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CENTENARY LECTURE*

Chemical Studies on Some Early Steps in the Biosynthesis of Squalene

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1 Introduction

The chemical problems that I want to discuss arise in the biosynthesis of squalene. It is well known that isopentenyl diphosphate (IPP) is isomerized into dimethylallyl diphosphate (DMAPP). The crucial step of attaching one molecule of each to the other, head-to-tail (1→4′), to produce geranyl diphosphate is carried out by prenyl transferase and this process is repeated to produce farnesyl diphosphate. Two molecules of farnesyl diphosphate are then attached to each other head-to-head (1→1′) by squalene synthetase to produce squalene. In an unexpected way this coupling goes through the remarkable three-membered ring intermediate presqualene alcohol, the cyclopropane ring of which is then reductively opened with introduction of a hydrogen atom from NADH to give squalene.[1a]

The stereochemistry of these processes has been elucidated beautifully, mainly thanks to the now classical investigations of Sir John Cornforth, Popjack, and others (Figure 1).

In order to account for these facts several hypotheses have been proposed. It has been suggested that the positive charge accumulating on C_3 in the prenylation step is neutralized by a nucleophilic group X from the enzyme coming in from the other side (*anti* addition). This would be followed by *anti* elimination with the *pro-R* proton. Alternatively, Rilling and Poulter made a strong case for ionization of the DMAPP. They reasoned that since the DMA cation comes in from underneath the IPP molecule so also does the counter ion PPO^- in the ion pair. If this is the base responsible for the proton removal, it is to be expected that the *pro-R* proton which points downwards will be removed preferentially. Recently the suggestion has been made that the process could be a concerted one (Figure 1).[1b] Presqualene formation would take place by electrophilic alkylation of one farnesyl PP molecule by another in the 1→2′ fashion with a nucleophilic X group stepping in as above. This would be followed by 1,3 elimination of the originally *pro-S* proton.

* Delivered at a Perkin Division Symposium on General and Synthetic Methods at the Scientific Societies' Lecture Theatre, London W1, on 25 January 1990.

[1] (*a*) C. D. Poulter and H. G. Rilling, in 'Biosynthesis of Isoprenoid Compounds', ed. J. W. Porter and S. L. Spurgeon, J. Wiley and Sons, 1983, Vol. 1, pp. 162–224; (*b*) J. W. Cornforth, in 'Structural and Functional Aspects of Enzyme Catalysis', ed. H. Eggerer and R. Heuben, Springer, 1981, p. 1.

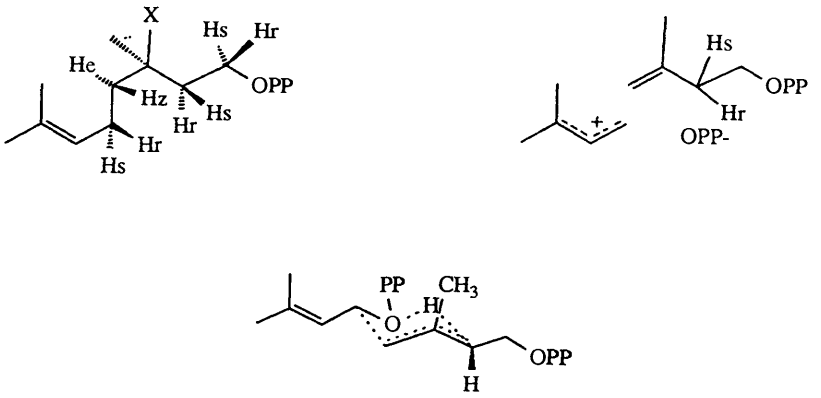


Figure 1

The chemical problems that we shall be concerned with in the prenyl transferase-catalysed reaction are: the alkylation of an sp^2 carbon atom (C-4) of IPP and the direction of the proton elimination in the intermediate, leading to geranyl diphosphate.

Two similar questions arise in the formation of presqualene alcohol: the alkylation of carbon atom C-2 of farnesyl diphosphate and the 1,3 elimination reaction leading to formation of a three-membered ring.

2 Prenyl Transferase

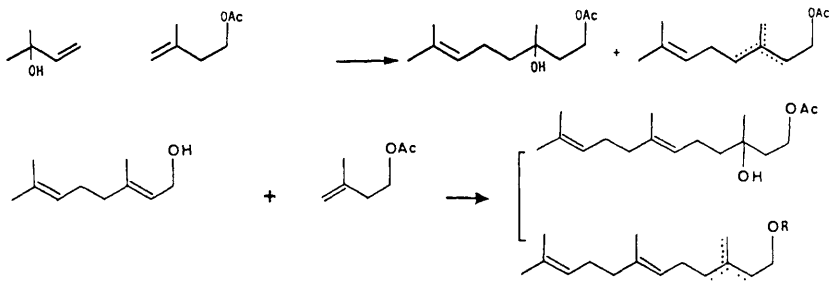
A. Allylation of sp^2Carbon Atoms.—Alkylation of alkenes is not as common as hydroxyalkylation or acylation. One reason is the difficulty of preventing further reaction with one or more molecules of alkene, leading to high molecular weight compounds.

H. Mayr[2] has nicely analysed the process and concluded that, in order to obtain the one-to-one adducts efficiently, the starting material must be more easily ionized than the product under the reaction conditions used. Since this is indeed the case when IPP is prenylated with DMAPP, it should be possible to avoid the formation of $1 + n$ adducts.

When the alkylating agent is allylic, however, another difficulty arises; since the first adduct will have a double bond in it, this will be a target for further allylation, leading to $n + 1$ adducts.

Two techniques have been developed to bring about the prenylation step. Dimethylallyl acetate or alcohol, with Lewis or Brønsted acids, could be used[3] as sources of electrophilic prenyl reagents, the formation of the one-to-one adduct being controlled by suitable choice of solvent and proportions of reagents.

