

A ¹³C-NMR study of squalene, part II : functionalized squalene-like compounds

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absence of π -delocalization of oxygen to phosphorus may explain the fast hydrolysis of III and IV into methyl α -D-ribose and methyl β -D-ribose, respectively. The increased basicity of the ester oxygen upon increased constraint catalyzes the nucleophilic attack of water on phosphorus with retention of configuration of the sugar moiety. It seems reasonable to assume that we are dealing with high-energy phosphites. One of the most fascinating observations for this supposition is based on the mass spectra which are fully presented under Results. The fact that for III the peak at m/e 191 corresponds with the proposed configuration as described in Scheme 1, leads to the assumption that the phosphite triester III (and IV) cannot be described as a pure chair configuration. X-ray diffraction measurements are in progress to elucidate both configurations unambiguously.

Experimental

All m.p.s were determined in a Buchi m.p. apparatus (designed by Tottoli) and are uncorrected. Electron impact mass spectra were produced with an AEI MS-12 magnetic sector instrument under the following conditions: 70 eV electron energy, 500 μ A trap current, 4 kV accelerating voltage. The samples III and IV were analysed after direct introduction and evaporation at 150° and 80°, respectively, at a source temperature of 250°. Coherent wave ^1H and pulsed ^{13}C and ^{31}P NMR spectra were run on a Varian HA-100 and/or a Bruker HX-90 spectrometer interfaced with a Digilab FTS-NMR-3 pulsing and data system. Samples were contained in 5 mm or 10 mm tubes for ^{13}C and ^{31}P measurements equipped with concentric capillaries containing the lock substance and/or the reference.

Methyl β -D-ribose and methyl α -D-ribose

Methyl β -D-ribose was prepared as described by Jackson and Hudson⁵. From 200 g of D-ribose we obtained 80 g of crystalline methyl β -D-ribose, m.p. 83–84°. The mother-liquor was distilled *in vacuo* and gave a thick, colourless syrup, b.p. 134–136/0.03 mm. This syrup exists mainly of methyl α -D-ribose, but still contains some methyl β -D-ribose. We used the crude syrup for further reactions.

Phosphite ester of methyl β -D-ribose (IV)

A 1000 ml five-necked flask was equipped with an efficient oil-sealed stirrer, two calibrated cylindrical dropping funnels, a thermometer and a drying tube filled with phosphorus pentoxide. The dropping funnels were respectively filled with a solution of methyl β -D-ribose (32.8 g = 0.2 mol) in dry redistilled pyridine (300 ml) and a solution of redistilled phosphorus trichloride (27.5 g, 0.2 mol) in dry carbon tetrachloride (300 ml) and protected with phosphorus pentoxide guard tubes. The flask now containing dry pyridine (30 ml) was cooled in a bath of ice and salt and both solutions were added at equal rates with stirring, the temperature being kept below 0°. After additions the mixture was stirred for 4 hrs at 20° and was filtered from the precipitated pyridine hydrochloride. The filtrate was evaporated and the residue was crystallized from sodium-dried hexane. The solution in hexane was cooled down to –5° and the crystals formed were filtered with suction on a Buchner funnel in a drying-box and washed with sodium-dried hexane at –5°.

Yield: 18 g of long (20–30 mm), colourless needles, m.p. 34.5–35°. The ester can be distilled in a vacuum, b.p. 82–84°/0.1 mm, and must be stored in a desiccator over phosphorus pentoxide (Found: C, 37.1; H, 4.7. Calc. for $\text{C}_6\text{H}_9\text{O}_5\text{P}$ (192.10): C, 37.51; H, 4.72). Subsequently we obtained IV more readily and in much higher yield by transesterification of trimethyl phosphite with methyl β -D-ribose.

Phosphite ester of methyl α -D-ribose (III)

III was prepared in exactly the same way as IV. From crude methyl α -D-ribose (32.8 g, 0.2 mol) we obtained only 1 g of III as small (1–2 mm), colourless needles, m.p. 119–120° (Found: C, 37.2; H, 4.9. Calc. for $\text{C}_6\text{H}_9\text{O}_5\text{P}$ (192.10): C, 37.51; H, 4.72).

III is less soluble in hexane than IV. The solution of III in hexane must be cooled down to 10° in order to get the pure compound. On concentration of the mother-liquor and cooling to –5° we were able to isolate 1.5 g of IV.

Acknowledgement

The authors thank Mr. H. Eding for carrying out the micro-analyses.

⁵ E. L. Jackson and C. S. Hudson, J. Am. Chem. Soc. 63, 1229 (1941).

A ^{13}C -NMR study of squalene. Part II. Functionalized squalene-like compounds.

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Abstract. ^{13}C -NMR chemical shifts of substituted squalenes and model compounds with a heteroatom in δ -position to a double bond show that a steric interaction exists between the heteroatom and the double bond. This points to the presence of "precoiled" conformations in the hetero-substituted squalenes and also in squalene itself. The nature of the interaction remains veiled, but it appears to be a general feature as is shown in a series of 2- and 3-substituted thiophenes with a double bond in the side chain.

