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# AN ASSESSMENT OF THE MOBILITY OF SQUALENE IN PART-AQUEOUS SOLUTIONS FROM CARBON MAGNETIC RESONANCE SPIN-LATTICE RELAXATION TIMES

## COMPARISON WITH SQUALENE AND 2,3-DIHYDROXY-2,3-DIHYDROSQUALENE

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**Abstract**—The $^{13}C$ NMR of squalene in tetrahydrofuran-$d_8$ is sufficiently resolved for $T_1$ measurements (by the inversion-recovery method) to permit the derivation of relative mobilities along the chain. On addition of water, no detectable changes occur to the point of saturation, consistent with the absence of conformational changes (coiling) with change in solvent polarity. On the basis of silver-ion induced carbon chemical shifts, complexation occurs unselectively at all three double bonds in THF, but with some preference for the terminal double-bond in part-aqueous solutions. Squalane shows similar mobility to the unsaturated compound but complications from line overlap are apparent. In squalene-2,3-glycol, the polar end of the molecule is of lower mobility, and this difference is accentuated on addition of water consistent with specific association of water at the OH terminus.

Squalene (1) has six trisubstituted olefinic double-bonds of formally similar electronic character. In consequence, the moderate site-selectivity towards reaction at the terminal double bond in most electrophilic additions is interesting, but the apparent complete lack of reaction other than at the terminal site with N-bromosuccinimide in aqueous glyme or THF is remarkable.[1] A similar pattern of selectivity has been noted for related substrates,[2] and the key factor[3] appears to be the presence of water in the reaction medium. Several explanations are possible, and in his earlier communications van Tamelen expressed the opinion that conformational changes associated with increasing solvent polarity led to reduced accessibility of the internal double-bonds. If this is valid, then the change in conformation of squalene with change in solvent water content should be detectable by physicochemical measurements. $^{13}C$ NMR spin-lattice relaxation times should be particularly useful, since the dipole–dipole relaxation mechanism predominates for large molecules.[4] The measured $T_1$ value for an individual carbon in the chain will reflect contributions from local motion and internal rotation about single bonds, and also from overall tumbling of the molecule. Changes in conformation which result in an alteration of molecular shape will affect the contributions to relaxation from tumbling motions, depending on the position in the chain. Naively, a rod-like molecule will have its global component of relaxation dominated by rotation about the long axis, and equivalent contributions to $T_1$ at all sites. A spheroidal molecule undergoing isotropic motion will have differential contributions to relaxation at the exterior and interior, since the rate at which a given C–H vector sweeps the field will vary with its distance from the centre. In the most favourable cases,[5] analysis of carbon $T_1$ data permits an assignment of preferred conformation.

In the present work, we have acquired $T_1$ data for squalene in tetrahydrofuran-$d_8$ at varying water concentrations. Similarly, squalane (2) and 2,3-dihydrosqualene-2,3-diol (3) were subjected to $T_1$ measurements under similar conditions, the latter at varying water concentrations. Recently, de Haan *et al.*[6] have carried out a related study in which $T_1$ values were obtained for squalene in a number of solvents of varying polarity.†

*Materials and methods.* Squalene was Sigma Grade II, and squalane BDH Chromatographic grade; both were used as supplied. 2,3-Dihydrosqualene-2,3-glycol was prepared by published methods,[7] with minor modifications. To squalene-2,3-epoxide (1 g) in THF (350 ml) and water (150 ml) there was added, at 0°, 3% aqueous perchloric acid (250 ml). The mixture was stirred under $N_2$ at 0° for 5 hr, worked up as described, and purified by preparative TLC (silica gel 2:1 petroleum ether/ether) giving 0.60 g (56%) of product as a colourless oil. THF-$d_8$ was distilled off LAH prior to use and stored under $N_2$. Europium nitrate (New England Nuclear Corporation) and silver hexafluoroantimonate (Alfa-Ventron) were commercial materials.