[2] H Mayr, *Angew Chem, Int Ed Engl*, 1981, **20**, 184
[3] M Julia and C Schmitz, *Tetrahedron*, 1986, **42**, 2485 and references cited therein



Scheme 1

Typically, the reaction of dimethyl vinyl carbinol (2-methyl-but-3-ene-2-ol) with isopentenyl acetate (4 moles) in nitromethane, with trifluoroacetic acid (2.6 mol), led after 90 minutes at 0 °C to conversion of nearly one mole of each reagent and formation of 3-hydroxy-3,7-dimethyloctyl acetate (73%) together with a mixture of diene acetates (7%). Oxidation of the diol led to citral (Scheme 1). In a similar way IPA could be alkylated at C-4 with geraniol, leading to a farnesol derivative. When nerol was used, alkylation of IPA was also observed together with the well-known cyclization.[3]

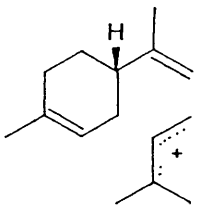
H. Mayr and his group[4] used prenyl chloride and ethereal $ZnCl_2$ to achieve efficient prenylation of a variety of olefins. In both cases an elimination reaction had to be carried out to produce the desired double bond; this will be discussed below.

These prenylation techniques could be used for a variety of syntheses. Prenylation of the terminal double bond of optically active limonene was easy but not very selective. The desired epimer could be separated. Optically active (+)- or (−)-bisabolol depending on the starting limonene could thus be prepared.[5] It is known that the corresponding carbonium ion is on the biosynthetic route leading from nerolidol to a series of polycyclic sesquiterpenes and eventually to cedrene. In other words, the natural sequence prenylation–prenylation (to farnesol)–cyclization(s) could be replaced by prenylation–cyclization–prenylation, followed eventually by another cyclization. Advantage could be taken of the ready availability of relay C_{10} compounds in high enantiomeric purity (Figure 2).

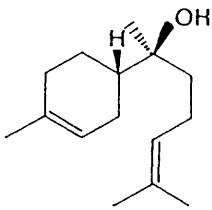
Prenylation of perillaldehyde was, as expected, much more selective. The epimeric one-to-one adducts with prenyl chloride could be separated and treated with Bu^{t}OK to form the cyclopropane ring. Further elaboration led to optically active sirenin.[6]

Other examples of prenylation at carbon are to be found in the biosynthesis of

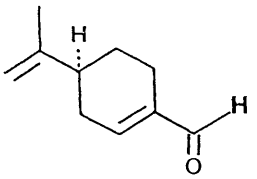
[4] H. Mayr and H. Klein, *J. Org. Chem.*, 1981, **46**, 4097; *Chem. Ber.*, 1982, **115**, 3528.
[5] D. Babin, J. D. Fourneron, and M. Julia, *Tetrahedron*, 1981, **37**, 1.
[6] P. Desbordes, Ph.D. Thesis, University of Paris VI, 1985.



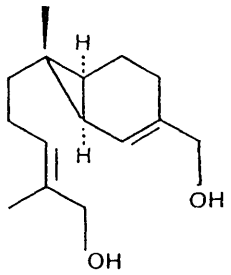
(-) - (S) - Limonene



(-) - (α) - (4S) - (8S) - Bisabolol



(-) - (S) - Perillaldehyde



(-) - Sirenin

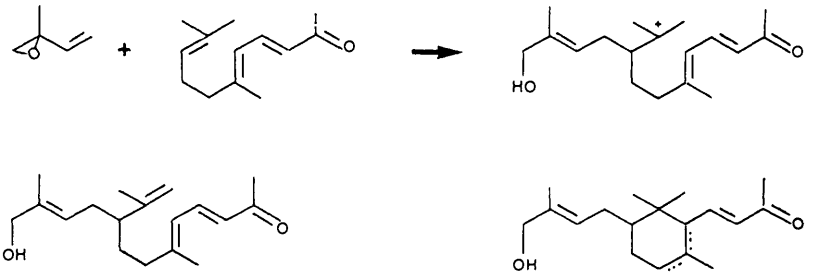
Figure 2

C_{45} and C_{50} carotenoids,[7] a number of representatives of which have been recently isolated from cultures of non-photosynthetic bacteria. They have one or two prenyl residues (often hydroxylated in the ω-position) attached to carbon atom(s) 2 and 2′ (carotenoid numbering). In some of them the polyene chain is acyclic, in others the usual cyclization has taken place leading the cyclohexane rings with the double bond in the usual α, β, or γ positions. This lengthening of the carotenoid molecules might be important to span the width of some membranes. Prenylation,[8] in particular hydroxyprenylation,[9] of pseudoionone (Scheme 2) led to a stereoselective synthesis of decaprenoxanthin and Cp450 (Scheme 3). Prenylation[10] resp. hydroxyprenylation[11] of geranylacetate was used for the synthesis of the isomeric sarcinaxanthin with a γ-double bond (Scheme 4).

Another question associated with the first step in the prenylation reaction is why should the 'X' nucleophile attack the C_{10} intermediate and not the prenylating reagent itself? Prenylation of IPA and Me_2S in competition showed that the sulphide is only moderately more reactive!

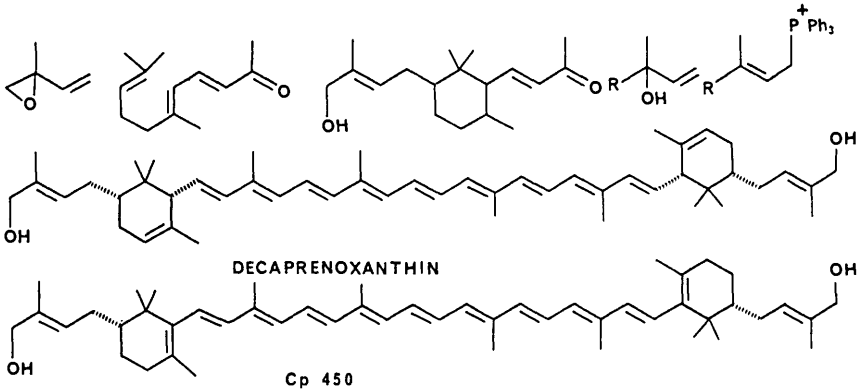
As by-products of this investigation it has been found that thioethers, even