Introduction

Recently, we investigated the behaviour of squalene (I) by means of ^{13}C -NMR in a number of media in order to check the existence of different degrees of coiling^{1a}. The main target of this study was to obtain additional information

regarding conformational aspects of steroid precursors. It was found that squalene itself assumes similar time-averaged conformations in the media tested at concentrations of

^{1a} M. E. van Dommelen, A. R. N. Wilson, J. W. de Haan and H. M. Buck, Recl. Trav. Chim. Pays-Bas 94, 206 (1975).

about five mol percent. Under these conditions, the motional pattern as judged from relative T_1 values is very much like that of alkanes, nearly independent of the solvent.

A detailed study of ^{13}C -NMR relaxation rates of squalene dissolved in media of varying polarity and different water contents has appeared recently^{1b}. The results are in accordance with the conclusions drawn in our previous work^{1a*}.

An important question which is still open pertains to the nature of the time-averaged conformations. Since our first ^{13}C -NMR study^{1a} appeared, Winnik² and Deno³ showed that the conformational equilibria of alkanes are sensitive towards the solvent and that polar media cause noticeable amounts of coiling. These experiments were carried out in concentrations in the order of 10^{-3} to 10^{-4} mol/l, a range inaccessible to ^{13}C -NMR without extensive labelling. It seems possible that at concentrations in the order of 5 mol percent, the solvation of squalene or derivatives will be only partial, especially in media of low molecular weight like hexane or "glyme"^{1a}.

There are a number of experimental⁴ and theoretical^{5,6} studies on the conformational behaviour of alkenes in general. A basic property is that usually, conformations with allylic hydrogens *syn* to the double bond are favoured over those with carbon atoms *syn*, which in turn are favoured over those with carbon atoms *anti*. A specific example is formed by 1-pentene⁶ as shown in Figure 1 (R = CH_3).

* We acknowledge a valuable interchange of information regarding the relaxation times with Dr. J. M. Brown.

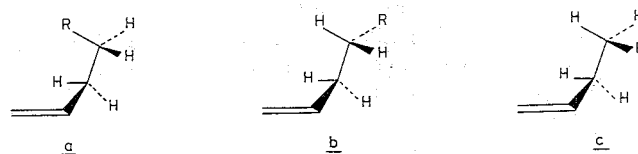


Fig. 1. Conformations of 1-alkenes.

The possible positions of the terminal methyl group are denoted with a, b and c. In 1,5-dienes, the basic element of most steroid precursors, this may yield stretched-like conformations by combination of "b-units" or "coiled" conformations by combination of "a-" and "c-units" (see Fig. 1). Actually, VFF calculations on 2,6-dimethyl-2,6-octadiene (III) demonstrate the feasibility of this situation⁷.

^{1b} J. M. Brown and D. R. M. Martens, *Tetrahedron* **33**, 931 (1977).

² M. A. Winnik, A. Lemire, D. S. Saunders and C. K. Lee, *J. Amer. Chem. Soc.* **98**, 2000 (1976).

³ N. C. Deno and E. J. Jedziniak, *Tetrahedron Letters*, 1259 (1976).

^{4a} S. Kondo, Y. Sakurai, E. Hiroto and Y. Morino, *J. Mol. Spectrosc.* **34**, 231 (1970).

^b N. Sheppard, *J. Chem. Phys.* **17**, 74 (1949).

^c F. H. A. Rummens, *J. Magn. Resonance* **6**, 550 (1972).

^d A. Almendingen, I. M. Anfinsen and A. Haaland, *Acta Chem. Scand.* **24**, 43 (1970).

