CHAPTER-III

FURTHER STUDIES ON BIOMIMETIC CYCLISATION OF ALLYLIC DIETHYL PHOSPHATE ESTERS

ABSTRACT

This chapter consists of two sections. Section-I discusses the effect of various divalent metal ions in biosynthesis of terpenoids and in biomimetic transformations. Section-II describes our attempts to enhance the formation of cyclic products in the reaction of farnesyl diethyl phosphate (2(Z) and 2(E) mixture) using various MgO/Al_2O_3 catalysts. These attempts were, however, unsuccessful.

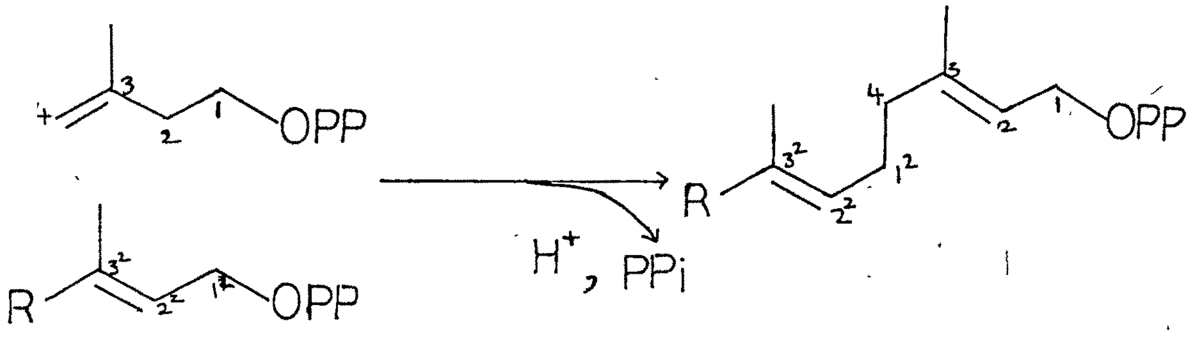
In an effort to evaluate the importance of a divalent metal ion in such biomimetic cyclisations, we have investigated these cyclisations with Grignard reagents. This in turn led to investigate organo-lithium compounds as reagents for such cyclisations. Both Grignard and organo-lithium reagents were found to be effective in such cyclisations. Particularly EtMgBr and n-BuLi have been found to induce cyclisation of allylic diethyl phosphate esters.

However, the reactions of allylic diethyl phosphate esters with other non-metallic organic bases (eg. pyridine and DBU), inorganic metallic bases (eg. CaH_2) and amphoteric salts (eg. benzothiazolium salt) were unsuccessful.

SECTION-I

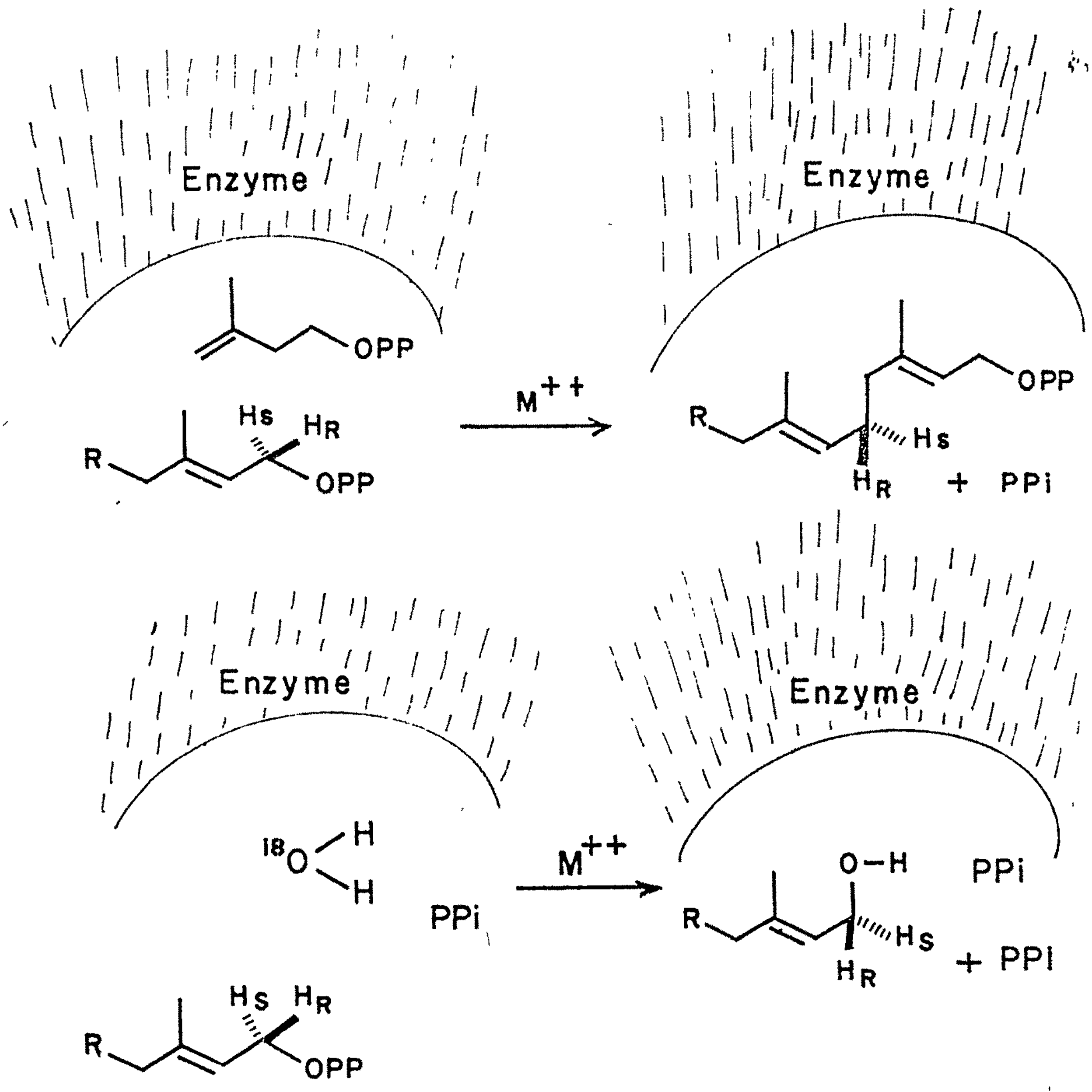
INTRODUCTION : EFFECT OF DIVALENT METAL IONS IN BIOMIMETIC TRANSFORMATIONS

Biosynthetically, terpenes are composed of 2-methyl butyl (isoprene) units, which may either be used to construct the entire carbon skeleton of a molecule or may be attached to a non-terpene fragment. The biological process for attacking a terpene fragment to another moiety is a prenyl transfer reaction - a bisubstrate reaction in which one of the partners is an allylic terpene pyrophosphate and the other can be one of a variety of terpene or non-terpene compounds. The basic polymerisation reaction of polyterpenoid biosynthesis is the condensation of C-4 of isopentenyl PP with the $C\text{-}1^2$ of an allylic pyrophosphate and the generation of the five carbon homologue of the allylic substrate. This reaction is catalyzed by prenyl transferase. Recently strong evidence was presented that this reaction proceeds by an ionization-condensation-elimination mechanism[1].



The enzyme requires a divalent cation, Mg^{+2} or Mn^{+2}, for activity, and since it was well established that the substrates for prenyl transferase bind to the enzyme in the absence of these cations, it was concluded that these ions are required for catalysis rather than binding[2]. Thus, it is possible that the role of metal ions is to assist in ionization of allylic substrate (Fig. 1). This consideration, along with the indication that the divalent cations enhance the solvolysis of allylic pyrophosphates[3], has provoked many investigators to undertake a more thorough examination of the solvolysis of allylic pyrophosphates in the presence of Mg^{+2} and Mn^{+2}.

Hydrolysis of GPP (geranyl pyrophosphate) is catalysed by salts of Mn^{+2} and involves C-O bond cleavage. GPP is a postulated precursor in the biosynthesis of cyclic monoterpenes such as limonene[4,5], although the E-configuration of GPP precludes direct cyclization[6]; the Z-isomer, neryl pyrophosphate (NPP), is a precursor of bicyclic monoterpenes in pinus radiata[7]. Recent enzymic studies with carbocyclases from citrus limonium[8] and Salvia officinalis[9] showed that GPP is also a precursor of cyclic monterpenes but because enzymic isomerization of GPP to NPP was excluded[8,9], rotation or interconversion of an enzyme bound carbocationic species derived from GPP was postulated[6,8].