[7] (*a*) O Isler, 'Carotenoids', Birkhauser, Basel, 1971, p 819, (*b*) G Britton, *Pure Appl Chem*, 1985, **57**, 701
[8] D Babin and M Julia, *Tetrahedron*, 1984, **40**, 1545
[9] J P Ferezou and M Julia, *Tetrahedron*, 1985, **41**, 1277
[10] M Julia and C Schmitz, *Tetrahedron*, 1986, **42**, 2491
[11] J P Ferezou and M Julia, *Tetrahedron*, 1990, **46**, 475



	α_T	α_C	β
E-ψ	38	35	27
Z-ψ'	95		
EQUIL.	41	4	55
IRONES, EQUIL.	37	10	53

Scheme 2



Scheme 3

diaryl thioethers, are efficiently converted into sulphonium salts when treated with alcohols, ethers, or alkenes in the presence of suitable acids.[12] The diphenyl alkyl sulphonium salts proved to be strong alkylating agents.[13] Conditions were found for the methylation of alkenes, which is carried out in living cells by methyl transferases using *S*-adenosyl methionine as a source of methyl groups.[14]

[12] B. Badet and M. Julia, *Tetrahedron Lett.*, 1979, **13**, 1101.
[13] (*a*) B. Badet, M. Julia, and M. A. Ramirez-Munoz, *Synthesis*, 1980, 926; (*b*) H. Mestdagh and M. Julia, *Tetrahedron*, 1983, **39**, 3, 433; (*c*) B. Badet, M. Julia, and C. Rolando, *Synthesis*, 1982, 291; (*d*) B. Badet, M. Julia, and C. Lefebvre, *Bull. Soc. Chim. Fr.*, 1984, **II**, 431.
[14] M. Julia and C. Marazano, *Tetrahedron*, 1985, **41**, 3717.

OH
O
OAc
OAc
OH
Z
Z = H
Z = OH
Z = OTMBz
X
Z
Z = H
Z = OTMBz
X = OAc
X = OH
X = OMs
X = SPh
X = SO2Ph
OSi(CH3)3
OTMBz
OR
Br
R
OTMBz
R = OH
R = Br
R = Ph3P+Br−
R = Si(CH3)3
R = Ac
OR
OR
SARCINAXANTHIN
R = TMBz
R = H
O
H
H
O

Scheme 4

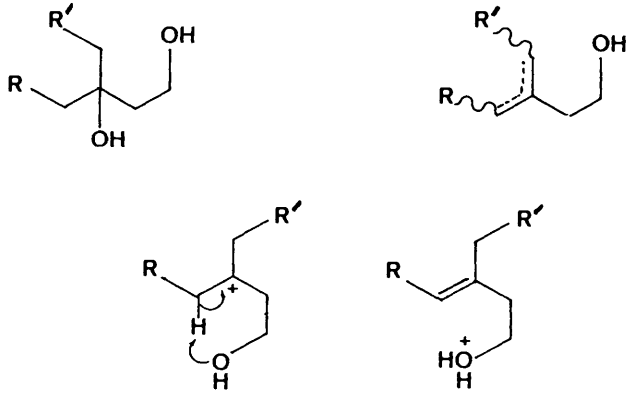


Figure 3

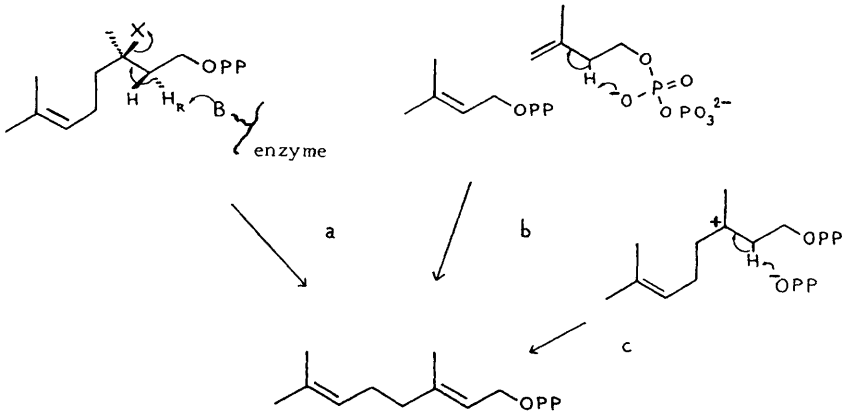
B. Regioselectivity of the Proton Elimination.—The problem of the *direction* of the proton elimination is not trivial: why is an allylic phosphate formed and not a *homo*allylic one; this would obtain if the proton had been removed from C-4 or the methyl group instead of from the 2 position. This is all the more a problem since in apparently similar situations the proton elimination has been found to take place in the other direction, leading to the β,γ-unsaturated alcohols (we will call them *retro*) instead of the α,β-isomers (which we will call *natural*) (Figure 3).

The Pfau–Plattner rule stated that in the acid-promoted dehydration of 1–3 primary-tertiary glycols, the homoallylic alcohols are formed.[15a] Arnold[15b] came up with an ingenious explanation in which the oxygen atom would act as an intramolecular basic relay to remove the proton through a very reasonable six-membered transition state. How is it then that the enzymic reaction gives the allylic derivatives?

A constructive suggestion has been made by E. Kosower:[16] the proton might be removed by the very diphosphate residue attached originally to the IPP, through a six-membered transition state. Phosphoric anions acting as intramolecular bases have been suggested in other cases (Scheme 5).[17,18]

Phosphoric esters with a C_5 or C_{10} terpene skeleton were selected as substrates.[19] The other residues in the phosphates were dimethyl (triesters), methyl hydrogen (diesters), or dihydrogen (monoesters). As leaving group, in the −3 position (X) a sulphonium group was introduced since these are known to go off reasonably easily under basic or acidic conditions, particularly the tertiary ones. (The basic site in the enzyme is supposed to contain an important thiol group, but a thiolate ion would be expected to be a pretty bad leaving group.)

[15] (*a*) A. St Pfau and P. Plattner, *Helv. Chim. Acta.*, 1932, **15**, 1250; (*b*) R. T. Arnold, *ibid.*, 1949, **32**, 134.
[16] E. Kosower, 'Molecular Biochemistry', McGraw-Hill, New York, 1962, p. 57.
[17] J. P. Richard, *J. Am. Chem. Soc.*, 1984, **106**, 4926.
[18] T. Widlanski, S. L. Bender, and J. R. Knowles, *J. Am. Chem. Soc.*, 1989, **111**, 2299.
[19] L. Jacob, M. Julia, B. Pfeiffer, and C. Rolando, *Bull. Soc. Chim. Fr.*, 1990, 719.



