A ^{13}C -NMR study of squalene : some aspects of selective terminal oxidation of squalene

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group were added and the resultant mixture was shaken mechanically in a tightly closed bottle. Potassium hydroxide pellets, divided into nine portions totaling 15.1 g, were added at two hourly intervals. After being shaken for an additional 48 hrs the mixture was filtered and the deposit was washed several times with methanol. The filtrate and the combined washings were evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml of water, 30 ml of conc. hydrochloric acid were added and this solution was heated to boiling. After removing the unreacted carbon disulfide by distillation, the solution was cooled, neutralized by a potassium hydroxide solution and then evaporated to dryness *in vacuo*. The solid product obtained was extracted with several portions of acetone and the extracts containing potassium thiocyanate were evaporated on a steam bath. The yield of **13** was 4.0 g.

2. ^{15}N -Thiosemicarbazide (**14**); ^{15}N -S-methylthiosemicarbazide (**15**); 3-(methylthio)-[4- ^{15}N]-1,2,4-triazine (**6**). These compounds were prepared as described in the literature for the unlabelled compounds^{11,18}.

b. Amination of **3** with potassium amide in liquid ammonia at -75°C

A solution of 500 mg (4 mmoles) of **3** in 5 ml of absolute ether was added to a well-stirred solution of potassium amide (16 mmoles) in 50 cc of liquid ammonia at -75°C . After 5 hrs the reaction was terminated by the addition of 0.85 g of ammonium chloride. The liquid ammonia was evaporated and the yellow-brown residue was extracted several times with boiling chloroform. Evaporation of the solvent yielded 150 mg of a yellow-brown product, which was separated by means of column chromatography (silica gel, benzene/ethyl acetate 1:1), yielding 60 mg of **5** ($\text{R}=\text{SCH}_3$, 12.1%), 15 mg of **5** ($\text{R}=\text{NH}_2$, 3.4%) and 39 mg of **4** (10.2%).

¹⁸ M. Freund, Chem. Ber. **34**, 3114 (1901).

¹⁹ W. W. Paudler and J. Lee, J. Org. Chem. **36**, 3921 (1971).

The structural assignments of **5** ($\text{R}=\text{NH}_2$) were made on the basis of the molecular weight (221, determined by mass spectrometry), IR data (CHCl_3): 3520 and 3420 cm^{-1} , being attributed to a NH_2 -group, and PMR data ($\text{DMSO}-d_6$): δ 2.75 (s, CH_3); δ 7.7 (s, broad, NH_2); δ 9.2 (s, (C_6 - or C_6)-H); δ 9.5 (s, (C_6 - or C_6)-H).

c. Conversion of 3-amino-[x - ^{15}N]-1,2,4-triazine (**16**) into potassium salt of [x - ^{15}N]-1,2,4-triazin-3-one (**17**)

This conversion was carried out by the same procedure as described for the unlabelled compound¹⁹.

d. Methylation of (**17**) into 2-methyl-3-oxo-2,3-dihydro-[x - ^{15}N]-1,2,4-triazine (**18**)

2-Methyl-3-oxo-2,3-dihydro-[x - ^{15}N]-1,2,4-triazine (**18**) was obtained from **17** by the same procedure as used for the unlabelled compound¹⁹. After the reaction, compound **18** was purified by GLC using a stainless steel column (length 200 cm, internal diameter 1/8 inch, filled with 2 g of Chromosorb W-HP 100-120 + 10% DC-430) at 130° . Carrier gas was nitrogen, velocity 75 ml/60 s.

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A ^{13}C -NMR study of squalene

Some aspects of the selective terminal oxidation of squalene

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Abstract. ^{13}C -NMR data (shifts and relaxation times) show that the conformational equilibrium and the intramolecular mobility of squalene are very similar in media of different polarity. This is inconsistent with one of *van Tamelen's* original proposals explaining the selective terminal epoxidation in terms of tight coiling of squalene in polar media. MINDO/2' calculations predict that terminal epoxidation is favoured over internal epoxidation by ~ 1.5 kcal/mole.