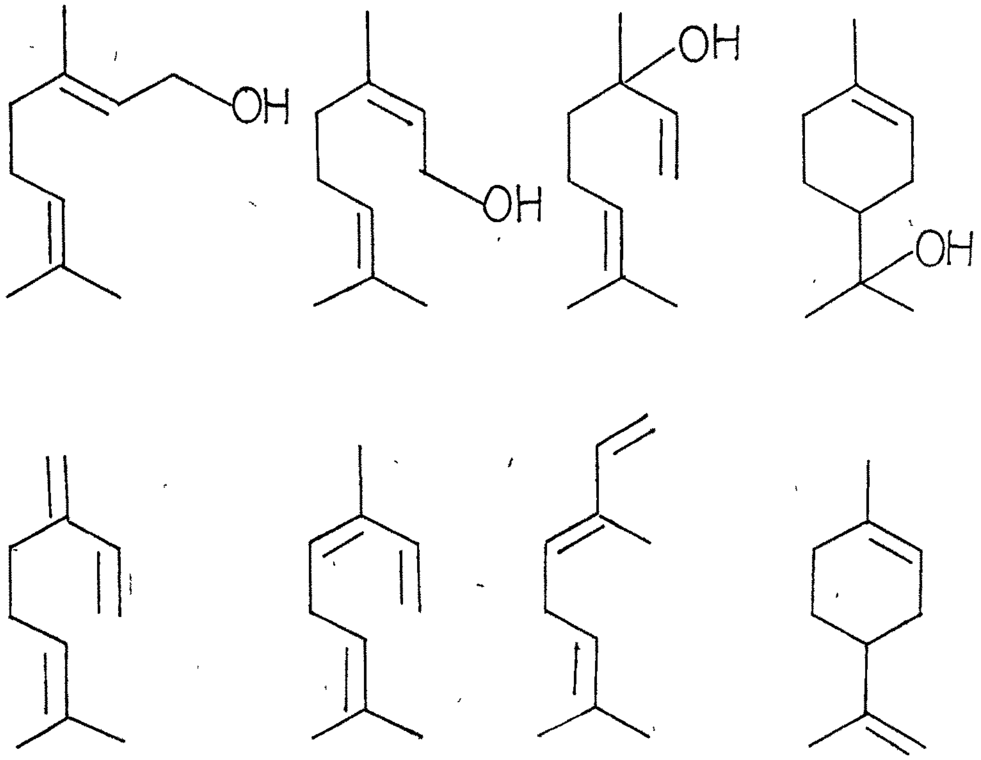


A schematic representation of the catalytic site of prenyl transfer catalyzing prenyl transfer to isopentenyl pyrophosphate or water

Fig.1

Carbocyclase is a Mn^{+2} dependent enzyme[8], and consistently non-enzymic hydrolysis of farnesyl[10], geranyl and chrysanthemyl pyrophosphate[11] are speeded, at neutral pH, by Mg^{+2} or Mn^{+2} ions. These observations suggest that metal catalyzed solvolysis of GPP could be a model for the enzymic reaction. The present introduction describes the effect of Mn^{+2} on the hydrolysis of GPP and geranyl monophosphate (GP), in an attempt to understand the role of Mn^{+2}, or the other divalent metal ions, in the biosynthesis of cyclic monoterpene hydrocarbons in plant tissues.

In the presence of excess Mn^{+2} at pH 7.0 and 40° GPP is transformed into a mixture of prenyl alcohols and terpene hydrocarbons (Scheme-I) and the overall reaction is of first order with respect to GPP.



SCHEME -I

Fig. 2 shows the dependence of the initial rate of formation of products upon (GPP), with a large excess of Mn^{+2}. If log (dp/dt) (where p is product) is plotted as a function of log GPP concentration, a stright line of unit slope is obtained.

The rate of hydrolysis of 10^{-4}M GPP at 40° and pH 7 is increased by a factor of, upto 50 by Mn^{+2}. The first order rate constants (K obs) are 3.4×10^{-7} S^{-1} in the absence of metal and 1.8×10^{-5} S^{-1} in 10^{-2} M Mn^{+2}. Complexing of Mn^{+2} with EDTA eliminates the catalysis.

Added acetone or dioxane has little effect on the spontaneous hydrolysis, but with 0.005 M Mn^{+2}, organic solvent at a mole fraction of 0.2 reduces the rate by approximately 80 % . These effects were not investigated further.

The products at pH 7 are markedly affected by Mn^{+2}. The spontaneous hydrolysis probably occurs largely with P-O rather than C-O bond fission, or by concerted nucleophilic substitution, and therefore gives only geraniol. Hydrolysis of GPP in dilute acid occurs with C-O bond fission[12,13], and the products are qualitatively similar to those of Mn^{+2} catalyzed hydrolysis, except that Mn^{+2} favours hydrocarbon formation. (Table-1)

TABLE-1 : PRODUCTS OF HYDROLYSIS OF GPP[a]

Products	10^{-2}M Mn^{+2}	0.42M H_2SO_4[b]
alcohols	94 (100)	98.3
hydrocarbons	6[c]	1.7
linalool	71.0 (29.0)	77.7
geraniol	19.0 (65.0)	17.1
nerol	0.6 (2.0)	0.9
α-terpineol	3.3 (3.3)	2.6
myrcene	2.7	0.3
ocimene (E+Z)	1.7	1.2
limonene	1.2	0.07
unidentified	0.3	-

(a) Moles % of products at 40°C with 10^{-4}M GPP and TES buffer, pH 7.0, unless specified. Values in parentheses are in the absence of Mn^{+2};

(b) at 20°C; (c) no hydrocarbons were detected in the absence of Mn^{+2}.

No buffer effects were observed on products or rates either in the presence or absence of metal. This is to be expected, because potentiometric[14] and EPR binding measurements have shown that there is no significant interaction between TES buffer base and Mn^{+2}. Increase of ionic strength with 0.3M KCl decreases the rate of reaction by 20 % in the presence of 10^{-4}M Mn^{+2} and by 50 % in its absence at pH 7 and 40°.

Analysis at partial reaction in the presence of Mn^{+2} showed that no geranyl monophosphate is formed, and solvolysis did not proceed stepwise as with the enzymic hydrolysis[15]. Control experiments with nerol, geraniol, linalool and α-terpineol showed that Mn^{+2} did not catalyze their interconversion or dehydrations. No other prenyl pyrophosphate was found in the reaction mixture, thus excluding isomerisation of the substrate.

Effect of [Mn^{+2}] on hydrolysis rate:

The dependence of the rate of hydrolysis of GPP on [Mn^{+2}] is shown in Fig. 3 for reaction using either $MnCl_2$ or $MnSO_4$. At high [Mn^{+2}] the rate constants reach limiting values so that GPP must be converted completely into a reactive species, x GPP- y Mn^{+2}. Because the association constant for formation of the 1:1 complex is 2.5×10^4 M^{-1} at 25°C[11], at 5×10^{-4} M Mn^{+2} and 10^{-4}M GPP over 90 % of the GPP is present as the 1:1 GPP-Mn complex.

Fig. 3 shows that the maximum rate of hydrolysis is obtained at a much higher concentration of Mn^{+2}, so that the reactive species is a complex with a Mn^{+2}/GPP ratio > 1.

Prenyl pyrophosphates and phosphates behave similarly with respect to hydrogen ion catalysis[13,16], but quite differently with respect to metal ion catalysis. At pH 7.0 Mn^{+2} retards the hydrolysis of GP (Fig.4). No hydrocarbons were formed and geraniol (94 %) is the major product, and the products are similar to those from spontaneous hydrolysis of the monoanion, via metaphosphate ion. Complexing of Mn^{+2} to the phosphate moiety will inhibit elimination of metaphosphate ion. The differences between GPP and GP as substrates are readily understandable because phosphate monoesters can not readily chelate with a metal ion.

The rate of hydrolysis of the 2,3-dihydro analog of GPP, citronellyl pyrophosphate (CiPP), is increased by only a factor of five by 10^{-2} M Mn^{+2} at pH 7.0 and 40°, and no hydrocarbons are formed. The allylic double bond in GPP favours formation of a carbocationic species and may also help to stabilize a reactive metal-substrate complex.