All $^{13}C$ NMR relaxation measurements were carried out at 308 K on a Bruker WH-90 spectrometer programmed for automatic $T_1$ data collection and storage. Spectra were accumulated using the conventional 180-$\tau$-90-T method or alternatively by the fast inversion-recovery method introduced by Levy.[8] Where comparisons could be made the two techniques gave identical results. Data were processed automatically (Bruker-Nicolet program facility) and frequently checked manually.

## RESULTS AND DISCUSSION

### (A) *Assignment of chemical shifts*

The $^{13}C$ NMR of squalene (1) has already been assigned[9] and entirely accords with our interpretation. In THF-$d_8$, Me carbons are deshielded by *ca.* 0.3 ppm and methylene or olefinic carbons by between 0.7 and 0.9 ppm relative to chloroform. Similar solvent dependencies have been noted for related cases.[10] Assignments to squalane (2) follow from the recorded spectrum of phytol[11] and 2-methylnonadecane.[12] Of particular interest is the 2 Hz differentiation between the two diastereotopic terminal Me carbons, even though the site of chirality is five C atoms away. In squalene glycol (3) half the molecule exhibits carbon chemical shifts identical with those of squalene, whilst the first seven carbons of the chain are differentiated to varying degrees of the influence of the OH groups. Thus $C_4$, $\beta$ to the 3-OH group is deshielded by 3.4 ppm relative to its counterpart at the non-polar

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†A detailed account of their work will appear separately. We acknowledge a valuable interchange of information with Dr. de Haan during the course of this work.

Table 1. ${}^{18}$C chemical shifts of squalene derivatives relative to TMS

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 2′ | 6′ | 10′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Squalene, CDCl$_3$ | 25.42 | 130.83 | 124.19<br>124.32 | 26.71 | 39.57 | 134.78 | As 3 | 26.49 | As 5 | 134.56 | As 3 | 28.12 | 17.40 | 15.73 | As 6′ |
| Squalene d$_8$, THF | 25.76 | 131.40 | 125.06 | 27.52 | 40.49 | 135.50 | As 3 | 27.35 | As 5 | 135.32 | As 3 | 28.94 | 17.70 | 16.08 | As 6′ |
| Squalane, CDCl$_3$ | 22.66 | 28.06 | 39.52 | 24.87 | 37.24<br>37.33<br>37.43 | 32.90 | As 5 | 24.58 | As 5 | 32.90 | As 5 | 27.55 | 22.75 | 19.81 | As 6′ |
| Squalane, d$_8$ THF | 23.02 | 28.88 | 40.28 | 25.66 | 37.99<br>38.08<br>38.30 | 33.70 | As 5 | 25.32 | As 5 | As 6 | As 5 | 28.30 | 23.10 | 20.14 | As 6′ |
| 2,3-dihydroxy-2,3-dihydro-squalene, CDCl$_3$, polar half | 23.22 | 73.14 | 78.28 | 29.94 | 36.89 | 135.02 | 124.92 | 26.71 | 39.78 | 134.81 | 124.32 | 28.30 | 26.45 | 16.00 | As 6′ |
| apolar half | 25.71 | 131.05 | 124.49 | 26.71 | 39.78 | 134.89 | 124.32 | 26.79 | As 5 | 134.81 | 124.32 | 28.30 | 17.64 | 16.00 | As 6′ |
| 2,3-dihydroxy-2,3-dihydro squalene, d$_8$ THF polar half | 24.69 | 72.65 | 78.70 | 30.87 | 37.75 | 135.02 | 125.04 | 27.36 | 40.49 | 135.50 | 124.65 | 28.98 | 25.73 | 16.18 | As 6′ |
| apolar half | 25.87 | 131.41 | 124.65 | 27.49 | 40.49 | 135.63 | As 3 | 27.49 | As 5 | 135.30 | As 3 | 28.98 | 17.74 | 16.18 | As 6′ |