One of the fascinating problems concerning the initial step in the oxidation-cyclization of squalene *in vivo* is the highly selective epoxidation of a terminal double bond. A similar non-enzymic selective oxidation was realized in polar media with charged agents. The explanation was given by *van Tamelen*¹ primarily in terms of solvent-induced coiling of squalene. It should be realized that a quantitative relation between the percentage coiling of squalene and the reactivity of the various double bonds is rather difficult to assess. Therefore, *van Tamelen's* picture can only be considered as preliminary. An alternative explanation presented by *van Tamelen* involved solvent clustering leading to a sterically hindered central part of the molecule. In order to investigate

these possible explanations, we initiated a ^{13}C -NMR study of squalene dissolved in media of different polarity. Partially following *van Tamelen's* original proposals, we focussed our attention on conformations I and II* (see Table I and Figure 1).

* VFF calculations² as well as MINDO/2' calculations³ show that the fragment C_2-C_5 (see Figure 1, II) with the C_4-C_5 bond perpendicular to the $\text{C}_2\text{C}_3\text{C}_4$ plane is lower in energy than the same fragment in a completely stretched conformation.

¹ E. E. van Tamelen, Acc. Chem. Res. **1**, 111 (1968).

² C. Altona, private communication.

³ M. E. van Dommelen, unpublished results.

Fig. 1. Coiled (I) and stretched (II) conformations of squalene.

Table I Atomic coordinates (Angstroms).

	Conformation I*			Conformation II*			Fragments IIIa, IIIb, IVa, IVb		
	X	Y	Z	X	Y	Z	X	Y	Z
C ₁	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C ₂	1.540	0.000	0.000	1.540	0.000	0.000	1.525	0.000	0.000
O							0.764	1.106	0.000
C ₃	2.242	1.141	0.000	2.242	1.141	0.000	-0.790	-0.278	-1.292
C ₄	3.782	1.141	0.000	3.782	1.141	0.000	-0.805	-0.283	1.282
C ₅	4.295	1.141	-1.452	4.295	1.141	1.452	2.421	-0.370	1.196
C ₆	4.467	-0.311	-1.936	5.835	1.141	1.452	2.719	-1.881	1.166
C ₇	3.468	-0.973	-2.537	6.538	2.283	1.452	3.615	-2.251	2.362
C ₈	3.640	-2.425	-3.021	8.078	2.283	1.452	3.096	-2.617	3.542
C ₉	4.153	-2.425	-4.473	8.591	2.283	0.000	3.992	-2.987	4.739
C ₁₀	4.324	-0.973	-4.957	10.131	2.283	0.000	4.290	-1.723	5.567
C ₁₁	5.478	-0.311	-4.797	10.833	3.424	0.000	5.186	-2.094	6.763
C ₁₂	5.649	1.141	-5.281	12.373	3.424	0.000	6.523	-2.066	6.675
C ₁₃	2.234	-1.375	0.000	2.234	-1.375	0.000	7.419	-2.437	7.871
C ₁₄	5.840	-0.965	-1.696	6.530	-0.233	1.452	5.137	-2.182	2.138
C ₁₅	3.105	-0.318	-5.633	10.825	0.908	0.000	4.472	-2.497	8.067
C ₁₆							-1.053	-1.790	-1.418
C ₁₇							-1.843	-2.068	-2.710
C ₁₈							-1.223	-2.318	-3.872
C ₁₉							-2.013	-2.596	-5.164
C ₂₀							0.313	-2.341	-3.978

* C₁-C₁₂ distance I: 7.8 Å.
II: 12.8 Å.

Coiling of squalene is expected to cause changing degrees of steric interaction within the molecule. This will cause chemical shift differences^{4,5}. The origin of ¹³C-NMR chemical shift differences has been frequently discussed⁶. The main sources are atomic charges (or bond orders) and excitation energies. We used the empirical relation for sterically induced chemical shifts developed by Cheney and Grant⁴:

$$\delta^{13}\text{C}(\text{ppm}) = +1680 \sum_i [\cos \theta_i \cdot \exp(-2.671 r_i)]$$

The summation is over all hydrogens H_i interacting with the atom H in a C-H bond; r_i is the distance between the repelling pair of hydrogens H_i ~ H; θ_i is the angle between r_i and the H-¹³C bond. This relation implicitly includes the various parameters mentioned above. The calculated differential sterically induced chemical shifts between the two postulated conformations I and II are tabulated in Table II.