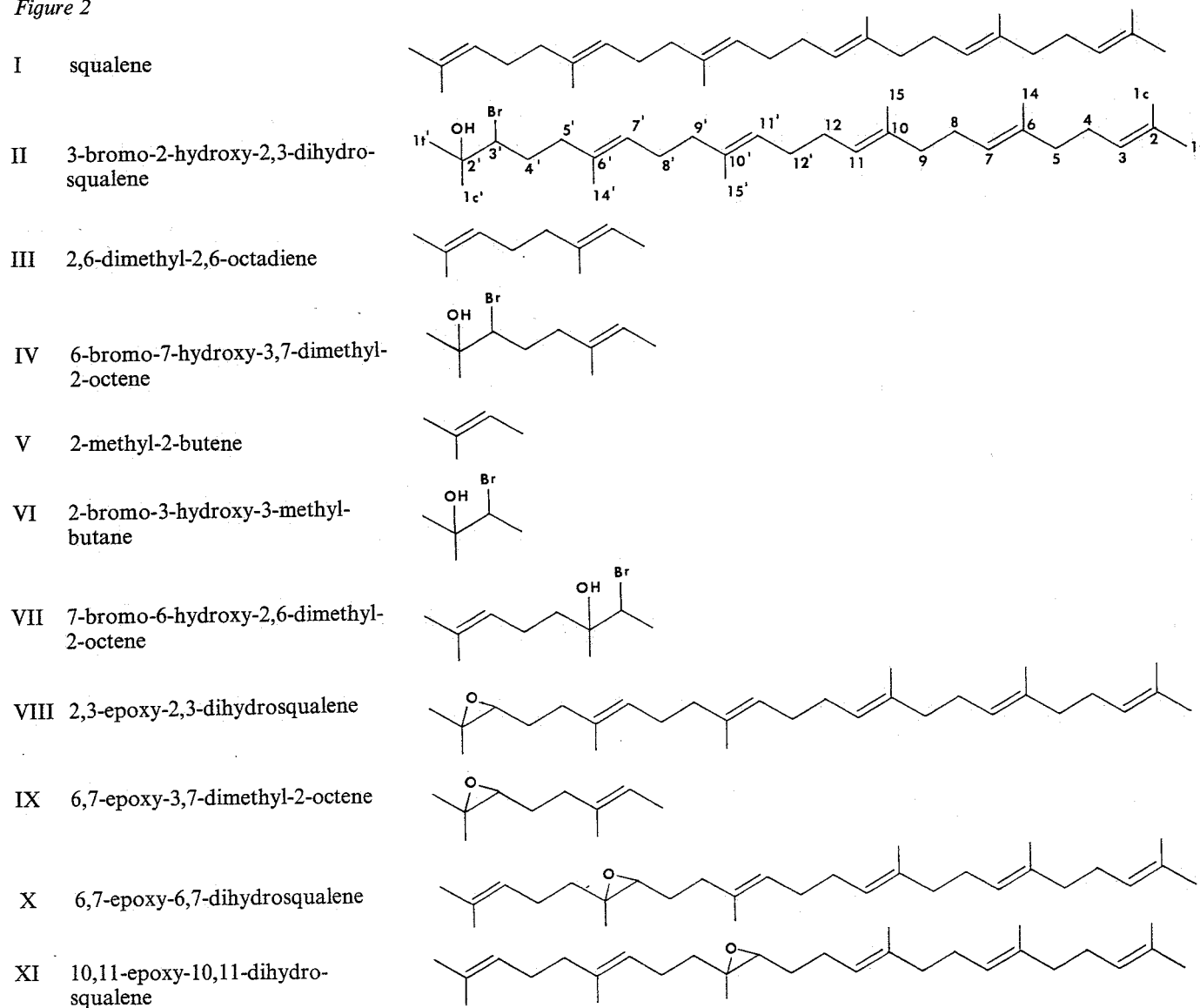
^{5a} O. Ermer and S. Lifson, *J. Amer. Chem. Soc.* **95**, 4121 (1973).

^b O. Ermer and S. Lifson, *Tetrahedron* **30**, 2425 (1974).

⁶ J. W. de Haan, L. J. M. van de Ven, A. R. N. Wilson, A. E. van der Hout-Lodder, C. Altona and D. H. Faber, *Org. Magn. Resonance* **8**, 477 (1976).

⁷ C. Altona and D. H. Faber, unpublished results.

Figure 2



These conformations are different, however, from the tight coiling proposed originally by *van Tamelen*⁸ in the way that the molecules still maintain a preferential axis of tumbling. It should be realized that these calculations do not account for differences in solvent-solute interactions in different media⁹.

Assuming that similar conformations occur in squalene derivatives, a hetero atom in δ -position with respect to the double bond could approach this bond. Thus, in the case of 3-hetero-substituted 2,3-dihydrosqualene, this kind of steric relation should be visible in ¹³C-NMR chemical-shift differences on C(6)=C(7) with respect to squalene. This should also enable one to study the solvent effects of the substituted part more closely. In view of its importance with respect to cyclization reactions, 2,3-epoxy-2,3-dihydrosqualene (VIII) and models seem logical starting points. As a precursor for VIII the 3-bromo-2-hydroxy-2,3-dihydrosqualene (II) was prepared. The latter compound is of particular interest with respect to the possible intramolecular interaction of the bromine atom with the neighbouring olefinic bond. All squalene derivatives and model compounds investigated in this study are listed in Fig. 2.

Experimental

The conversion of the alkenes I, III and V to the monobromohydrins II, IV and VI was carried out by oxidation with *N*-bromosuccinimide in aqueous *t*-butanol under such conditions of temperature (<23°) and concentration that the solution at the start of the reaction was saturated with alkene¹⁰. Products were separated by chromatography on silica gel. Compound VII was present as a minor product in IV.

The epoxides VIII and IX were prepared by conversion of the corresponding bromohydrins II and IV with K₂CO₃ in DMF.

The epoxides X and XI were synthesized from squalene upon epoxidation with *m*-chloroperbenzoic acid in CH₂Cl₂. After chromatography a 50/50 mixture of X and XI was isolated²³.