terminus, $C_{23}$ whilst $C_5$, $\gamma$ to the 3-OH group is shielded by 2.7 ppm in comparison with $C_{22}$. On transference from $CDCl_3$ to THF, the tertiary OH-carbon $C_2$ becomes more shielded, but the secondary OH-carbon $C_4$ is deshielded. The two prochiral Me groups $C_1$ and $C_2'$ are differently affected by solvent change. In conformation **4a** which is likely in chloroform since it permits intramolecular H-bonding the pro-*R̲* Me group in the *R̲* enantiomer is *gauche* to the alkyl-chain and *trans* to OH, both expected to cause shielding.[13] In conformation **4b** both the pro-*R̲* and pro-*S̲* Me groups are *gauche* to OH, and the latter is *gauche* to the chain. We thus interpret the pronounced dowfield shift of $C_1$ and the minor upfield shift of $C_2'$ on changing from chloroform to THF to imply that conformation **4b** becomes much more important in the latter solvent. Data are recorded in Table 1.

### (B) *Squalane relaxation times*

Although the detailed interpretation of squalane $T_1$ data (Table 2) is complicated by overlap of several methylene carbons the gradation of values is quite similar to those reported for other non-polar long-chain compounds. The data may be treated by the methods of Lyerla and Torchia,[12] making the assumption that relaxation is dominated by the dipole–dipole mechanism, as is normal for protonated carbons in larger organic molecules. If relaxation times are converted into effective correlation times $\tau_{\rm eff}$, then this latter term represents contributions derived from overall molecular motion $\tau_0$ and internal or segmental motion, $\tau_i$, according to:

$$[\tau^{\rm eff}]^{-1} = [\tau_i]^{-1} + [\tau_o]^{-1}. \qquad (1)$$

The efficiency of relaxation increases along the chain as the frequency of segmental motion decreases, the relative importance of the two terms in (1) for given values of $\tau_{\rm eff}$ being shown in Fig. 1. It is possible to estimate that the overall correlation time is in the region of 40–60 picoseconds.† In support of this it is seen that the tertiary carbons $C_6$ and $C_{10}$, where relaxation is likely to be dominated by overall motions with relatively restricted segmental motion, have an effective correlation time of *ca.* 36 psec.

### (C) *Squalene relaxation times*

It is likely that squalene is more mobile in solution than its saturated counterpart. Unfortunately, the resonances of $C_7$ and $C_{11}$ are insufficiently resolved from that of $C_3$, otherwise the relaxation times of these relatively rigid protonated olefinic carbons might give definitive evidence on the dynamics of overall molecular motion. We see no compelling reason why the overall mobility of squalene and squalane should be drastically different and take the longer relaxation time at C12 in squalene to reflect on increased contribution of segmental motion. This is in line with the lower rotation barrier for $sp^2$–$sp^3$ C–C bonds than their $sp^3$–$sp^3$ counterparts.[14]

More significantly, the observed relaxation times are quite unaffected by dilution of anhydrous $d_8$-THF with varying quantities of water to the point of saturation. Visual comparison of the spectral arrays confirms that any computed differences reflect experimental error rather than systematic trends. Thus it is unlikely that there are significant changes in conformation on going to more aqueous media. Presumably the variation from an essentially rod-like to an essentially coiled structure would affect both $\tau_o$ and $\tau_i$ although the detailed consequences might be difficult to interpret. Inspection of models suggest that if the preferred sub-unit conformation is as **5**, then the main contribution to internal segmental motion is a *trans–gauche* interconversion followed by a *gauche–trans* motion which restores the initial geometry. In a coiled conformation it may be anticipated that the increased internal rigidity would decrease the mobility, leading to shorter relaxation times for internal carbon atoms. Without more detailed insight

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†As it stands the data does not provide information on the molecular conformation, due to the difficulty in separating the contributions from different components to $T_1$.