Table II Differential calculated ¹³C-NMR chemical shifts (ppm) of squalene in a coiled conformation I with respect to a stretched conformation II*.

C ₃	C ₇	C ₁₁	C ₄	C ₅	C ₈	C ₉	C ₁₂	C ₁	C ₁₃	C ₁₄	C ₁₅
+0.1	-0.8	-2.5	+0.4	+0.7	+1.7	+1.4	+0.7	-0.0	-2.9	-0.6	-8.7

* Positive numbers relate to downfield shifts.

It should be realized that the induced chemical shift differences depend rather strongly on interatomic distances in the coiled situation. The same applies for conformational energies. In Table III we report the ¹³C-NMR shifts of squalene in media of different polarity with reference to the neat liquid.

Table III Solvent effects in ppm on ¹³C-NMR chemical shifts of squalene*.

	Carbontetrachloride ^a	n-Hexane or 1-Hexene ^b	Glyme ^c	tert-Butyl alcohol/water ^d
=C	+0.30 ± 0.05	-0.35 ± 0.10	-0.10 ± 0.20	-0.10 ± 0.10
CH ₂	+0.15 ± 0.05	-0.30 ± 0.10	-0.35 ± 0.05	-0.20 ± 0.10
CH ₃	+0.55 ± 0.05	-0.55 ± 0.05	-0.60 ± 0.05	-0.10 ± 0.05

* Positive numbers relate to downfield solvent shifts with reference to the neat liquid.

^a 5.3 mole % in Carbon tetrachloride.

^b 3.5 mole % in n-Hexane, 3.4 mole % in 1-Hexene.

^c 3.5 mole % in Glyme.

^d 2.6 mole % in tert-Butyl alcohol/water (70:30 mole %).

The shifts in hexene are analogous to those reported by some of us⁷ for a number of binary hydrocarbon mixtures. The shifts in carbon tetrachloride are comparable to those reported by Mantsch and Smith⁸ and Maciel⁹, very similar effects are found for comparable carbon atoms (e.g. CH₃, CH₂ and C= carbons) in squalene and cholesterol⁸. No explanation for the downfield shifts in carbon tetrachloride has been presented. From the calculations as presented in Table II it is clear, that even small changes in the conformational equilibrium should have been detectable^{*}, taking into account an experimental error in our chemical shifts of about 0.1 ppm. The conclusion at this point is that squalene assumes similar time-averaged conformations (as detectable by NMR) in all media tested in this study.

* Conformational changes in small mono-, di- and tri-substituted ethylenes caused by changing temperatures over a range of 80°, yield relative shift differences of ~1 ppm for various carbon atoms depending on the specific substitution pattern. For instance, the shifts induced in 2,3-dimethyl-1-pentene are: C₁: -0.77 ppm, C₂: +0.78 ppm, C₃: -0.08 ppm, C₄: +0.34 ppm, C₅: -0.34 ppm, C₂(¹³CH₃): +0.79 ppm, C₃(¹³CH₃): -0.37 ppm¹³.

⁴ B. V. Cheney and D. M. Grant, J. Amer. Chem. Soc. **89**, 5319 (1967).

^{5a} E. Lippmaa, T. Pehk and J. Past, Eesti NSV Teaduste Akad. Toimetised XVI, Koide Füüsikalise Mat., 345 (1967);

^b E. Lippmaa, T. Pehk, J. Paasivirta, N. Belikova and A. Platé, Org. Magn. Resonance **2**, 581 (1970);

^c J. B. Grutzner, M. Jautelat, J. B. Dence, R. A. Smith and J. D. Roberts, J. Amer. Chem. Soc. **92**, 7107 (1970).

⁶ A. B. Strong, D. Ikenberry and D. M. Grant, J. Magn. Resonance **9**, 145 (1973) and references cited therein.