The syntheses of the 2- and 3-substituted thiophenes have been described elsewhere¹¹.

¹³C-NMR spectra were measured at 25.1 MHz: Compounds I to XI in 5 mol % solutions in hexane and in "glyme", the thiophenes neat.

Spectral assignments

The assignments of I and V are in agreement with those published previously¹² with the exception of C(6) and C(10) in I which are reversed on account of *T*₁ measurements. The assignments of III from I are straightforward. In the aliphatic part of II, many signals are found equivalent to squalene. Extra signals are found at 72.56 (singlet), 70.05 (doublet), 32.6 and 38.86 (triplets), 26.95 and 26.56 (quadruplets), each representing one carbon. This amounts to two signals, due to the substituted carbons [C(2') and C(3')], two triplets and two quadruplets, clearly influenced by the substitution [C(4'), C(5'), C(1't), C(1'c)]. The olefinic part of II contains four tertiary *sp*₂ signals, namely 124.97 (two carbons), 125.15, 125.28 and 126.60. The assignments of C(3) and C(7) rest on relaxation times, 126.60 to C(7) (shortest *T*₁ value), 125.15 as C(3) (longest *T*₁ value). The assignment of C(7), C(11) and C(11') is based on two

⁸ E. E. van Tamelen, Acc. Chem. Res. 1, 111 (1968).

⁹ J. H. McCreery, R. E. Christoffersen and G. G. Hall, J. Amer. Chem. Soc. 98, 7191 (1976).

¹⁰ E. E. van Tamelen and T. J. Curphey, Tetrahedron Letters 121 (1962).

¹¹ A. Corvers, J. H. van Mil, M. M. E. Sap and H. M. Buck, Recl. Trav. Chim. Pays-Bas 96, 18 (1977).

¹² J. B. Stothers, Carbon-13 NMR Spectroscopy, Academic Press, New York (1972).

Table I ¹³C NMR chemical shifts in ppm downfield from TMS of compounds I to VII in hexane.

	I	II	III	IV	V	VI	VII ^a	VII ^b
C(1't)	25.67	26.95 ^c	25.59	26.56 ^d	25.41	26.69 ^e	26.80	
C(1'c)	17.56	26.56 ^c	17.42	26.42 ^d	16.90	25.54 ^e	18.70	
C(2')	130.66	72.56	130.66	72.30	131.32	72.21	132.16	131.59
C(3')	125.01	70.05	124.93	70.14	118.57	62.90	125.37	124.79
C(4')	27.39	~32.6	27.31	32.69	*	21.44	24.26	
C(5')	40.36	38.86	40.32	38.77			40.05	
C(6')	134.72	133.31	135.51	134.01			75.08	
C(7')	124.93	126.60	118.44	120.25			61.05	
C(8')	27.26	27.31	13.15	13.28			23.20	
C(9')	40.36	40.50						
C(10')	134.89	134.81 [†]						
C(11')	124.93	125.28 [†]						
C(12')	28.81	28.94						
C(12)	28.81	28.94						
C(11)	124.93	124.97 [†]						
C(10)	134.89	135.12 [†]						
C(9)	40.36	40.50						
C(8)	27.26	27.31						
C(7)	124.93	124.97 [†]						
C(6)	134.72	134.89 [†]						
C(5)	40.36	40.50						
C(4)	27.39	27.48						
C(3)	125.01	125.15						
C(2)	130.66	130.88						
C(1c)	17.56	17.82						
C(1t)	25.67	25.94						
C(14')	16.01	16.06	*	15.40			21.79	
C(15')	16.01	16.19						
C(15)	16.01	16.19						
C(14)	16.01	16.19						

* Coinciding with the signal of C(1) of hexane.

† See text.

^a As minor product in IV, measured "pure".

^b As minor product in IV, measured in hexane.

^{c-e} Assignments may be reversed.

Table II ^{13}C -NMR shifts in ppm downfield from TMS of some squalene epoxides in hexane.

	VIII	IX	X	XI
C(1't)	25.01	24.79	25.59	25.72
C(1'c)	19.01	18.79	17.60	17.60
C(2)	56.82	56.55	131.10	130.84
C(3)	63.30	63.12	124.88	124.97
C(4')	28.32	28.23	24.26 ^a	27.35
C(5')	37.14	36.97	39.44	40.36
C(6')	134.19	134.94	59.51 ^b	135.60 ^c
C(7)	125.32	118.88	62.46	124.53 ^d
C(8')	27.22	*	28.14	24.35 ^a
C(9')	40.41		37.10	39.44
C(10')	134.76 ^e		134.41	59.42 ^b
C(11')	124.84		125.28	62.46
C(12')	28.81		28.81	29.73
C(12)	28.81		28.81	25.72
C(11)	124.84		124.75	124.18 ^d
C(10)	134.98 ^e		135.07	135.07 ^e
C(9)	40.41		40.36	40.36
C(8)	27.22		27.17	27.17
C(7)	125.01		124.75	124.75
C(6)	134.76		134.76	134.89
C(5)	40.41		40.36	40.36
C(4)	27.39		27.35	27.35
C(3)	125.01		124.97	124.97
C(2)	130.70		130.75	130.75
C(1c)	~17.6		17.60	17.60
C(1t)	25.72		25.72	25.72
C(14')	16.01	*	16.94	16.06
C(15')	16.01		16.06	16.94
C(15)	16.01		16.06	16.06
C(14)	16.01		16.06	16.06

* Coinciding with the signal of C(1) of hexane.