Table 2. $^{13}C$ spin-lattice relaxation times of squalene derivatives in seconds at 308 K. Values for (1) and (3) are consistent with duplicate or triplicate experiments, and in the case of (1) with experiments at lower $H_2O$ concentrations. Values for (2) accord with an experiment at half the quoted concentration

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 2′ | 6′ | 10′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Squalane, 0.45M | 1.57 | 4.71 | 2.32 | 1.99 | 0.99 | 1.43 | As 5 | 1.86 | As 5 | As 6 | As 5 | 0.85 | 3.0 | 1.57 | 1.57 |
| Squalene, 0.45M | 4.81 | * | 2.45 | 1.84 | 1.49 | * | As 3 | 1.51 | As 5 | * | As 3 | 1.19 | 9.5 | 7.1 | As 6′ |
| Squalene + 2.4M $H_2O$ | 4.05 | * | 2.71 | 1.84 | 1.61 | * | As 3 | 1.43 | As 5 | * | As 3 | 1.05 | 8.6 | 6.5 | As 6′ |
| 2,3-Dihydroxy-2,3 dihydrosqualene 0.45M | | | | | | | | | | | | | | | |
| polar half | 1.17 | 9.8 | 1.90 | 0.84 | 0.72 | * | 1.54 | 1.26 | 1.32 | * | 1.98 | 0.82 | 2.8 | 5.06 | As 6′ |
| apolar half | 4.04 | * | ‡ | 1.26 | 1.32 | * | 1.98 | 1.26 | 1.32 | * | 1.98 | 0.82 | 11.0 | 5.06 | As 6′ |
| 1.8 M in $H_2O$ | | | | | | | | | | | | | | | |
| polar half | 1.17 | 7.8 | 1.38 | 0.71 | 0.73 | * | 1.43 | 1.25 | 1.16 | * | 1.64 | 0.75 | †† | 5.13 | As 6′ |
| apolar half | †† | * | ‡ | 1.25 | 1.16 | * | 1.64 | 1.25 | 1.16 | * | 1.64 | 0.75 | 10.0 | 5.13 | As 6′ |
| 3.6 M in $H_2O$ | | | | | | | | | | | | | | | |
| polar half | 1.14 | 8.2 | 1.29 | 0.73 | 0.68 | * | 1.35 | 1.21 | 1.12 | * | 1.65 | 0.66 | 2.9 | 4.75 | As 6′ |
| apolar half | 3.40 | * | 3.14‡ | 1.21 | 1.12 | * | 1.66 | 1.21 | 1.12 | * | 1.65 | 0.66 | 10.2 | 4.75 | As 6′ |

*Quaternary carbons. ‡Overlapping with $C_{11}$. ††Not separated in this run.

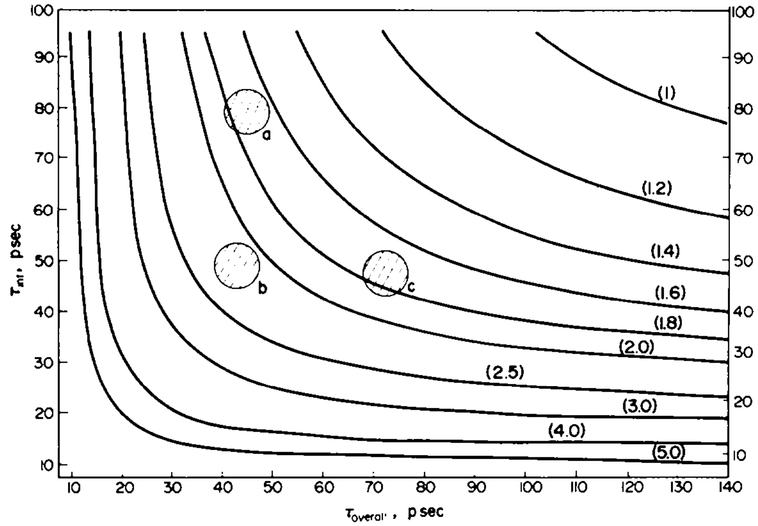
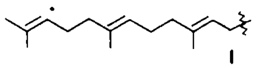