Effect of pH :

Fig.5 shows that a change in pH from 6.5 to 7.0 has little effect on the rate of solvolysis, and a further change to pH 7.6 reduces the rate by only one third, over a wide range of [Mn^{+2}]. A wider range of pH could not be used, because at pH 7.6 a visible precipitate was formed, and at pH < 6.5 the spontaneous hydrolysis is fast, relative to the Mn^{+2} catalyzed reaction.

Temperature dependence :

Table-2 compares the temperature dependence of the reaction rate in spontaneous and metal catalyzed hydrolysis of GPP, under conditions in which the predominant species is GPP $(Mn^{+2})_2$. The Arrhenius plots are linear. The activation parameters for the metal ion catalyzed hydrolysis include terms for complex formation and breakdown, including alcohol, and hydrocarbon formation, and the catalysis is due to an increase in log A (more positive Δ s) which offsets the higher activation energy (Table-2). The percentage of hydrocarbon formation increases with increasing temperature, as is generally found in elimination - substitution reactions and the difference in activation energies is Ca. 4 K.cal mole^{-1}, which agrees with the difference observed between elimination and substitution reactions[17].

TABLE-2 : TEMPERATURE EFFECTS UPON OVERALL REACTION AND HYDROCARBON FORMATION FROM GPP$^{(a)}$

T°C	$10^{-7} K_{obs}\ S^{-1}$	
	without Mn^{+2}	Mn^{+2} present
15°C	0.20	2.21 (2.2)
25	1.44	7.4 (3.2)
37.8	4.0	76.0
46.5	19.0	258.0 (6.0)
54.0	-	683.0 (6.0)
E, K cal $mole^{-1}$	25	31
log A	11, 3	16, 7

(a) at pH 7, 0.1M TES buffer and 0.005M Mn^{+2}.

Values in parentheses are moles % hydrocarbon.

Effects of other metal ions :

Fig.6 shows that other metal ions catalyze hydrolysis of GPP. Transition metal ions are the most effective in the sequence. $Cu^{+2} > Mn^{+2} > Zn > CO^{+2}$, but Ca^{+2} nor Mg^{+2} are relatively ineffective. There is no obvious relation between Lewis acidity[18] and catalytic effectiveness of the metal ion. (The experiments with Cu^{+2} were in HEPES buffer to avoid complexation).

The apparent high reactivity of Cu^{+2} may be due to catalysis by colloidal aggregates because a precipitate was formed above 0.002 M Cu^{+2}. However, the sequence of effectiveness depends on temperature, for example at 30° the sequence of catalytic effectiveness is $Mn^{+2} > Cu^{+2} > CO^{+2}$. This question was not explored in detail.

The product compositions are similar for reactions in the presence of the various metal ions. (Table-3). However, the amount of cyclic products were largest with CO^{+2}, Zn^{+2} and Mg^{+2}. Although Ca^{+2} and Mg^{+2} give little overall rate enhancement (Fig. 6) the products are derived by C-O bond cleavage.

Nature of the metal ion catalyzed hydrolysis :

Both hydrogen and metal ions catalyze the formation of carbocationic intermediates from GPP by neutralizing the negative charge of the phosphate moiety[19]. But

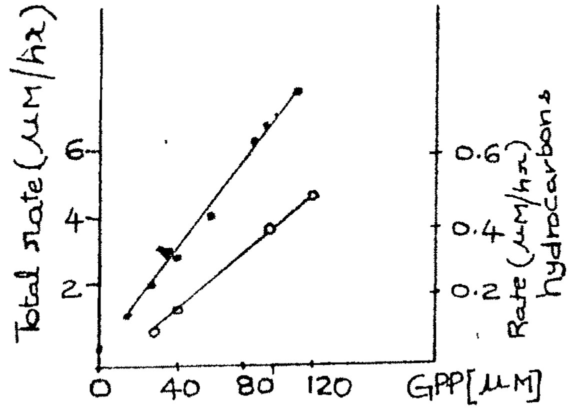


Fig.2: Effect of [GPP] on the rate of Mn^{+2} catalyzed hydrolysis at 40°C and 0.1 M TES, pH:7 and 0.03 M $MnCl_2$. ●-● , formation of total products, ○-○ , formation of hydrocarbons

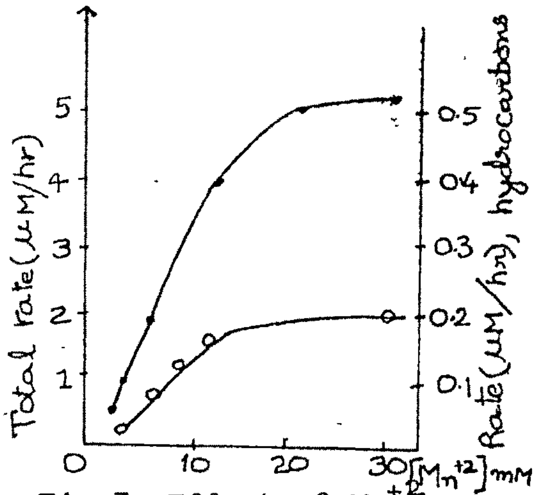


Fig.3: Effect of Mn^{+2} on GPP hydrolysis at 40°C, 0.1 M TES, pH:7 and 10^{-4} M GPP. ●-● , formation of total products, ○-○ , formation of hydrocarbons.

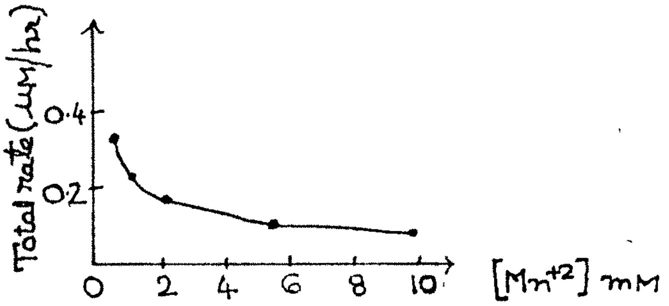
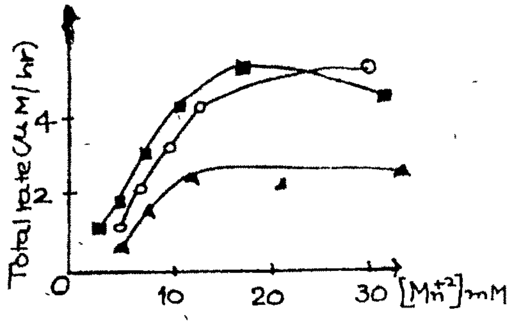


Fig.4: Effect of Mn^{+2} on GP hydrolysis



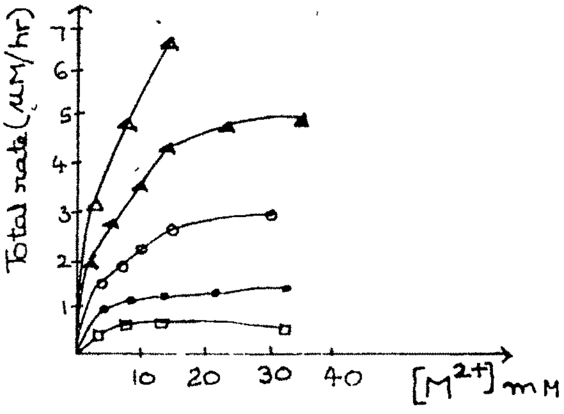


Fig.5: Effect of pH and $[Mn^{+2}]$ on GPP hydrolysis ■-■ , pH:6.5, ○-○ , pH:7, ▲-▲, pH:7.6.

Fig.6: Effect of concentration of metal ions on GPP hydrolysis at 40°C, △-△, Cu^{+2}; ▲-▲, Mn^{+2}; o-o, Co^{+2}; ●-●, Mg^{+2}; □-□ , Ca^{+2}

divalent metal ions also increase the amounts of elimination, and cyclization, relative to the hydrogen ion catalyzed reaction.