^{a-e} Assignments may be reversed.

criteria*: First, as in squalene C(7) should be upfield from C(3), second the shift differences of C(3), C(7) and C(11) with respect to squalene should show a monotonous path. This leads to the assignment given in Table I. The assignments of the five quaternary sp_2 carbons have been done in the same way. This means that C(2) and C(6') are based on the longest and shortest T_1 value, respectively. Further application of criterion 1 [that is C(6) downfield from C(2), C(10) downfield from C(6')] and criterion 2 [a monotonous path with respect to squalene for C(2), C(6) and C(10)] leads to two possible assignments for C(6), C(10) and C(10'), respectively 134.89, 135.12, 134.81 and 134.81, 134.89 and 135.12.

The assignments of IV, VI and VII from II and the unsubstituted olefins are straightforward.

The spectrum of VIII showed three almost "unperturbed" double bonds (including one of the terminal type) as well as one "perturbed" double bond with respect to I. See Table II.

The ^{13}C -NMR spectrum of the mixture X and XI clearly revealed ten double bonds: four terminal double bonds, one of which was "perturbed" with respect to squalene and six internal double bonds, three being "perturbed". Apart from the terminal double bonds and a few shifts of the perturbed internal double bonds, no specific assignments were made in view of the small mutual shift differences. The internal double bonds could be assigned with a rather large degree of mutual consistency.

The assignment of IX follows straight from VIII and III. The assignments of the 2- and 3-substituted thiophenes were based on multiplicities and model compounds. 2-Methylthiophene and 3-methylthiophene, published previously¹², formed the bases for a and b.

The assignments of the double bonds in the chain in c to f rest on the known γ -substituent effect and the downfield γ' effect^{13,14} and on data obtained for substituted thiophenes with a CH_3 group in 2-position of the side chain.

* Both criteria are based on the assumption that the C(12')-C(1) part of II shows the same relative shifts as in squalene.

¹³ D. E. Dorman, M. Jautelat and J. D. Roberts, *J. Org. Chem.* **36**, 2757 (1971).

¹⁴ J. W. de Haan and L. J. M. van de Ven, *Org. Magn. Resonance* **5**, 147 (1973).

Table III Solvent effects in ppm on ^{13}C -NMR chemical shifts of compounds I to IX[⊙].

	I	II	III	IV	V	VI	VIII	IX
C(1't)	-0.04	-1.50 [†]	-0.05	-1.42 [†]	0	-1.10 [†]	-0.04	-0.04
C(1'c)	0	+2.02 [†]	0	+2.12 [†]	+0.08	+1.85 [†]	-0.13	-0.13
C(2')	+0.49	-0.26	+0.53	-0.13	+0.58	-0.13	+0.70	+0.84
C(3')	-0.08	-1.41	0	-1.68	+0.14	-0.92	+0.35	+0.45
C(4')	-0.08	-0.3	-0.09	-0.44	*	-0.09	-0.04	-0.13
C(5')	-0.04	+0.09	-0.09	0			-0.13	-0.14
C(6')	+0.35	+0.83	+0.40	+0.93			+0.53	+0.44
C(7')	0	-0.57	+0.13	-0.53			-0.13	+0.05
C(8')	-0.09	-0.05	0	-0.05			0 ^b	*
C(9')	-0.04	-0.09					-0.05	
C(10')	+0.36	+0.44					+0.5 ^a	
C(11')	0	-0.22					+0.10	
C(12')	-0.05	-0.09					-0.01	
C(12)	-0.05	-0.09					-0.01	
C(11)	0	+0.09					+0.10	
C(10)	+0.36	+0.22					+0.3 ^a	
C(9)	-0.04	-0.09					-0.05	
C(8)	-0.09	-0.05					+0.13 ^b	
C(7)	0	+0.09					0	
C(6)	+0.35	+0.27					+0.36	
C(5)	-0.04	-0.09					-0.05	
C(4)	-0.08	-0.09					+0.05	
C(3)	-0.08	-0.09					0	
C(2)	+0.49	+0.40					+0.53	
C(1c)	0	-0.09					~0	
C(1t)	-0.04	-0.14					0	
C(14')	-0.04	-0.09	*				0	*
C(15')	-0.04	-0.04					0	
C(15)	-0.04	-0.04					0	
C(14)	-0.04	-0.04					0	

⊙ Positive numbers refer to downfield shifts in glyme compared with hexane.

