbon at 70.6 p.p.m. (Figure 4). In several reference compounds containing the mid-chain hydroxyl group as in (10) and (11)¹¹, the relevant chemical shifts in contrast had been in the range 72.6–73.4 p.p.m.

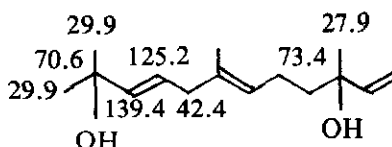


(10)



(11)

This slight but significant difference is best summarised by the assignments for the stress metabolite (12)¹².

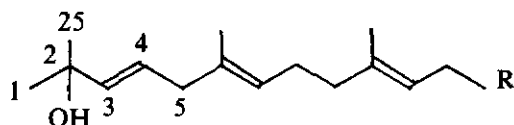


(12)

Thus structures (8) and (9) ought to have the chemical shift for the hydroxyl-bearing carbon in the 73 p.p.m. region while the terminal tertiary alcohol should show this signal 2–3 p.p.m. upfield and share shifts similar to those for the analogous carbons of (12). The latter situation prevails.

Support lent by the mass spectral data included the expected loss of methyl ($M^+ - CH_3$) weakly at m-equiv. 411 absent in the mass spectrum of (2). The *trans* nature of the rearranged double bond was suggested by the prominent infra-red peak at 960 cm^{-1} . The

partial proton and C-13 n.m.r. assignments may be summarised as:



R = Farnesyl

(7)

Chemical shift assignments

C-13, $CDCl_3$, p.p.m. (TMS)*

| | |
|-----------|--------|
| C-1, C-25 | 29.88 |
| C-2 | 70.61 |
| C-3 | 139.27 |
| C-4 | 125.48 |
| C-5 | 42.39 |

Proton, $CDCl_3$, p.p.m. (TMS)

| | |
|----------------------|---------------------|
| sat. $-CH_3$ | 1.2 (6Hs) |
| bis-allylic $-CH_2-$ | 2.5–2.6 (2H) |
| 4-H and 3-H | 5.5 (2Hm) |
| $>C=CH-$ | 4.9–5.4 (5H, env.) |
| $=C-CH_3$ | 1.5–1.7 (18H, env.) |
| $=C-CH_2-$ | 1.8–2.2 (16H, env.) |

*The remaining signals [and their equivalent in squalene (1)] are 16.02 (16.10), 17.60 (17.67), 25.59 (25.68), 26.77 (26.88), 28.31 (28.41), 39.74 (39.93), 124.33 (124.51), 131.16 (131.04), 133.59 (134.86), 134.96 (135.05)

i.r., (liq. film) cm^{-1} : 3400(–OH),
960(*trans* $-CH=CH-$)

EIMS, m-equiv. (% intensity): 426 (M^+ , 0.3), 408(9), 137(16), 136(13), 135(27), 121(19), 109(23), 107(22), 105(13), 95(27), 94(11), 93(41), 91(19), 82(10), 81(77), 80(10), 79(19), 77(12), 69(100), 68(25), 67(25), 55(22), 53(21), 43(30), 41(46), 39(24).

CONCLUSIONS

The isolation of the mono-ols and the characterisation of their structures support the contention that initial reaction of squalene with singlet oxygen occurs by allylic hydroperoxide

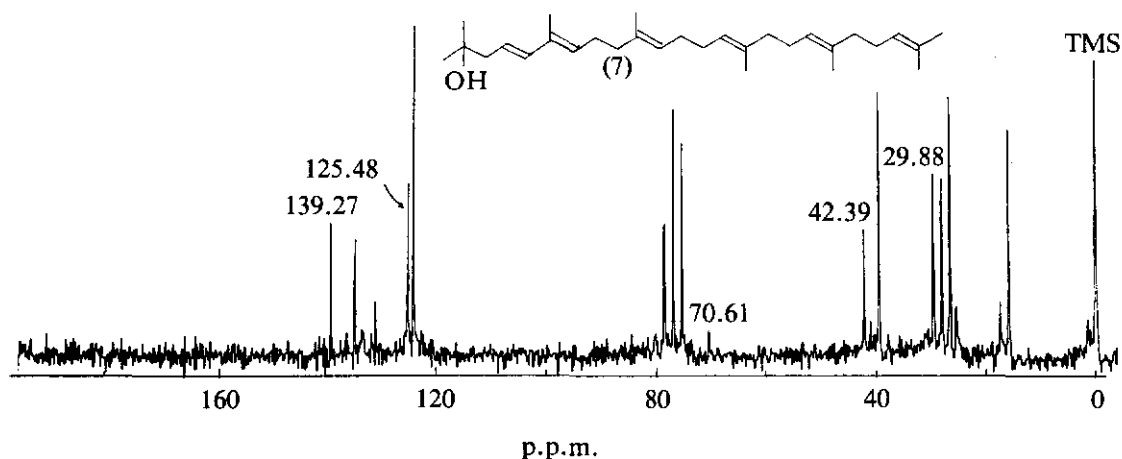


Figure 4. Proton noise decoupled 20 MHz carbon-13 spectrum of (7) recorded in $CDCl_3$ with TMS as internal standard.

formation at the terminal double bonds in polar solutions. The low yields of these mono-ols may be in part due to the greater reactivity of these mono-ols with singlet oxygen compared to squalene thus leading to a small steady state concentration or it may point to a ready decomposition of the first formed hydroperoxides under the experimental conditions (strong ultra-violet irradiation) which then promoted consumption of squalene by alternative reactions which may include auto-oxidation. If the latter is the case, then singlet oxygen attack on polyene systems such as rubber could conceivably cause or initiate polymer degradation as we have suggested in our earlier paper. A thorough investigation of the accompanying products in this singlet oxygen reaction to characterise products of further singlet oxygen attack or of concurrent auto-oxidation promoted by these first-formed hydroperoxides should settle the point.

ACKNOWLEDGEMENTS

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Universiti Sains Malaysia. Finally the authors acknowledge also the gift of a GEC Solarcolor lamp from Dr Oon Hiang Hock.

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