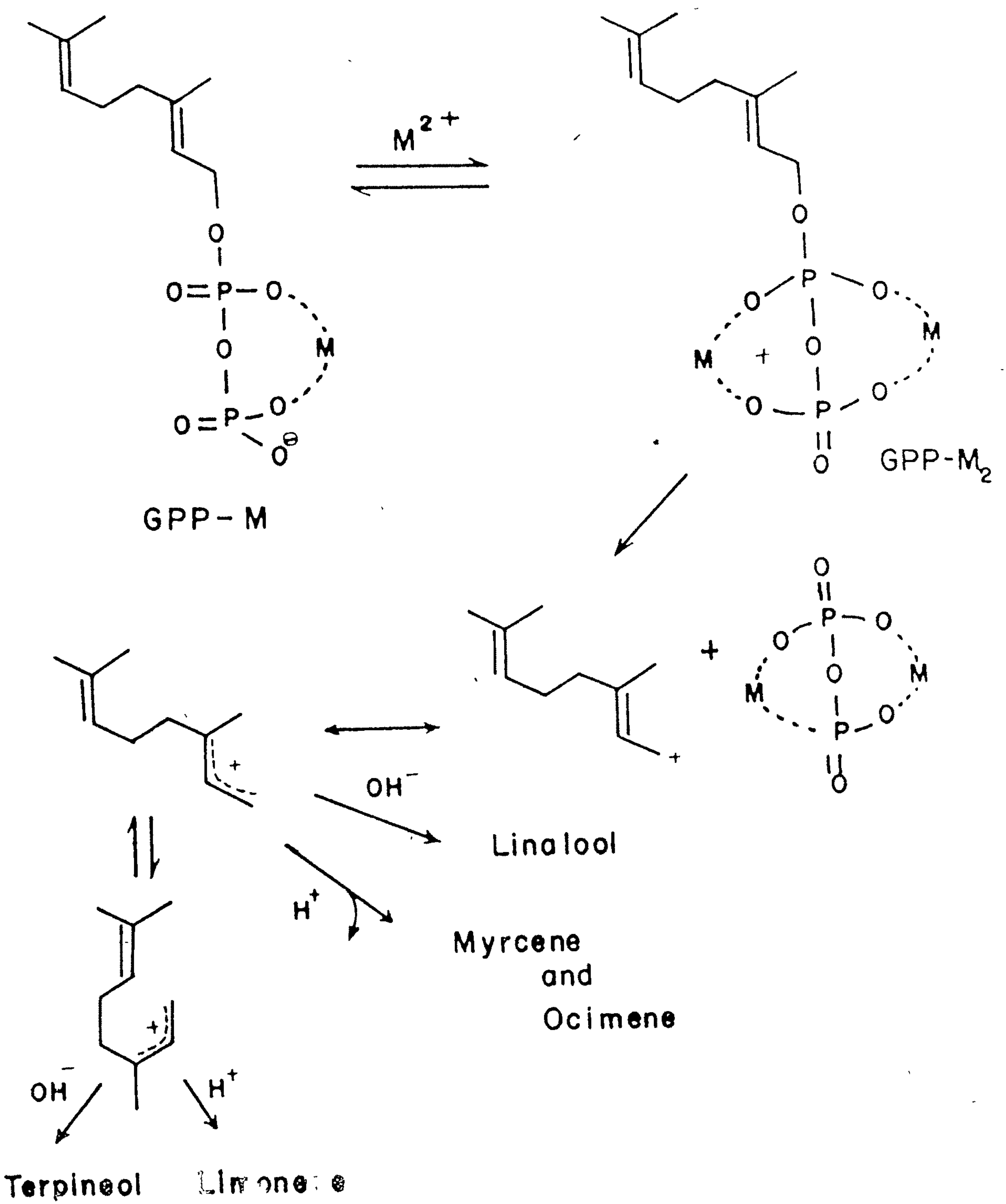
The fact that more limonene was formed in metal ion rather than in acid catalyzed hydrolysis deserves comment. The transformation of GPP, with its E-conformation, into cyclic products requires rotation about the delocalized 2,3-double bond of the intermediate carbocation. This interconversion is not rapid with other allylic carbocations in an aprotic solvent[20-22]. Several Investigators found no evidence of E-Z interconvension of the pyrophosphates via the tertiary linalyl pyrophosphate although it may be too reactive under our conditions to be detected. In addition Cramer found no isotopic scrambling in the hydrolysis of ^{18}O neryl monophosphate[23]. Another difference between hydrogen ion and Mn^{+2} catalyzed hydrolyses of prenyl substrates is that for the hydrogen ion catalyzed reactions, product distribution and rates are essentially independent of the leaving group[13,16], which is clearly not so for hydrolyses of GPP and GP in the presence of Mn^{+2}. Metal ions can participate in enzymic reactions of phosphorylated substrates by shielding negative charges in the phosphate moiety[24]. But, the absence of a relationship between Lewis acidity[18] and catalytic effectiveness of metal ions, and the differences between mono- and pyrophosphate hydrolyses suggest that the metal ion may play a more complicated role. Two metal ions could cordinate with a geranyl pyrophosphate trianion, giving a cationic complex which should readily break a C-O bond (Scheme-II). Formation of the neutral PPi-Mn_2 complex by break down of a cationic complex should provide much of the driving force for the reaction. Therefore, GPP $(Mn^{+2})_2^+$ makes the major contribution to reaction, although the equilibrium is in favour of GPP (Mn^{2+}). Such complexes can not be formed from monophosphate esters, which is consistent with the marked differences in behaviour of the mono- and pyrophosphate esters.

The increased formation of cyclic products in metal ion, relative to the hydrogen ion, catalyzed hydrolyses of GPP, suggests that the carbocationic species formed from the metal ion complex has a longer life than from the acid catalyzed reaction. At the same time interactions between the double bonds and the metal ions may favour a conformation compatible with cyclization.

Bond cleavage of a GPP $(Mn^{2+})_2^+$ complex will give the carbocation and the formally neutral PPi complex (Scheme-II). This neutral complex should however be strongly hydrated and therefore, deactivate adjacent water molecules, so that the cation has a longer life time, and can more easily change its conformation from <u>anti</u> to <u>syn</u> and then lose a proton, forming an alkene, rather than undergo addition of water. There are, however, examples of high nucleophilicity of metal bound OH[25,26].

Therefore, metal ion catalysis seems to be better than acid hydrolysis as a model for enzymic reactions. Although, enzymic reactions occur in aqueous media, the active sites of enzymes are typically in hydrophobic regions and shielded from the solvent, a purpose which might be served by the PPi-Mn-leaving group.

Similar effects were observed that Mn^{+2} and Mg^{+2} promote the solvolysis of FPP to farnesol and nerolidol even at pH 8.0[27].


M^{2+}
GPP-M
GPP-M_2
M
OH^-
H^+
Linalool
Myrcene and Ocimene
Terpineol
Limonene

SCHEME-II

TABLE-3: EFFECT OF METAL IONS ON THE PRODUCTS[a]

Product moles %	Metal ion				
	3 mM Cu^{+2}[b]	10 mM Mn^{+2}	10 mM CO^{2+}	10 mM Zn^{2+}	30 mM Mg^{+2}
alcohols	93.3	94.3	94.7	96.7	96.8
hydrocarbons	6.7	5.9	5.3	3.3	3.2
linalool	69.5	71.0	65.4	71.0	66.5
geraniol	17.1	19.9	19.9	18.0	21.3
nerol	-	0.6	1.0	0.5	-
α-terpineol	3.3	3.3	8.4	7.2	9.0
myrcene	3.6	2.7	1.9	1.6	1.9
ocimene (E+Z)	3.1	1.7	1.9	1.2	1.4
limonene	-	1.3	0.6	0.5	-

(a) At 40°C, pH 7.0, TES buffer, except where specified; 10^{-4}M GPP.

(b) HEPES buffer.

Conclusion :

That the divalent metal ions have an important role to play in the biosynthesis of various terpenes from allylic phosphate esters is a well established and documented fact. Interest in this area ranges from mechanistic considerations to synthetic utility. An interesting and probably at some future date viable development has been the use of divalent metal ions to prepare, biogenitically, terpenic molecules.

The work described in the following section discusses our investigations into the effect of 5 % MgO and 10 % MgO, impregnated over alumina, those known to catalyze allylic pyrophosphates, in the cyclization of these esters.

Other investigations include reactions of EtMgBr with allylic diethyl phosphate esters, in order to simulate the interactions between the phosphate oxygen and Mg^{+2} of RMgx thereby inducing cyclizations and with other organo metallic compounds (eg n-BuLi) to induce biomimetic cyclizations of these esters.

SECTION-II

PRESENT WORK

A. CYCLIZATION STUDIES OF FARNESYL DIETHYL PHOSPHATE ESTER ON MgO IMPREGNATED ALUMINAS

In the preceding chapter, it was observed, when terpene allylic diethyl phosphate esters are exposed to active alumina, facile cyclization takes place. Thus, the active alumina, as a template and as an activator resembles the role played by some enzymes in biological systems. And it was well-established that enzymes in the biological systems need divalent cation for catalytic activity to assist ionization of allylic pyrophosphate esters. This consideration along with the indication that the divalent cations enhance the solvolysis of terpene allylic pyrophosphates has led us to undertake, a more thorough examination, in an effort to improve the percentage of cyclic products in the reactions of terpene allylic diethyl phosphate esters, by using active alumina, impregnated with various percentages of MgO on it.