Fig. 1. Contributions of internal and overall motion to spin-lattice relaxation times according to eqn (1). Numbers in parentheses are $NT_1$ values in secs. Assuming that (i) relaxation of tertiary carbons $C_6$ and $C_{10}$ in squalane are dominated by overall motion (ii) squalene and squalane are similar in respect of overall motion and (iii) squalene and squalene glycol are similar in respect to internal motion, a, b and c represent the contributions to relaxation of the central carbon $C_{12}$ in squalane, squalene and squalene glycol respectively, in dry THF-$d_8$.



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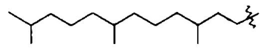

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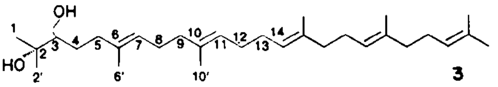

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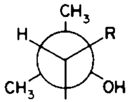

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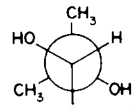

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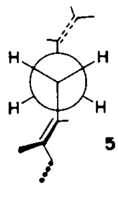

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into molecular geometry, however, attempts at the kind of quantitative treatment applied elsewhere[5] are not justified.

### (D) *Squalene glycol relaxation times*

Hydroxylation of squalene at the 2- and 3-positions has two evident consequences—there is an overall reduction in mobility affecting all chain carbon relaxation times, together with a more localised and more dramatic reduction in the region of the OH groups. Some complications arise from coincident chemical-shifts, the clearest pattern being shown by the allylic carbons $C_4$, $C_{12}$ and $C_{21}$, with $T_1$ values respectively 0.46, 0.69 and 0.71 those of the corresponding carbons in squalene. Since the glycol is likely to be H-bonded to at least two THF molecules, a substantial reduction in overall motion is to be expected, and the latter two values seem reasonable reflections of this. The further reduction in relaxation time at $C_4$ must reflect an additional constraint on internal motion in the region of the OH groups. If relaxation at $C_3$ and $C_4$ is dominated by $\tau_0$ then a minimum rotational correlation time of *ca.* 28 psec is suggested.

On addition of water there are considerable changes in the observed relaxation times, the most well-defined effects being in the region of the OH groups at $C_3$. In addition to this local effect, it appears that there is a general shortening of $T_1$ values evident, for example at $C_{12}$ but in many cases the differences are close to the level of experimental error. It also appears that the bulk of the effect operates on addition of the first 50$\mu$l of water, equivalent to a 4 molar excess over squalene glycol and adding a further 90$\mu$l produces small incremental changes. This suggests that the glycol is now H-bonded to water, and the local solvent structure is consequently much more ordered in that region. The saturation solubility of water in the solution is much greater than in the case of squalene.

### (E) *Metal-ion probes of solution structure*

Since addition of water to solutions of squalene in $d_8$-THF does not alter its mobility (and by implication its averaged conformation) an alternative approach was attempted. Silver-ion complexation affects olefinic carbon chemical shifts, the more substituted carbons becoming deshielded, and tertiary or secondary carbons shielded.[15] Incremental addition of aliquots of silver hexafluoroantimonate in THF to an excess of squalene in the same solvent (external $D_2O$ lock) affects all three pairs of olefinic C atoms to the same extent, suggesting a similar degree of complexation. In the presence of water, the extent of complexation is clearly much less (Fig. 2) due to competitive ligation of water, and there is definite selectivity in favour of the terminal double bond, although this is not exclusive at the highest attainable water concentrations.