* Coinciding with the signal of C(1) of hexane.

† See text.

^a Solvent shifts may be reversed.

^b Solvent shifts may be reversed.

Discussion

As can be deduced from Table I, the chemical shifts of II are equal to those of I within 0.4 ppm with the exception of the C(1')-C(7') part. The differences between II and I are reproduced reasonably within the pair 2,6-dimethyl-2,6-octadiene (III) and its bromo, hydroxyderivative IV. This means that the conformations of IV and the C(1')-C(7') part of II will be similar, assuming that the unsubstituted systems will have similar conformational equilibria as well. In this stage, the discussion is thus confined to *local changes* upon 2,3-disubstitution of I. This is confirmed also by the solvent effects which, for the C(1')-C(7') part of II, are very much like those of IV, while the rest undergoes similar solvent effects as I^{1a}. See Table III.

The literature contains a number of data concerning attractive or repulsive steric interactions between alkyl groups, hydroxyl groups and bromine atoms in vicinal position in substituted alkanes¹⁵⁻¹⁸. The same is true for sterically induced ¹³C chemical shifts¹⁹⁻²¹. This opens the possibility of translating the solvent effects and shift differences between II and I or IV and III into conformational information. We shall examine the possible conformations around C(2')-C(3'), C(3')-C(4'), C(4')-C(5') and C(5')-C(6') separately and in combination. Around C(2')-C(3') the possible conformations are designated A, B, C as depicted in Fig. 3.

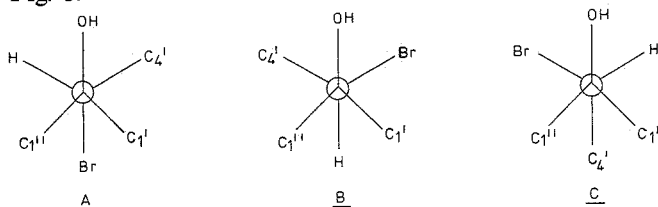


Fig. 3. Conformations around C(2')-C(3') in II, IV and VI.

Assuming approximate additivity of steric interaction energies of vicinal alkyl groups, hydroxyl groups and bromine atoms in the more crowded situation of II (as compared with disubstituted ethanes), leads to the result that conformer B will presumably have the lowest energy. Comparison of B and C yields a *gauche* CH₂-OH interaction in B versus a *gauche* CH₃-CH₂ in C. As the first action is attractive^{16,17} and the second one repulsive^{16,18}, the difference being approximately 1 kcal, conformer C will occur only in low concentrations. Comparison of B and A yields a *gauche* Br-OH interaction in B versus a *gauche* Br-CH₃ in A. Presumably, the first interaction is favoured, although no exact energies are known^{15,16}. In polar media, conformer B with *gauche* Br-OH will be more stabilized due to its larger dipole moment. The major change using polar instead of apolar media, will be from A to B. The ¹³C-NMR chemical shifts concomitant with such a conformational change can be estimated as follows. In Table IV the 1,4 *gauche* and *anti*-partners of both methyl groups C(1') and C(1'') as well as C(4') are given.

The change A → B will have no appreciable effect on C(4'), which is in agreement with the experiment. For C(1') a downfield shift is expected going from A to B, C(1'') will shift upfield by a smaller amount. It is assumed that the

Table IV

	A	B
C(1')	gBr + gR	gBr + aR
C(1'')	gBr + aR	aBr + gR
C(4')	gOH + gCH ₃ + aCH ₃	gOH + gCH ₃ + aCH ₃

R denotes C(5') through C(1) of compound II.

difference (*γ gauche* CH₃-CH₂)-(γ *anti* CH₃-CH₂) is about -6 ppm^{19,20}, the difference (*γ gauche* Br-CH₃)-(γ *anti* Br-CH₃) about -3 ppm*.

There is no *a priori* criterion, by which the two methyl signals near 27 ppm in II, IV and VI can be assigned specifically to C(1') and C(1''). Therefore, two sets of solvent effects are obtained for these compounds which should, however, be similar for each of them. The resulting effects lead to the prediction of a change from A to B in polar media**. The conformations around C(3')-C(4') can be visualized as depicted in P, Q, R of Fig. 4.

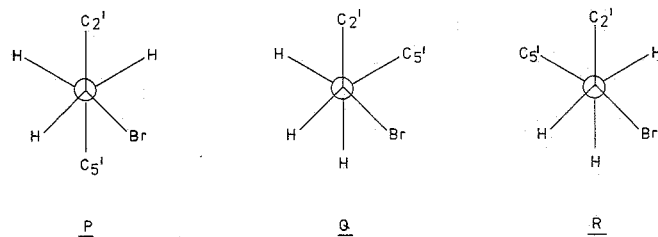


Fig. 4. Conformations around C(3')-C(4') in II and IV.