For the present study, to investigate the effect of Mg^{+2} ions on alumina - triggered cyclizations, farnesyl diethyl phosphate (mixture of 2(Z) and 2(E) isomers) was taken as substrate. It was prepared from the farnesol (2(Z) and 2(E) mixture, freshly distilled, 6.0 gm, 0.027 mol), diethyl chlorophosphate (freshly prepared, 6.25 gm, 0.036 mol) dry pyridine (5.48 gm, 0.069 mol) using dichloromethane as solvent under the similar reaction conditions, used for geranyl diethyl phosphate.

Catalysts 5 % MgO on Al_2O_3, 10 % MgO on Al_2O_3 and 10 % $MgCl_2$ on Al_2O_3 were prepared in the laboratory (see experimental).

(a) Cyclization of farnesyl diethylphosphate [2(Z) and 2(E) mixture] on 5 % MgO/Al_2O_3 catalyst

To a stirred suspension of 5 % MgO/Al_2O_3 (10 gm) in dry petroleum ether [(60-80°), 10 ml], farnesyl diethyl phosphate (2(Z) and 2(E) mixture, 0.5 gm) was added slowly and refluxed the reaction mixture, under exclusion of atmospheric moisture, for 4 hrs and kept at room temperature (~25°C) for 12 hours. After usual filtration work up, it afforded a mixture of compounds consisting of trans-β-farnesene, (11.96 %), β-bisabolene (46.29 %), trans-α-bisabolene + ar-curcumene (31.19 %). These results were tabulated in Table-1.

(b) Cyclization of farnesyl phosphate (2(Z) and 2(E) mixture) on 10 % MgO/Al_2O_3 catalyst

To a stirred suspension of 10 % MgO/Al_2O_3 (activity = grade I, pH = 7, 10 gm) in dry pet ether (60-80°, 10 ml), farnesyl diethyl phosphate (2(Z) and 2(E) mixture, 0.5 gm) was added slowly and refluxed the reaction mixture for 4 hrs and kept at room temperature (~25°C) for 12 hours. After usual filtration work up, the GLC of the product shows the formation of four major compounds and these are identified as trans-β-farnesene (14 %), β-bisabolene (48.23 %), trans-α-bisabolene + ar-curcumene (28.9 %). These results are tabulated in Table-1.

(c) Cyclization of farnesyl diethyl phosphate (2(Z) and 2(E) mixture) on 10 % $MgCl_2/Al_2O_3$ catalyst

The cyclization reaction of farnesyl diethyl phosphate (2(Z) and 2(E) mixture, 0.5 gm) was carried out on 10 % $MgCl_2$- Al_2O_3 catalyst (10 gm) in dry petroleum ether using the same reaction condition described above. After usual filtration work up, the GLC of the product shows the formation of four major compounds, which were identified as trans-β-farnesene (12.84 %), β-bisabolene (46.17 %), trans-α-bisabolene + ar-curcumene (31.8 %). These results are tabulated in Table -1.

From these studies, it is observed that there was no net improvement in the formation of total cyclic products in the reactions of farnesyl diethyl phosphate (2(Z) and 2(E)) mixture) with the reagents 5 % MgO/Al_2O_3, 10 % MgO/Al_2O_3 and 10 % $MgCl_2/Al_2O_3$ when compared the total cyclic products of reaction with active alumina. This implies that under the effect of metal ions, ionization of allylic diethyl phosphate esters by Al^{+3} ions is predominant, compared to the effect of Mg^{+2} ions. This fact can be rationalized in the structural differences of allylic pyrophosphates and allylic phosphate esters because phosphate esters can not readily chelate with divalent metal ion when compared to pyrophosphate esters.

Thus, active alumina acts the dual role of enzyme and metal ions in the cyclization reactions of terpene allylic diethyl phosphate esters.

TABLE-1 : EFFECT OF Mg^{+2} IONS ON CYCLIZATION OF FARNESYL DIETHYL PHOSPHATE[a]

Sr. No.	Substrate	Reagent	Product distribution(% GLC)[b]				Total % cyclized products
			trans-β-farnesene	β-bisabolene	ar-curcumene+ trans-α-bisabolene[c]	Unidentified products	
1.	Farnesyl diethyl phosphate (2(Z) and 2(E) mixture)	5 % MgO/Al_2O_3	11.96	48.29	31.19	8.54	79.48
2.	,,	10 % MgO/Al_2O_3	14	48.23	28.9	8.80	77.13
3.	,,	10 % $MgCl_2/Al_2O_3$	12.34	46.17	31.8	9.16	77.97
4.	,,	Pure Al_2O_3	9.56	47.63	33.4	9.43	81.03

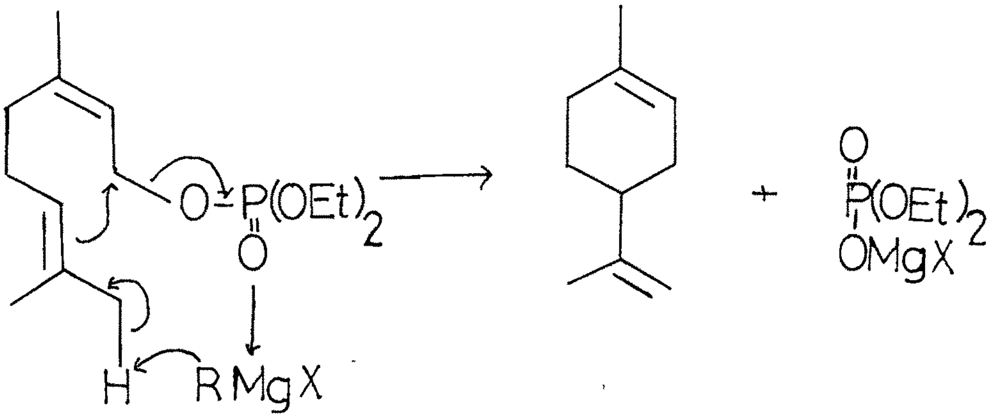
a. Farnesyl diethyl phosphate (2(Z), 2(E)- 65:35 mixture)+ reagent, dry pet. ether were refluxed under stirring.

b. Column: 360 cm x 0.37 cm stainless steel column packed with 10 % carbowax on 60-80 mesh chromosorb W; temp. 150°C; carrier gas: 60 ml H_2/min.

c. It has been observed that under the above conditions used for GLC, these two compounds do not separate clearly, that is why percentage of each was not calculated separately.

B. ACTION OF ORGANO METALLIC REAGENTS ON TERPENE ALLYLIC DIETHYL PHOSPHATE ESTERS

Since the importance of Mg^{+2} ions in the ionization of C-O bond of allylic pyrophosphate esters in biological processes was unequivocally established, it was of interest to use other related organo magnesium compounds as reagents to induce biomimetic transformations of allylic diethyl phosphate esters. The choice fell on alkyl magnesium halides (Grignard reagents) to study the effect of these reagents on allylic phosphate esters. Neryl diethyl phosphate was chosen as a substrate for the present study. It was prepared from freshly distilled nerol, diethyl chlorophosphate, pyridine using the experimental conditions described in earlier chapter. It was thought to use Grignard reagents with a view that the oxygen of the phosphate coordinates with Mg of the RMgX thereby facililating the ionization of C-O bond with concommitant attack of alkyl anion to abstract an allylic proton from neryl diethyl phosphate, in the formation of limonene, as follows.



Literature survey of the reactions of Grignard reagents with allylic phosphate esters has revealed the following examples. H.C.S. Wood et al.[28] reported the reaction of neryl diphenyl phosphate with PhMgBr in ether in the formation of monterpene hydrocarbon mixture (25 %) and a mixture of cis- and trans-3,7-dimethyl-1-phenyl-octa-2,6-diene (55 %). When the same reaction applied to geranyl diphenyl phosphate, it yielded hydrocarbon mixture (10 %) and cis- and trans-3,7-dimethyl-1-dimethyl-1-phenyl-octa-2,6-diene (25:75, 69 %). however, these authors did not analyse the hydrocarbon mixture and the mechanistic implications of these reactions were not discussed. After several years in 1982, Yasuo Butsugan et al.[29] reported the regio- and stereo-specific allylation of Grignard reagents using allyl phosphates, thus these results were contrasting the earlier ones. These two contradicting results, further, made us to undertake a more thorough study of the reactions of allylic diethyl phosphate esters with EtMgBr and to study their mechanistic pathways in order to explore the synthetic utility of organo metallic compounds in biomimetic transformations of these esters.