Scheme 5

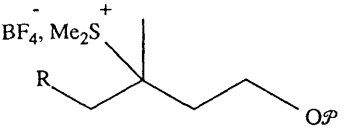
The mono-and di-esters were well equipped with an oxyanion for the removal of the proton, the triesters were included for comparison These were to be submitted to some elimination conditions, and the proportions of *natural/retro* elimination determined (Figure 4)

They were prepared starting from prenal resp citral through addition of MeSH, dimethylphosphorylation, suitable demethylations, and ternarization of the thioether groups with trimethyloxonium tetrafluoroborate A reference compound without any phosphoric ester was made for comparison

The sulphonio phosphoric esters were then submitted to basic conditions (Table 1) The reference compound gave mainly the *retro* olefin, as expected from a 'bad' leaving group undergoing the Hofmann-type elimination The rates of elimination went down from the tri- to the di- to the mono-ester The triester gave practically only the *natural* isomer, the diester gave 75% and the monoester about 40% It thus indeed appears that the phosphoric residue has a tremendous influence on the direction of the elimination This influence is the more marked with the tri-ester and fades away when going to the di- and the mono-ester This is not in agreement with Kosower's suggestion the triester has no oxyanions, the diester has one whereas the monoester has two with higher pK_a

Some elimination reactions were also performed under solvolytic conditions (Table 2), in the presence of Hunig's base in trifluoroethanol or methanol All esters reacted more slowly than the reference compound except the monoester which reacted much faster

All esters, like the reference compound, gave more substitution than elimination, however the monoester gave elimination exclusively The direction of elimination which now was mainly *natural* for the reference compound was now *retro* for the alcohol (Arnold effect) but also for the diester and even more so (90%) for the monoester These results again are not in agreement with the Kosower model



R=H , C_5

R=prenyl , C_{10}

$\mathcal{P}$ = $-P(OMe)_2$ triester

$-P(OMe)OH$ diester

$-P(OH)_2$ monoester

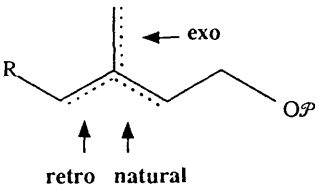


Figure 4

(i) *Proximity Effect.* The intramolecular basic relay mechanism was checked by moving the basic atom down the chain: the Arnold mechanism would then lead to formation of the *natural* isomers. The prenylation of mono and bis homologues of IPA, *i.e.* 4-methyl-4-pentenylacetate and 5-methyl-5-hexenylacetate gave indeed much higher (67% and 86% respectively) proportions of the *natural* isomer in the diene fraction.[3]

When the bis homologous sulphonium phosphoric tri- and particularly monoesters were however solvolysed[19] the elimination proved as easy as in the C_5 monoester, but now the *natural* isomer predominated to the extent of 90% in the product. That the effect was intramolecular was checked by comparison with a mixture of the reference compound and 2-octyl phosphate (Table 2).

The conclusion would be that a monophosphate ion can influence the direction of elimination but the transition state involved would have to be eight-membered. On the Kosower model a slight change would have to be made in that a terminal oxy-anion would be responsible for the proton removal. Such eight-membered transition states are not uncommon in proton transfers.[20]

[20] (*a*) B. Capon and S. P. McManus, 'Neighbouring Group Participation', Vol. 1, 56, Plenum Press, 1976; (*b*) H. Dugas and C. L. Penney, 'Bioorganic Chemistry', 206, Springer, 1981; (*c*) C. L. Penney and B. Belleau, *Can. J. Chem.*, 1978, **56**, 2396; (*d*) J. Hine, *Acc. Chem. Res.*, 1978, **11**, 1; (*e*) J. Jankowska and J. Stawinski, *Synthesis*, 1984, 408; (*f*) H. Eggerer, *Liebigs Ann.*, 1963, **657**, 212; (*g*) B. K. Tidd, *J. Chem. Soc., B*, 1971, 1168.

Table 1 *Basic eliminations*

Substrate (0 2 M)	*Solvent*	*Base*	*Time*/h	*Conversion*/%	*Total yield*/% *Nat + Ret*	*Natural/ Retro*[a]
Triester	CD_3OD	NaOD 10 N (3 eq)	0 5	79	72	100/0
C_5	$CHCl_3$/ MeOH(4/1)	NaOH 10 N (3 eq)	0 5	90	80	100/0
Diester C_5	CD_3OD	NaOD 10 N (4 eq)	30	95	90	75/25
Monoester C_5	CD_3OD	NaOD 10 N (4 eq)	96	95	95	42/58
Reference	$CDCl_3$/ CD_3OD	NaOD 10 N (3 eq)	24	90	86[b]	11/89
Triester C_{10}	CH_2Cl_2/ MeOH(4/1)	NaOD 10 N (3 eq)	0 5		88(68)[b]	95/5 (*E Z* = 27 68)
Diester C_{10}	MeOH	NaOH 10 N (4 eq)	30		90(77)[b]	85/15 (*E Z* = 27 58)

[a] Measured by ^{1}H NMR [b] Measured by GLC

Table 2 *Solvolysis of* C_5 *compounds* (0 2 M) *in* MeOH, $EtN(Pr^i_2)$ (1—1 5 *eq*) *at* 20 °C

Substrate	*Time/ Days*	*Conversion/ %*	*Yield*		*Natural/ Retro*
			Substitution	*Elimination*	
Triester	21	17			
Diester	21	35			
	1 year 5 °C	75	51	34	30/70
Monoester	7	83	0	100	8/92
Alcohol	100	85	70	18	20/80
Reference	45	90	84	13	70/30
Triester $N + 2$	7	70	80	20	58/42
Monoester $N + 2$	3	90	15	80	90/10
Reference + 2 octylphosphate	7	90	68	32	50/50

The very strong effect of the triphosphate group under basic conditions might be reconciled with Kosower's model by considering a five-coordinated phosphorous derivative formed by addition of base as in the first step of nucleophilic substitution at tetracoordinated phosphorus derivatives. Such an addition elimination mechanism would be expected to lead to some incorporation of CD_3O when using CD_3O^- in CD_3OD (or of EtO in EtOH, EtO^-) which was not observed.