Presumably, P will be favoured because it minimizes R-R' interactions. Also, Anderson and Pearson¹⁵ showed that in 2-bromobutane conformer P is energetically favoured. Combination of conformations P with A, B or C will not lead to any significant extra steric interaction. Q and R combined with A, B or C will always lead to *syn* diaxial 1,5 interaction between C(5') and either C(1'), C(1'') or the OH group depending upon the combination. Therefore, Q and R will lead to significantly higher conformational energies. The conformations around C(4')-C(5') are X, X', Y, Y' and Z, Z', as given in Fig. 5. For reasons already discussed, the

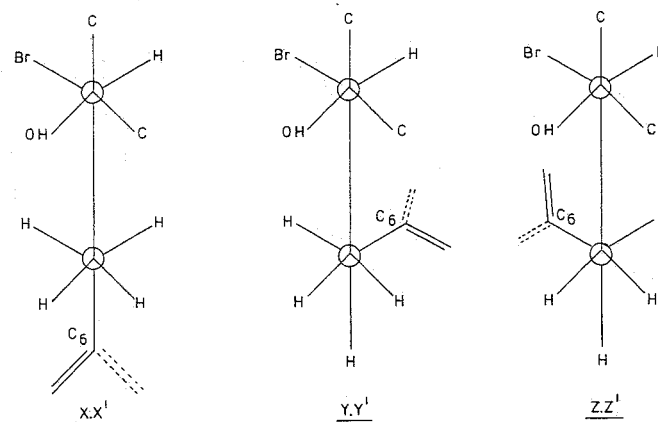


Fig. 5. Conformations around C(4')-C(5') in II and IV.

* Calculated via approximate energy differences between *gauche* and *trans* Br-CH₃¹⁵ using the Boltzmann equation and the known shift difference between propane and propyl bromide¹².

** The solvent shifts of C(2') and C(3') probably have to be ascribed to intrinsic properties of C atoms directly attached to either a hydroxyl group or a bromine atom. This became clear after a study of the solvent effects of 2-hydroxy-2-methylbutane and 2-bromobutane where appreciable effects were observed on C(2), but not on C(1) and C(4).

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C(1')-C(4') part is given in the B-P conformation. The conformation around C(5')-C(6') is drawn with one of the allylic hydrogens at C(5') *syn* with respect to the C(6')=C(7') double bond (see below). As C(5') carries two such hydrogens, there are also two possibilities for H *syn* to C(6')=C(7'). One of these possibilities, as viewed along C(4')-C(5') is drawn as full lines in Fig. 5, the other set is symbolized by the dashed lines, again viewing along the C(4')-C(5') bond.

Combination of either X or X' with P, Q, R is possible without extra steric interactions, apart from those already described (see above). Combinations of Y, Y', Z and Z' with Q and R are to be excluded for steric reasons. Moreover, the combination Z'P would involve a rather small distance between the bromine atom and C(15'). Finally, YP causes 1,5 diaxial positions of C(3') and C(15'). Thus in apolar media the system will be involved in a conformational equilibrium involving conformations XP, X'P, Y'P and ZP, in combination with A and B. Independent studies have led to the conclusion that in simple 1-alkene fragments situations a, b, c (see Fig. 1) are favoured over those with C(4) *syn* to the double bond (see Introduction). It can be shown that conformations (X,X'), (Y,Z') and (Z,Y') correspond to b, c and a of Fig. 1, respectively. In 1-pentene, conformation "a" has the lowest energy with "b" and "c" at slightly higher energies⁶. In the present case we are dealing with tri-substituted double bonds. Relative conformational energies cannot simply be transferred from 1-pentene to the molecules under study. However, it seems justified to assume that *syn*-H structures will still be favoured over *syn*-C analogues.

The differential shielding of C(6')=C(7'), with respect to the unsubstituted squalene means that in II conformations occur, in which steric interactions between this double bond and the substituted C(2')-C(3') part exist. In our view, it is mainly the contribution of conformers ZPA and ZPB that are responsible, because in ZP the bromine atom approaches the double bond C(6')=C(7') (see Fig. 5).

In principle, it would be possible that hetero-substitution introduces "precoiled" conformations like ZP. We believe, however, that in the unsubstituted olefins these "precoiled" conformers also occur, for two reasons. VFF calculations on 2,6-dimethyl-2,6-octadiene (III) predict the existence of "precoiled" conformers by combination of two "a-pentene" units (see Introduction). Secondly, from Table V it is obvious that the double bond C(10')=C(11') of II also shows a small [compared with C(6')=C(7')], but distinct deviation from the other double bonds.

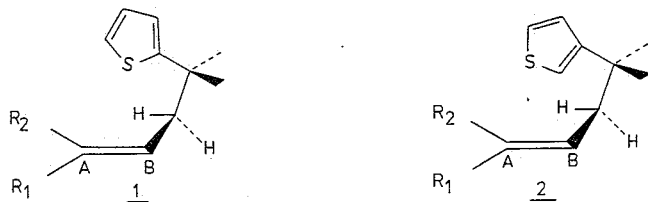
Upon using an apolar solvent instead of a polar one, no clear differences can be found between the observed changes in the chemical shifts of C(8') through C(1) in compound II and the corresponding solvent shifts in squalene itself. Probably, these solvent shifts represent the average intrinsic (van der Waals) solvent shifts, not due to conformational changes of squalene. Similar solvent shifts were also observed in model compounds^{1a}.