Reaction of neryl diethyl phosphate with EtMgBr

To a stirred solution of neryl diethyl phosphate (freshly prepared from nerol using diethyl chlorophosphate, pyridine); (2.32 gm, 0.008 mol), in solvent ether, ethyl magnisium bromide (freshly prepared, estimated 89.0 % yield, 1.064 gm, 0.008 mol) in solvent ether was slowly added, during a period of five minutes, at room temperature (~30°C) under dry nitrogen atmosphere. It was further stirred for 3 hours at room temperature (~30°C). After usual work up, the crude product was obtained. It was distilled to furnish 0.95 gm of distillate and 0.18 gm of residue. GLC of the distillate showed the presence of atleast 7 compounds. Major compounds were separated by preparative GLC and identified as cyclic monoterpene hydrocarbon mixture [(21 %); α-terpinene (1), limonene (2), p-cymene (3)], and cis-3,7-dimethyl-1-ethyl-octa-2,6-diene (5) and 3-ethyl-3,7-dimethyl-octa-1,6-diene (4) and are shown in Scheme-I. These were characterised by their spectral properties. GLC of this product mixture is shown in Fig.1. The compounds

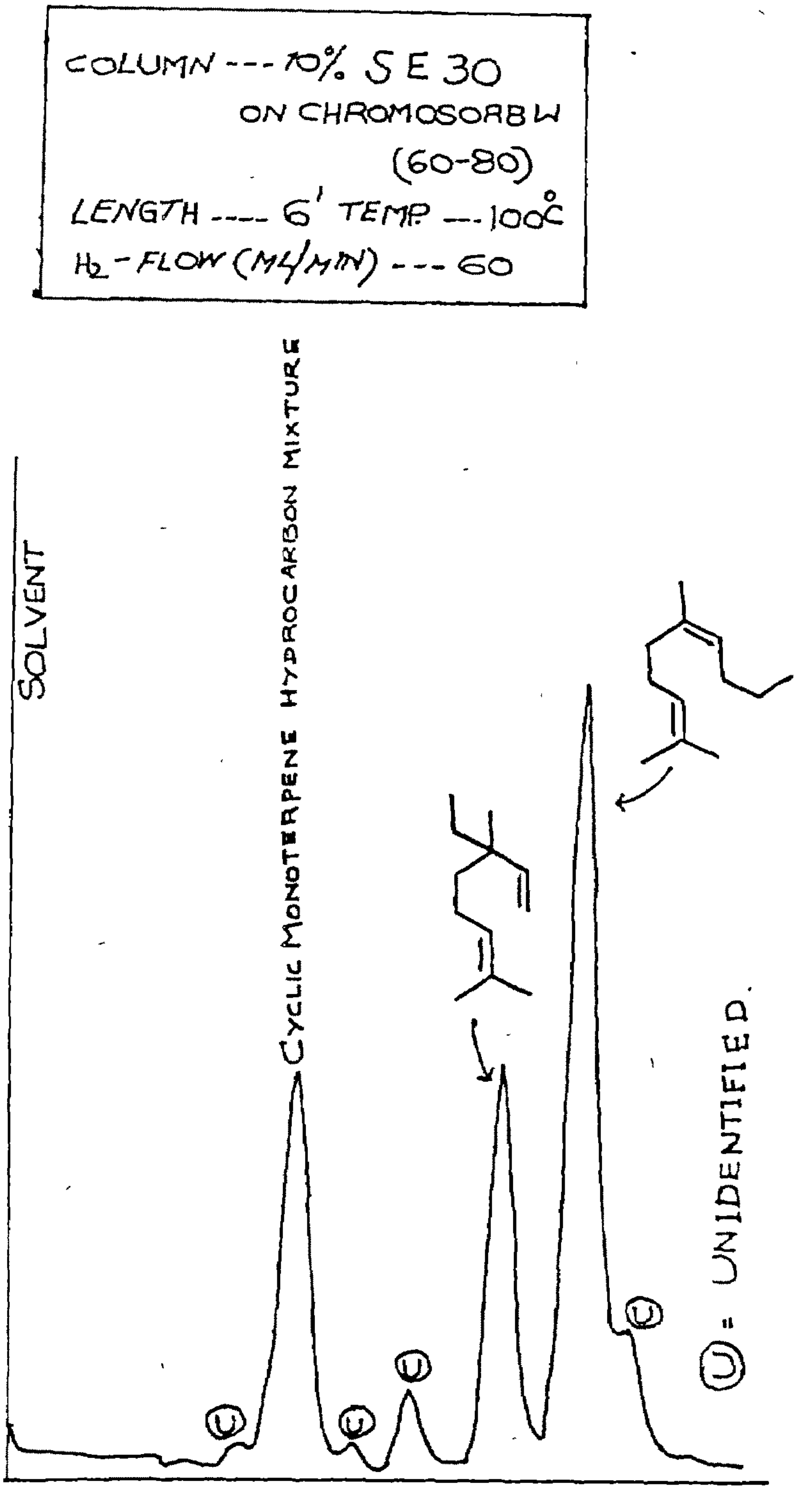


Fig.1 : GLC of reaction products from the reaction neryl diethyl phosphate with EtMgBr

formed and identified are tabulated in the Table-2. PMR of compounds (4) and (5) are given in Fig.2 and Fig.3. IR of compounds (4) and (5) are given in Fig.2a and Fig.3a.

Reaction of geranyl diethyl phosphate with EtMgBr

A reaction of geranyl diethyl phosphate (freshly prepared from geraniol and diethyl chlorophosphate, 8 gm, 0.027 mol) was carried out with EtMgBr (freshly prepared, estimated 90% yield, 4.392 gm, 0.033 mol) in solvent ether using the same experimental procedure described in the case of neryl isomer. After usual work up, it afforded a crude light yelllow oil. It was distilled to furnish 3.6 gm of distillate and 0.3 gm of residue. GLC of the distillate showed the presence of two major compounds (to an extent of 98%) and monoterpene hydrocarbon mixture (~1.5 %).(Scheme-II). These two major compounds were separated by 15 % $AgNO_3$-Silica gel-G, column chromotography to afford pure *trans*-3,7-dimethyl-1-ethyl-octa-2,6-diene (6) and 3-ethyl-3,7-dimethyl-octa-1,6-diene (4). These were characterised by their spectral properties. GLC of the total reaction product is shown in Fig.4.

Reaction of neryl diethyl phosphate with EtMgI

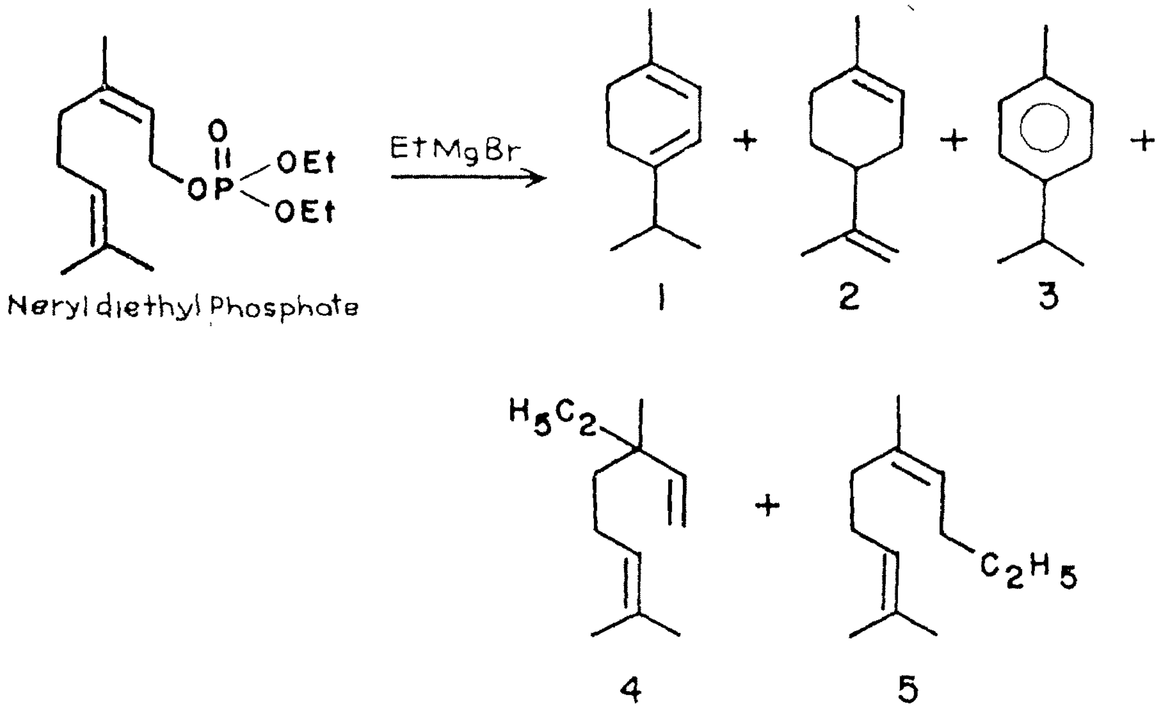
In an effort to increase the amount of cyclized products in these reactions, a reaction of neryl diethyl phosphate