(ii) *Electronic Inductive Effect.* It is known that in basic eliminations of 'bad' leaving groups in the Hofmann direction the deciding factor is the relative acidity of the protons to be removed on the respective β and β′ carbon atoms.

The phosphoric triester might be expected to be more strongly electron attracting than the diester and the monoester. Unfortunately no information could be found in the literature on the σ_I value of the phosphate esters groups. In order to check on the effect, a series of tertiary sulphonium salts was prepared with isopentane skeleton, substituted in the 1-position with groups of widely different electron attracting power (Table 3). The eliminations performed under two sets of basic reaction conditions showed the ratio of natural isomers to increase markedly with the σ_I value of the terminal group: from 12 to 97% when σ_I varied from 0 to 0.55.[21a]

Reasoning that the rate of abstraction of the remote hydrogen leading to the *retro* compound should be little influenced by the terminal group, a correlation was sought between the ratio *natural/retro* olefin and the σ_I value of the terminal group and a good straight line was obtained.

The ratio observed above with the phosphate triester would correspond to a σ_I value of that group of roughly 0.5 which is pretty high. Considering the tremendous importance of phosphoric esters in biochemistry, the desired σ_I value was determined by several methods.[21b]

Correlation with the pK_a of $Z\text{–}CH_2\text{–}COOH$; the pK_a value measured (2.4) gave for σ_I 0.57 for the dimethyl ester group. Correlation with the δ value of $Z\text{–}CH_2\text{–}COOR$ in ^1H NMR gave 0.5. Correlation with the pK_a of $Z\text{–}CH_2\text{–}CH_2\text{–}NMe_2$ gave 0.4. The preferred method used the F NMR of *m*-fluorophenyl phosphates and diphosphates which were prepared by standard methods.

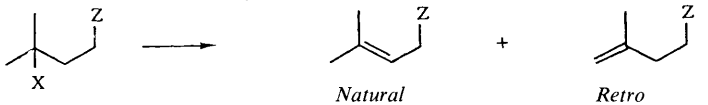
Dimethylphosphate 0.51; dihydrogen 0.38; Mg salt 0.28; Na salt 0.24.

The diphosphate after correction for change of solvent had: Mg salt 0.39; Na salt 0.34 (for comparison the values for F and $PhSO_2$ are 0.52 and 0.55 respectively).

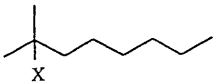
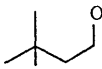
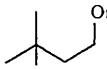
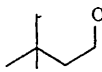
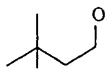
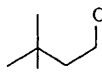
The phosphoric triester thus appears to be an extremely strong electron attracting group. The monoester diphosphate is much weaker, even with a magnesium counter ion (which is known to favour the ionization of allylic

[21] (*a*) B. Badet, M. Julia, J. M. Mallet, and C. Schmitz, *Tetrahedron*, 1988, **44**, 2913; (*b*) M. Julia and J. M. Mallet, *Tetrahedron Lett.*, 1986, **27**, 5851.

Table 3 *Elimination in t-amyl derivatives*



$X = SMe_2^+, BF_4^-$

		NaOH/MeOH, CH_2Cl_2(1/4) r.t. 24h		Bu^tOK(1 eq.)DMSO r.t. 24h	
	σ_I	*Total yield*	*Natural / Retro*	*Total yield*	*Natural / Retro*
	−0.08	86	12/88	82	9/91
	0.16	37	1/99	45	1/99
	0.29	87	63/37	58	43/57
	0.33	Hydrolysis		55	54/46
	0.37	99	82/18	94	52/48
	0.55	99	97/3	24	71/29

derivatives).[22] However, this effect might contribute to the regioselectivity of the proton removal.

3 Squalene Synthetase, Presqualene Alcohol (PSA) Formation

Four suggestions have been made in the literature for this part of the biosynthesis[23] (Figure 5). The first assumed alkylation 1,2′ of one farnesyl PP by the other followed by 1,3 elimination.[24] The difficulty associated with this 1,3 elimination was circumvented in the second suggestion[25] in which the product formed in the first alkylation would undergo a double bond shift; a homoallylic rearrangement would then lead to formation of the three-membered ring. In the

[22] M V Vial, C Rojas, G Portika, L Chayet, L M Perez, O Cori, and C A Bunton, *Tetrahedron*, 1981, **37**, 2351
[23] Ref 1, pp 413–441, C D Poulter, *Acc Chem Res*, 1990, **23**, 70
[24] H C Rilling, C D Poulter, W W Epstein, and B Larsen, *J Am Chem Soc*, 1971, **93**, 1783
[25] E E van Tamelen and M A Schwartz, *J Am Chem Soc*, 1971, **93**, 1780

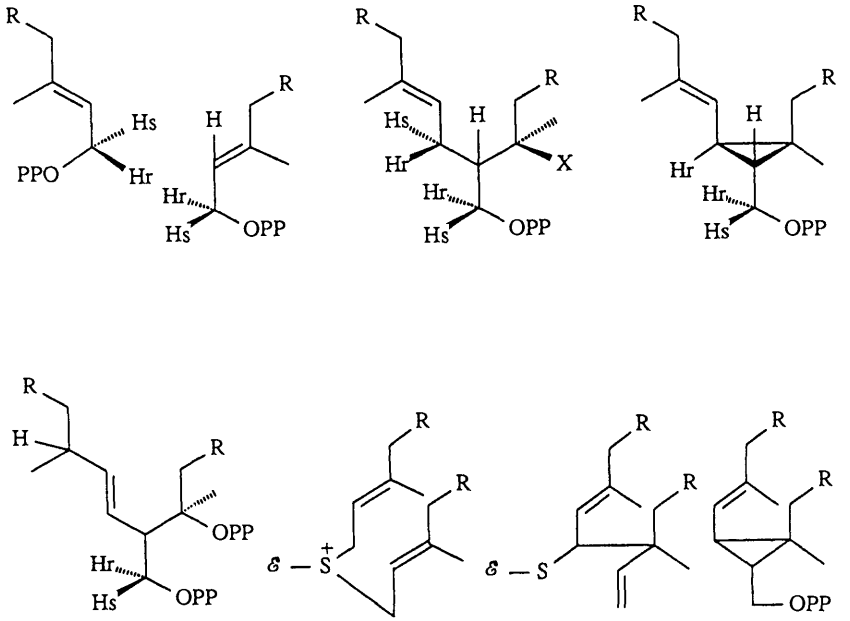
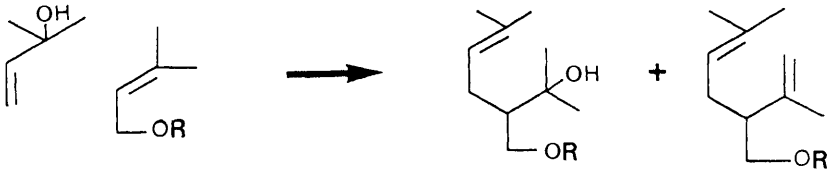