The solvent shifts of the C(1')-C(4') part of II and IV have already been discussed (see above). Marked changes in the chemical shifts of C(6') and C(7') in II and IV are observed going from hexane to "glyme". In both compounds the differences of these carbon atoms with respect to the unsubstituted olefins I and III decrease. Several explanations are

Table V Shift differences of the sp² carbons of II compared with I (see spectral assignments).

C(3)	+0.14	C(7)	+0.04	C(11)	+0.04	C(10')	-0.08 (+0.23)	C(6')	-1.41	C(2')	-OH
C(2)	+0.22	C(6)	+0.17 (+0.09)	C(10)	+0.23 (+0.0)	C(11')	+0.35	C(7')	+1.67	C(3')	-Br

Table VI ¹³C NMR chemical shift differences of C(A) and C(B) in 2- and 3-substituted thiophenes.



	R 1	R 2	(δ _{AB}) ₁	(δ _{AB}) ₂	Δ(δ _{AB})
a	H	H	-21.92	-22.89	0.97
b	CH ₃	CH ₃	8.46	7.36	1.10
c		H	2.21	1.10	1.11
d	H		2.31	1.23	1.08
e		H	1.41	0.35	1.06
f		H	1.45	0.24	1.21

possible. One of them pertains to the turning-away of the bromine atom from the double bond*. Lambert et al.²² showed that the interaction between a hetero-atom and a double bond is strongly solvent dependent. Even without a turning-away process the chemical shifts of C(6') and C(7') could still change as a consequence of the different media.

In the epoxides VIII and IX the chemical shift differences of C(6')=C(7') with respect to squalene are considerably smaller than in the corresponding monobromohydrins (see Table II). This is presumably caused by the fact that the interaction is now caused by the smaller (with respect to bromine) oxygen atom. Moreover, the epoxy oxygen is situated less favourable for interaction with the C(6')=C(7') double bond in a ZP-like conformation.

The double bonds adjacent to the epoxy groups in X and XI also deviate from squalene. Comparison of the epoxides VIII, IX, X and XI shows that the magnitude, but not the sign, of the deviation is dependent on the substitution pattern of the double bonds.

A rather clear example of chemical shift changes of a double bond with a δ -hetero-substituent may be found in a series of 2- and 3-substituted thiophenes with a double bond in the

side chain (see Table VI). The chemical shift differences of C(A) and C(B), δ_{AB} , in these systems show rather consistent differences, in the order of 1.0 to 1.2 ppm between the 2-substituted thiophenes and their 3-substituted counterparts. In 2-substituted thiophenes, δ_{AB} is more negative, the differences in δ_{AB} are practically independent of the mode of substitution of the C(A)=C(B) double bond [mono-, (Z)- and (E)-di- or tri-alkylated]. The spectra of 2- and 3-*n*-butylthiophenes were also recorded. Here, the chemical shift differences between the terminal methyl groups and the adjacent methylene signals are equal within the limits of accuracy. This proves that the effects described above require the presence of a double bond and a suitably positioned hetero-atom. This is in agreement with the small but consistent effects on C(6')=C(7') in VIII and IX and the larger effects on C(6')=C(7') in II and IV. Thus, although the nature of the interaction remains veiled, heterosubstitution at C(3') of squalene enables us to observe the specific steric relations which are also present in squalene itself.

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* More information regarding this point would require the complete analysis of the ¹H-NMR spectra of the C(3')-C(5') part of II and IV. Such a study is in progress.

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Reverse-phase high-performance liquid chromatography of protected peptides in the sequential synthesis of secretin and analogues

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Abstract. The synthesis of the gastrointestinal hormone secretin, a heptacosapeptide, and analogues by the Repetitive Excess Mixed Anhydride (REMA) method was monitored by reverse-phase high-performance liquid chromatography (HPLC). All intermediate protected peptides were thus purified on a micro-preparative scale (100–200 μ g, \geq 20 nmole).

The method allowed the separation of the protected diastereoisomeric [L-Ala⁴]- and [D-Ala⁴]-des-His¹-secretin.

An aspartyl-glycyl rearrangement could be detected in the protected pentacos-, hexacos-, and heptacosapeptide (secretin).

Introduction

In this Laboratory the gastrointestinal hormone secretin, a linear peptide amide of 27 amino acid residues, was synthesized by the Repetitive Excess Mixed Anhydride (REMA) method^{1,2}. This served two purposes: firstly, an investigation of the scope and limitations of the REMA method; secondly, the production of the precious secretin for urgent chemical and biological research.

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