(1.13 gm, 0.0038 mol) was carried out using ethyl magnesium iodide (0.841 gm, 0.0046 mol) under the similar reaction conditions described earlier. After usual work up, it afforded a crude light yellow oil which on distillation gave 0.312 gm of distillate and 0.4 gm of residue. GLC of this product mixture showed the formation of only 7 % cyclic monoterpene hydrocarbons and (4) (37.61 %), (5) (46.33 %) contrary to our expectation. The compounds formed and identified are tabulated in Table-2.

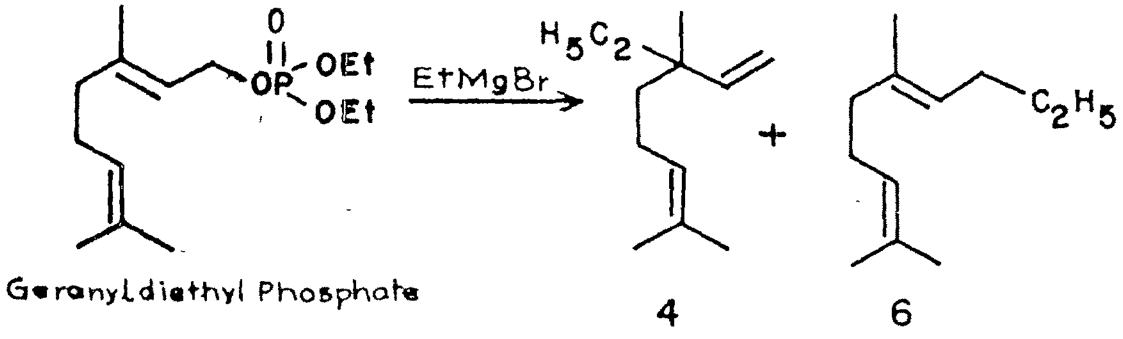
Reaction of geranyl diethyl phosphate with EtMgI

A reaction of geranyl diethyl phosphate (1.13 gm, 0.0038 mol) with ethyl magnisium iodide (0.841 gm, 0.0046 mol) was carried out using the same experimental procedure described above. After usual work up, it afforded a crude light yellow oil which on distillation gave 0.42 gm of distillate and 0.1 gm of residue. GLC of this product mixture shows the formation of (4) (51.7 %) and (6) (43.7 %). The compounds formed and identified are tabulated in Table-2.

In order to support our view that the cyclized products formation is due to the Grignard reagent and not because of $MgBr_2$, present in the reaction mixture, a reaction of neryl diethyl phosphate with magnesium bromide-etherate was carried out.



SCHEME - I



SCHEME - II

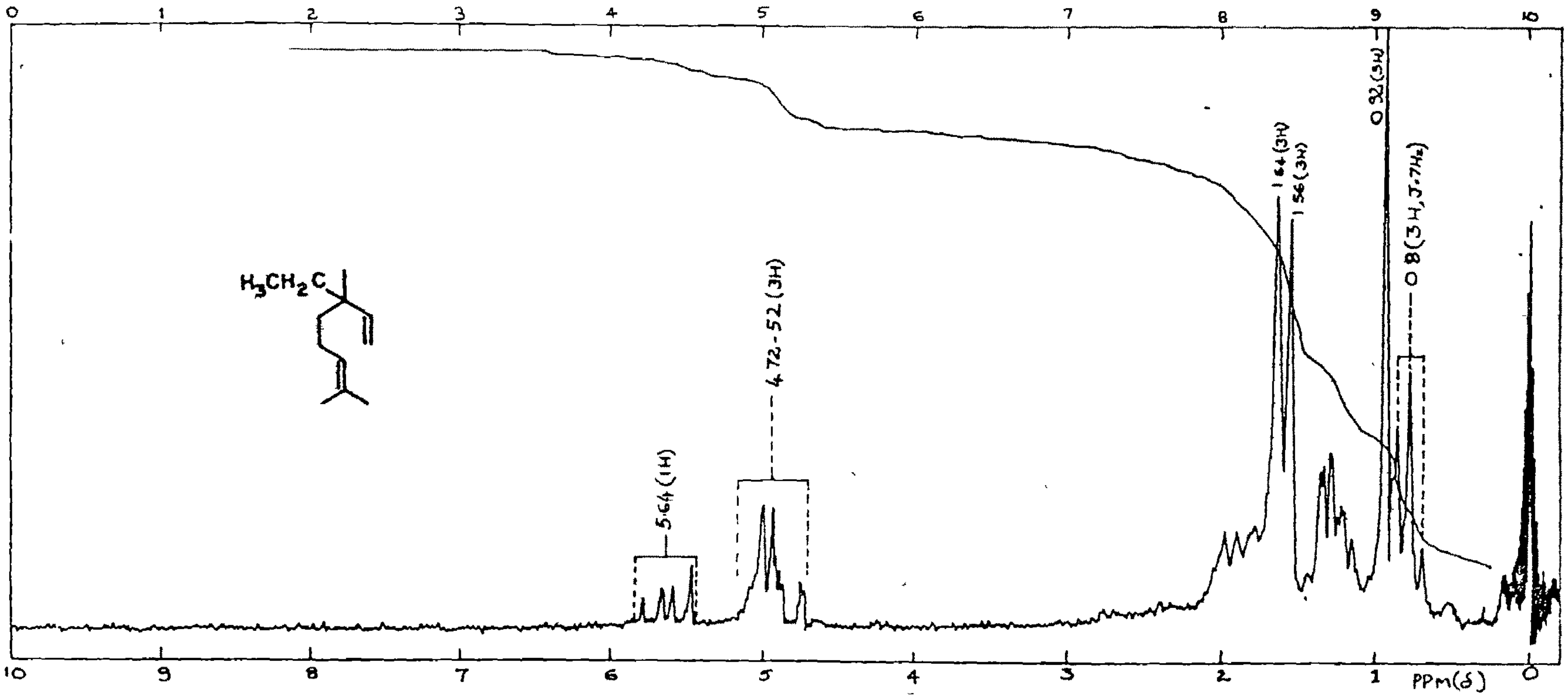


Fig.2 : PMR spectrum of 3-ethyl-3,7-dimethyl-octa-1,6-diene (4)