Figure 5

third suggestion,[26] the thiol group would be alkylated by the two farnesyl units, the sulphonium salt would undergo a base-promoted (2,3) shift to give a homoallylic thioether which could cyclize to form a cyclopropane. Interestingly, the use of chiral bases led to enantioselectivity. Lastly, the ylid derived from diphenyl (but not dialkyl) methyl sulphonium salts was shown to convert unactivated olefins into cyclopropanes under copper catalysis, which could be a model for PSA formation.[27]

Several syntheses of PSA have been published: on treatment of farnesol (*t,t*) with 'diazofarnesane' in the presence of zinc iodide[28] the desired alcohol was obtained in admixture (70:30) with an isomer in 25% yield. The tosylhydrazone of glyoxylyl chloride was converted[29] into farnesyl diazoacetate which was cyclized (20%); the CH_2 branch of the lactone formed was elaborated into a C_{13} terpene chain.[29] Reaction of farnesyl sulphone with methylfarnesoate produced the corresponding ester which was reduced to PSA.[30]

[26] (*a*) J. E. Baldwin, R. E. Hackler, and D. P. Kelley, *J. Am. Chem. Soc.*, 1968, **90**, 4758; (*b*) G. M. Blackburn, W. D. Ollis, C. Smith, and I. O. Sutherland, *J. Chem. Soc., Chem. Commun.*, 1969, 99; (*c*) B. M. Trost and W. G. Biddlecom, *J. Org. Chem.*, 1973, **38**, 3438 and references cited therein.
[27] T. Cohen, C. Herman, T. M. Chapman, and D. Kuhn, *J. Am. Chem. Soc.*, 1974, **96**, 5627.
[28] L. J. Altman, R. C. Kowerski, and H. C. Rilling, *J. Am. Chem. Soc.*, 1971, **93**, 1782.
[29] R. M. Coates and W. H. Robinson, *J. Am. Chem. Soc.*, 1971, **93**, 1785.
[30] R. V. M. Campbell, L. Crombie, D. A. R. Findley, R. W. King, G. Pattenden, and D. A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 1975, 897.



Scheme 6

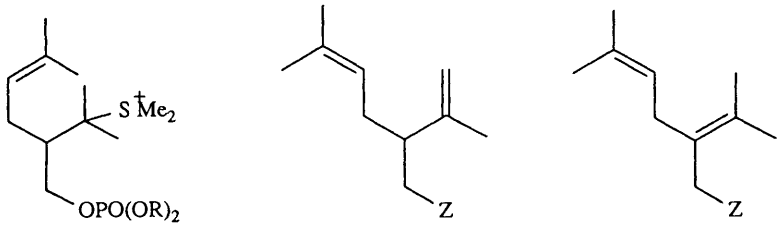


Figure 6

A. 1,2′ Alkylation.—Prenylation of DMA acetate with 2-methyl-but-3-en-2-ol took place readily in the 2-position[31,3] to give 2-(2′-hydroxy-2′-methylethyl)-5-methyl-hex-4-ene-1-ol, in which the trisubstituted double bond proved more reactive than DMA itself so that the conversion had to be kept low. Some DMA ethers however proved more reactive than an isolated trisubstituted double bond. This suggests a way in which the selectivity in the alkylation of farnesyl diphosphate in the C-2 rather than the C-6 or the C-10 position might be achieved. Dehydration of the diol led to lavandulol[32] (Scheme 6).

B. 1,3′ Elimination to a Cyclopropane Ring.—(i) In order to find out whether phosphoric residues might favour the 1,3 ring closure we prepared the necessary sulphonio phosphates and treated them with base as above: only lavandulol and isolavandulol were detected,[9] no favourable influence of the phosphoric residues was noted (Figure 6).

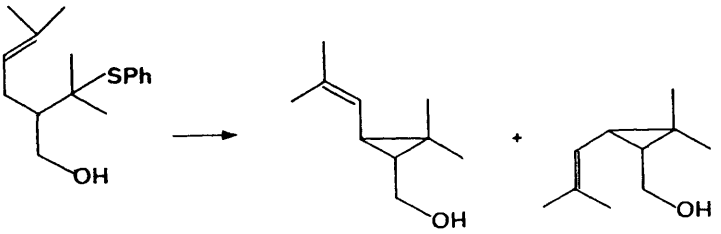
(ii) It was then reasoned that a very bad leaving group might not undergo the 1,2 elimination easily, in which case, a 1,3 elimination, favoured by allylic activation of the proton, might become the preferred pathway. The necessary starting material was made with phenylthio as a leaving group, using the information provided by the fundamental studies on elimination reactions.[33] When the phenylthio alcohol was treated with BuLi, chrysanthemol (*c* + *t*) (15%) was indeed formed together with 50% starting material.[34] The hydroxy

[31] (*a*) M Julia and L Saussine, *J Chem Res*, 1978, (S), 269, (M) 3420, (*b*) M Julia, C Perez, and L Saussine, *J Chem Res*, 1978, (S), 311, (M) 3877, (*c*) D Babin, J-D Fourneron, and M Julia, *Soc Chim Fr*, 1980, II, 588, 600

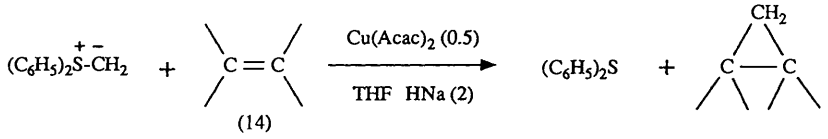
[32] H Schinz and G Schappi, *Helv Chim Acta*, 1947, **30**, 1483

[33] C J M Stirling, *Acc Chem Res*, 1979, **12**, 198

[34] B Babin, J D Fourneron, L M Harwood, and M Julia, *Tetrahedron*, 1980, **37**, 325



Scheme 7



Scheme 8

group is not inert since the desoxy analogue reacted more slowly.[35] So indeed chrysanthemol (specifically PSA) could be produced in this way but it is unlikely that a strong enough base would be available in living cells (Scheme 7).