⁷ A. R. N. Wilson, L. J. M. van de Ven and J. W. de Haan, Org. Magn. Resonance **6**, 601 (1974).

⁸ H. H. Mantsch and I. C. P. Smith, Can. J. Chem. **51**, 1384 (1973).

⁹ M. R. Bacon and G. E. Maciel, J. Amer. Chem. Soc. **95**, 2413 (1973).

¹³ J. W. de Haan, L. J. M. van de Ven and A. R. N. Wilson, unpublished results.

In order to explore the molecular motions of squalene in the different media, we also measured T_1 values. Results are summarized in Table IV for a characteristic series of analogous carbon atoms: C_4 , C_8 , C_{12} .

Table IV Some longitudinal relaxation times (in secs) in CMR spectra of neat squalene and of squalene in some solvents.

	C_4	C_8	C_{12}
neat squalene	0.43	0.25	0.19
1.5 mole % in <i>tert</i> -Butyl alcohol/water (60-40 mole %)	0.72	0.43	0.3
4.1 mole % in Glyme	1.7	1.2	0.9
3.4 mole % in 1-Hexene	2.3	2.0	1.2

Similar patterns of T_1 values were found for other series of corresponding atoms. Our results reflect the variable overall mobility of the squalene moiety, as a consequence of solvent viscosity. Also, variation of the relative importance of segmental motions is observed. Since these two phenomena are interrelated¹⁰, our experiments do not allow us to support or reject the idea of selective solvent clustering near the center of squalene. More detailed consideration of the experimental T_1 values is hampered by the lack of sufficiently suitable model studies. So far, the T_1 values in normal alkanes have been discussed in detail¹¹. The model developed for these compounds contains too many restrictions to be useful for squalene, since the latter molecule contains branching and rigid double bonds in addition to alkane-like fragments. Models have also been developed for normal 1-halogeno-alkanes, the halogen atom serving as a molecular anchor¹¹. In a way, squalene qualifies as an example of this kind since one half of the molecule serves as an anchor for the other half. Our experimental results yield a similar motional pattern in all media, as is demonstrated by the essentially invariant ratio of T_1 values at C_4 , C_8 , C_{12} . Also, the mobility increases markedly towards the ends of the chain. This is consistent with a rather rapid exchange between conformations with relatively small energy differences, similar to normal alkanes. Tight coiling of the molecule would make its motional behaviour more isotropic and would tend to equalize relaxation times of analogous carbon atoms.

A different and, in our opinion, far more attractive explanation of van Tamelen's results is offered by the possibility of specificity of the reaction products, based on a thermodynamically favoured epoxidation. We calculated by MINDO/2¹² the heats of formation (HOF) of the fragments IIIa, IIIb, IVa and IVb (see Table I, Figure 2).

Fig. 2. Heats of formation of various squalene-epoxide fragments.

Comparison of the pairs IIIa, IIIb and IVa, IVb yields a difference of ~ 1.5 kcal/mole in favour of the terminal epoxides. Apparently, the rather subtle difference in substitution pattern of the terminal double bond and the internal double bond is sufficient to cause this difference. Although the difference of 1.5 kcal/mole is rather small, it is sufficient to cause a very high specificity, which is in good agreement with the experimental values of van Tamelen¹. Our final conclusion is that the clear preference for terminal epoxidation of squalene is best explained by assuming a thermodynamically favoured epoxidation. It should be kept in mind, that our calculations do not include solvent effects and therefore do not explain the different degrees of selectivity in polar and apolar media. We prefer to ascribe this last phenomenon to solvent clustering as already suggested¹.

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¹⁰ D. Doddrell and A. Allerhand, *J. Amer. Chem. Soc.* **93**, 1558 (1971).

¹¹ Y. K. Levine, N. J. M. Birdsall, A. G. Lee, J. C. Metcalfe, P. Partington and G. C. K. Roberts, *J. Chem. Phys.* **60**, 2890 (1974).

¹² N. C. Baird and M. J. S. Dewar, *J. Chem. Phys.* **50**, 1262 (1969).