Addition of 50 $\mu$l 1M aqueous europium nitrate to solutions of squalene glycol in THF causes perceptible

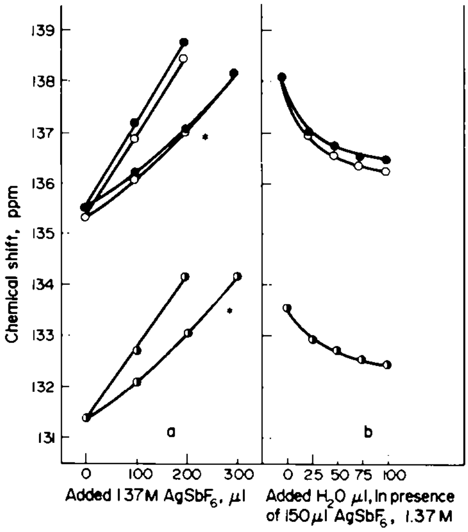

Fig. 2. Effects of water and $AgSbF_6$ on the chemical shifts of quaternary olefinic carbons $C_2$ ◑, $C_6$ ● and $C_{10}$ ○. (a) Addition of increments of 1.37 M $AgSbF_6$ to 1.2 ml 0.5 M squalene in -THF; asterisked lines record similar experiments after additon of 0.5 ml $H_2O$. (b) Addition of increments of water to a solution containing 1.2 ml 0.5 M squalene to which 150μl 1.37 M $AgSbF_6$ in THF had been added.

chemical shifts only to the OH bearing carbons and their nearest neighbours. The negative incremental shifts (at $C_2$ and $C_3$) indicate the operation of a contact rather than pseudo-contact mechanism.[16] No other carbons are affected, and thus folded conformations of the "hairpin" type[17] are effectively ruled out.

*Selective terminal electrophilic additions to squalene.* In the classic papers of van Tamelen *et al.*, it was demonstrated that selective addition to a terminal olefinic bond occurred with N-bromosuccinimide in part-aqueous THF or glyme.[1] In similar solvent mixtures, other polyolefins show comparable terminal selectivity in electrophilic addition reactions.[2] It appears that the reagent must operate so that the transition-state bears net positive charge and thus mercuric acetate, but not peracids show terminal selectivity.[3] In addition the terminal epoxide is selectively hydrated in acidic aqueous t-butanol in the presence of its two internal isomers.[18] van Tamelen considers that in aqueous organic solvents "squalene assumes a more highly coiled compact conformation... the system of H-bonding in the medium is disrupted as little as possible." Thus "solvent clustering at the reacting centre of the reagent discourages reaction at the sterically encumbered central portion...." Selectivity is ascribed to a steric effect limiting access of the electrophile to the olefinic double bond. de Haan *et al.* have suggested an alternative view that the terminal double-bond in squalene is more stabilised than internal double bonds and produced CNDO/2 calculations in support of this view, although it is difficult to see that this explanation could cover the whole range of terminal-selective reactions with complete generality.

Our CMR relaxation results, together with those of de Haan, make it implausible that squalene abruptly changes conformation on transfer from organic to part-aqueous solvents. Alternative explanations of the selectivity based on aggregation or micellisation[19] of squalene in aqueous organic solvents are equally incompatible with the experimental $T_1$ results. There remains a simple straightforward suggestion which is compatible with all observations, taking into account the much greater selectivity observed in those cases where the transition-state bears a formal positive charge. The rate of such reactions will be highly dependent not only on the overall solvent polarity, but more particularly on the polarity of the reaction micro-environment. It is reasonable to assume that the effective polarity in the region of the terminal olefin during the approach of a cationic reagent is higher than in the region of an internal double-bond under comparable conditions. That is to say, the cybotactic region of the transition-state for internal attack contains a substantial contribution from the non-polar hydrocarbon chain, and this is correspondingly less at the terminal site. Consequently the latter is the favoured point of electrophilic attack.

This explanation may also hold for the acid-catalysed hydration of squalene monoepoxides under conditions where specific acid catalysis would be expected.[20] Here the internal epoxides react much more slowly because the transition-state involves approach of a water molecule to a solvated oxonium ion, and local polarity will be crucially important.

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