In a similar reaction limonene could be converted into car-2-ene.[36]

(iii) Since we had a very convenient access to sulphonium salts we investigated the cyclopropane formation described by Cohen *et al.*[27] (Scheme 8).

Among the many modifications of the reaction conditions which we tried, substitution of an alkyl chain in the 3-position of acac did lead to a substantial improvement, the yield with cyclohexene increasing to 80—85%. A methyl substituent already had some favourable influence: H, 28; Me, 57; Am, 86; Oct, 77; Lauryl, 77% with only 1 eq. of NaH and 0.25 eq. of catalyst.[37]

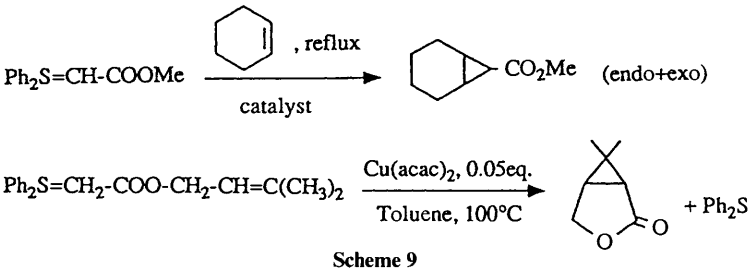
A number of olefins could be efficiently converted into the corresponding cyclopropanes. The reagent seems to be *electrophilic* in that electron rich olefins react better. However, tetramethyl ethylene was unchanged and prevented the reaction of another olefin . . .

The methoxycarbonyl-substituted sulphonium ylid was next prepared by a new route and converted cyclohexene into methyl norcarane carboxylate in 70% yield. It thus could offer a safe alternative to the use of methyl diazoacetate (Scheme 9).

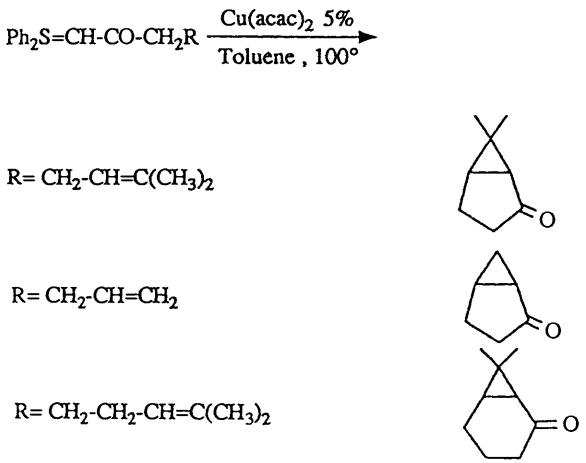
Intramolecular versions of this cyclopropanation proved very efficient: the cyclopropane bicyclic lactone is a key compound for the synthesis of *cis*-pyrethroids. The necessary starting material was produced by transesterification of methyl diphenylsulphonio acetate with prenol. Deprotonation followed by treatment with $Cu(acac)_2$ led to the lactone in 72% yield.[37]

In a similar way, acylation of diphenylsulphonium methylid by the appropriate

[35] G. N. Klumpp, *Recl. Trav. Chim. Pays Bas*, 1986, **105**, 1.
[36] J. D. Fourneron, L. M. Harwood, and M. Julia, *Tetrahedron*, 1981, **38**, 693.
[37] B. Cimetière, Ph.D. Thesis, University of Paris VI, 1989.



Scheme 9



Scheme 10

unsaturated acid chloride followed by $Cu(acac)_2$ treatment provided (50—70%) the bicyclic cyclopropane lactones which are usually[38] produced by cyclization of the diazoketones (Scheme 10).

It is known from the work of E. J. Corey[39] that electron-deficient olefins can be converted into cyclopropanes by sulphonium ylids (nucleophilic attack). This raises the question of how the copper ion manages to convert a nucleophilic reagent into an electrophilic one.

It is known that in heteroatom-stabilized carbanions the hetero substituent often facilitates the metallation in the α-position but the nucleophilic properties are somewhat weakened. On the other hand, the conversion of the α-carbon into a carbanion, more or less closely associated with its counter ion, sometimes facilitates the departure of the hetero group and is responsible for enhanced *electrophilic* properties compared with the non-metallated species.

[38] (*a*) G Stork and J Ficini, *J Am Chem Soc*, 1981, **83**, 4678, (*b*) S D Burke and P A Grieco, *Org React*, 1979, **26**, 361

[39] E J Corey and M Jautelat, *J Am Chem Soc*, 1967, **89**, 3912

It is not necessary for the metal M in a carbenoid RR′CMZ[4] to be a transition metal: cases are known where electrophilic behaviour was displayed by lithium species with Z = halogen[40a] or Z = oxygen,[40b,c] or magnesium species with Z = oxygen,[41] or zinc species with Z = halogen[42] (for a review see reference 43).

Now taking into account the fact that phosphoric esters are strongly electron withdrawing groups (see above) and would therefore make the protons on the α-carbon atom very acidic, the conversion of farnesyl diphosphate into a carbenoid seems feasible (Scheme 11). The enzyme is known to contain magnesium ions.[44] Such a carbenoid would be expected to convert an olefin (particularly another molecule of farnesyl diphosphate) into a cyclopropane derivative: after electrophilic attack at carbon atom C-2 the much discussed 1,3-elimination would be straightforward since the proton involved would have been removed already! As in the case of the sulphonium ylid discussed above,[26] the chirality could be controlled by the chriality of the enzymic base.

It should be pointed out that diazoalkanes in the presence of metallic salts behave as electrophilic carbenoids[45] so that the Altman–Kowerski–Rilling synthesis[28] is closely related to the proposed biosynthesis.

Two more points must be discussed. Although the diphosphate mono esters have a moderate electron attracting power the formation of the carbenoid by removal of the allylic proton might be considerably helped by the assistance which a suitably placed OLi group can bring.[35,46] On the other hand, it is known that allylic phosphates on treatment with strong base undergo facile Wittig rearrangement to give the isomeric hydroxy allyl phosphonate esters.[47] Preliminary experiments[48] showed however that such a carbanion can be trapped.

4 Concluding Remarks

In summary I have tried to say what happened when we asked simple chemical questions about a couple of steps in the biosynthesis of terpenes. As usual, things turned out to be much more complicated than first envisioned. Some understanding of the processes involved has emerged together with a number of new reaction possibilities which might be of use in various fields of organic chemistry. It is of course realized that many questions still remain to be answered.

[40] (*a*) G. Wittig and H. Witt, *Chem. Ber.*, 1941, **74**, 1474; (*b*) G. Wittig and L. Lohmann, *Liebigs Annalen*, 1942, **550**, 260; (*c*) A. Lüttringhaus, C. von Saëf, E. Sucker, and G. Berth, *ibid.*; 1947, **557**, 46.

[41] B. Castro, *Bull. Soc. Chim. Fr.*, 1967, 1533.

[42] (*a*) H. E. Simmons and R. D. Smith, *J. Am. Chem. Soc.*, 1958, **80**, 5323; (*b*) H. E. Simmons, T. L. Cairns, S. A. Vladuchik, and C. M. Hoiness, *Org. React.*, 1973, **20**, 1.

[43] (*a*) G. Köbrich, *Angew. Chem., Int. Ed. Engl.*, 1972, **11**, 413; (*b*) J. Villieras, M. Rambaud, B. Kirschlager, and R. Tarhouni, *Bull. Soc. Chim. Fr.*, 1985, 837.

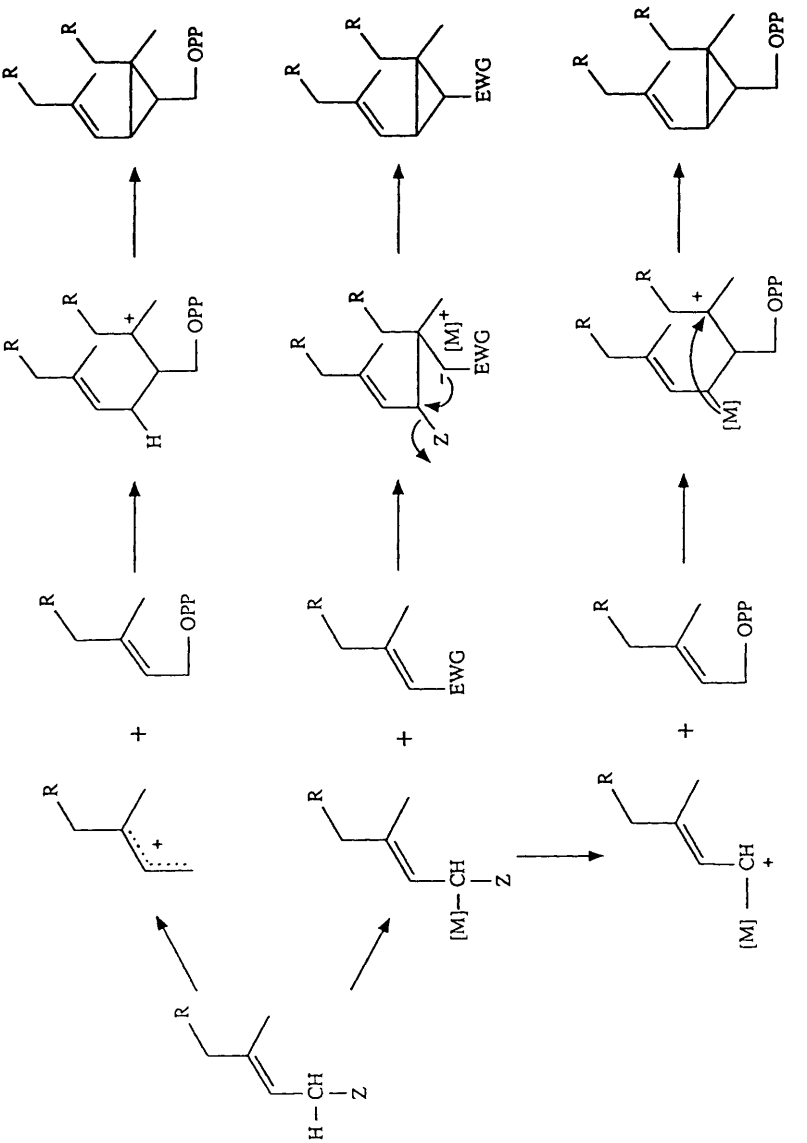
[44] T. Nishino, H. Takatsuji, S. Hata, and H. Katsuki, *Biochim. Biophys. Res. Commun.*, 1978, **175**, 867.

[45] (*a*) G. Wittig and K. Schwarzenbach, *Liebigs Annalen*, 1961, **650**, 1; G. Wittig and F. Wingler, *ibid.*, 1962, **656**, 18; *Chem. Ber.*, 1964, **97**, 2139, 2146; (*b*) H. Hoberg, *Liebigs Annalen*, 1962, **656**, 1, 15.

[46] A. R. Katritzky, W. Q. Fan, and K. Akutagawa, *Synthesis*, 1987, 415.

[47] A. Sturtz and B. Corbel, *C.R. Acad. Sci., Paris*, 1973, **276**, 1807.

[48] P. Mulot, A. Puechberty, and J. N. Verpeaux, unpublished results.



Scheme 11

Acknowledgements.—All this would not have been possible without the skill, ability and enthusiasm of the young colleagues whose names appear in the references. They contributed very much to the results and deserve my sincerest thanks. I also acknowledge with gratitude the generous support of the E.N.S., the U.P.M.C., the C.N.R.S., and the Rhône-Poulenc Company.