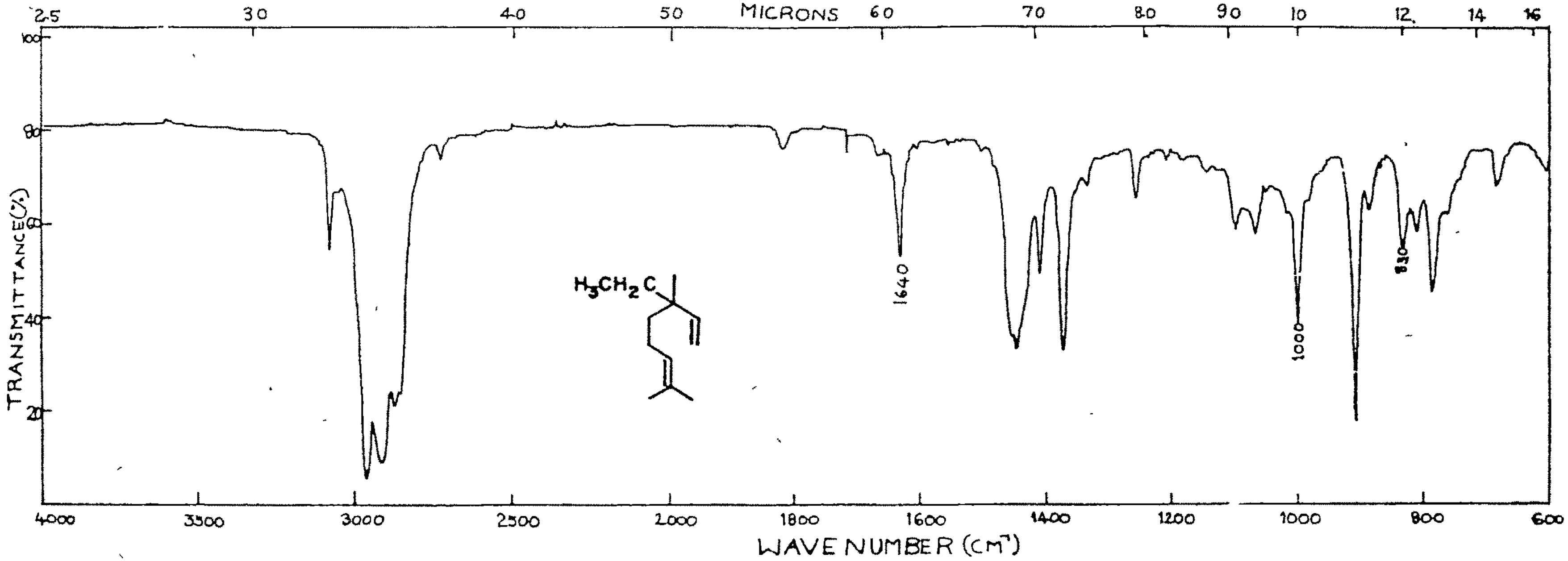


Fig.2a : IR spectrum of 3-ethyl-3,7-dimethyl-octa-1,6-diene (4)

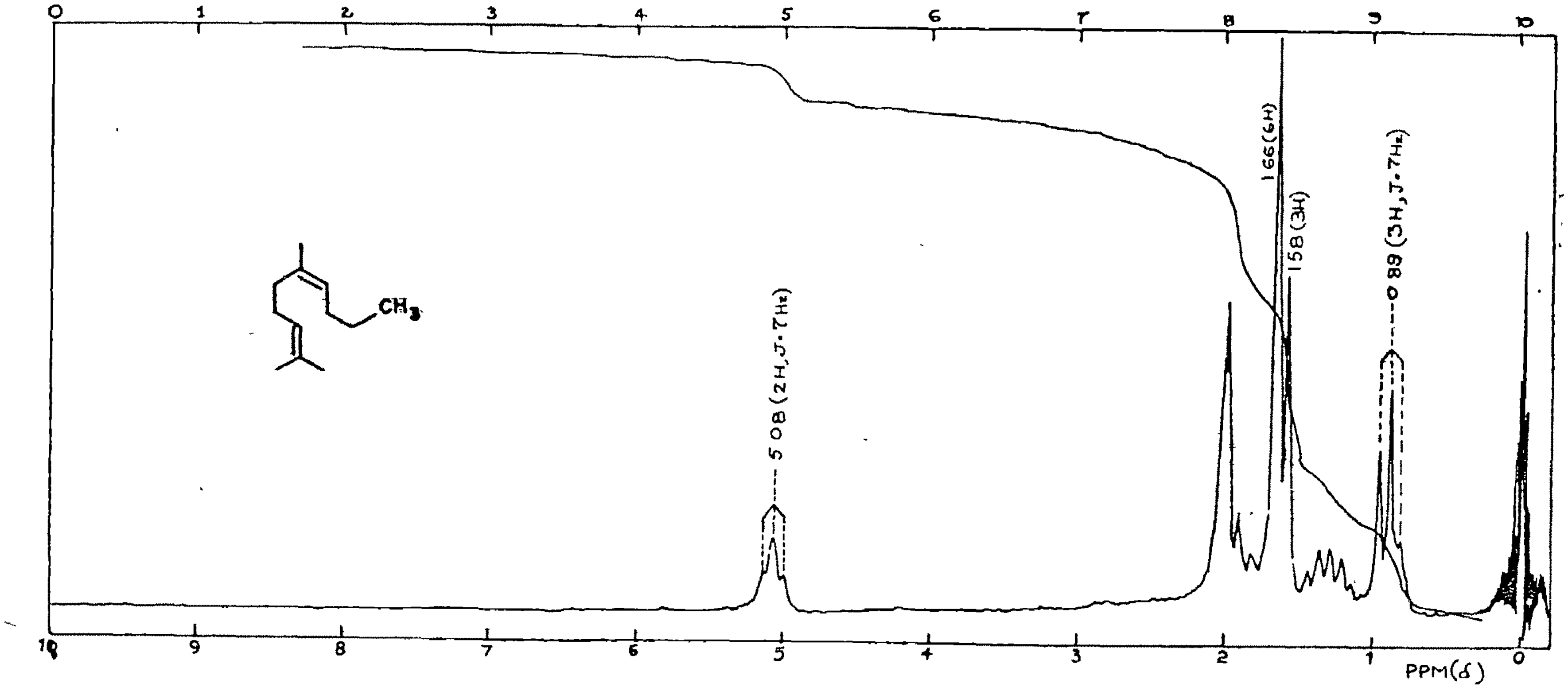


Fig.3 : PMR spectrum of cis-3,7-dimethyl-1-ethyl-octa-2,6-diene (5)

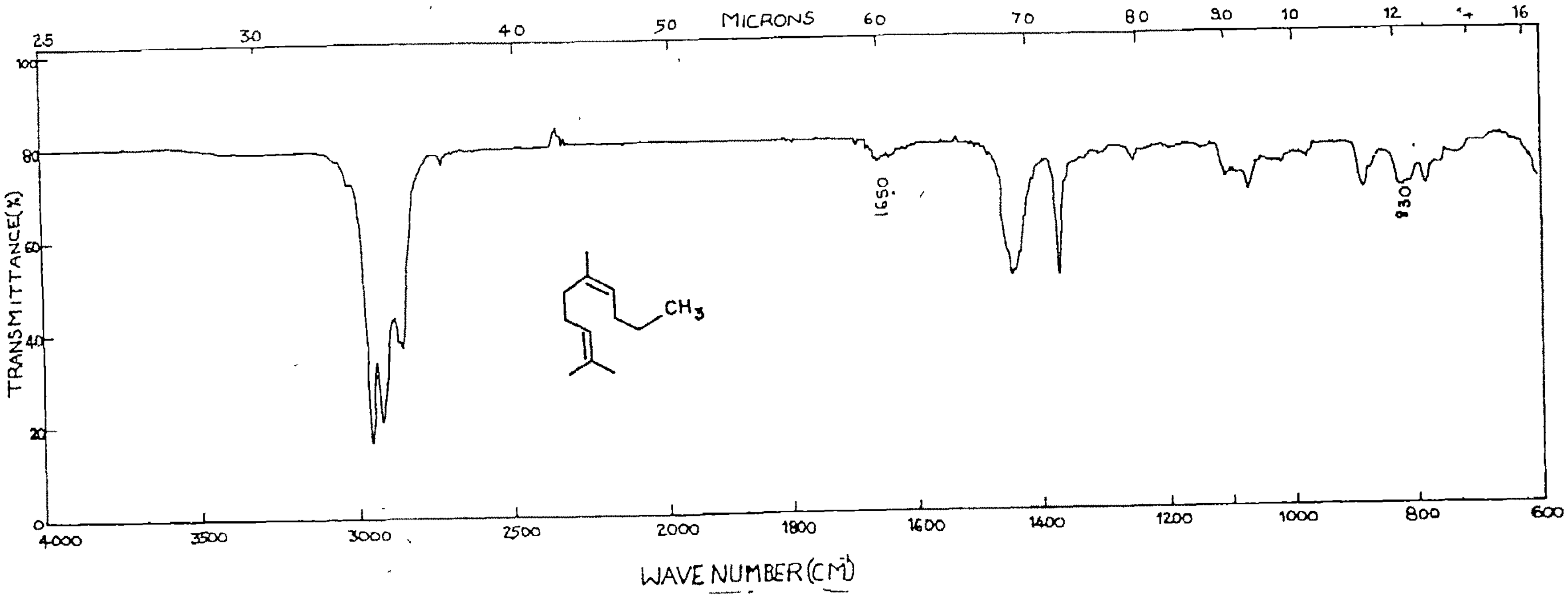


Fig.3a : IR spectrum of cis-3,7-dimethyl-1-ethyl-octa-2,6-diene (5)

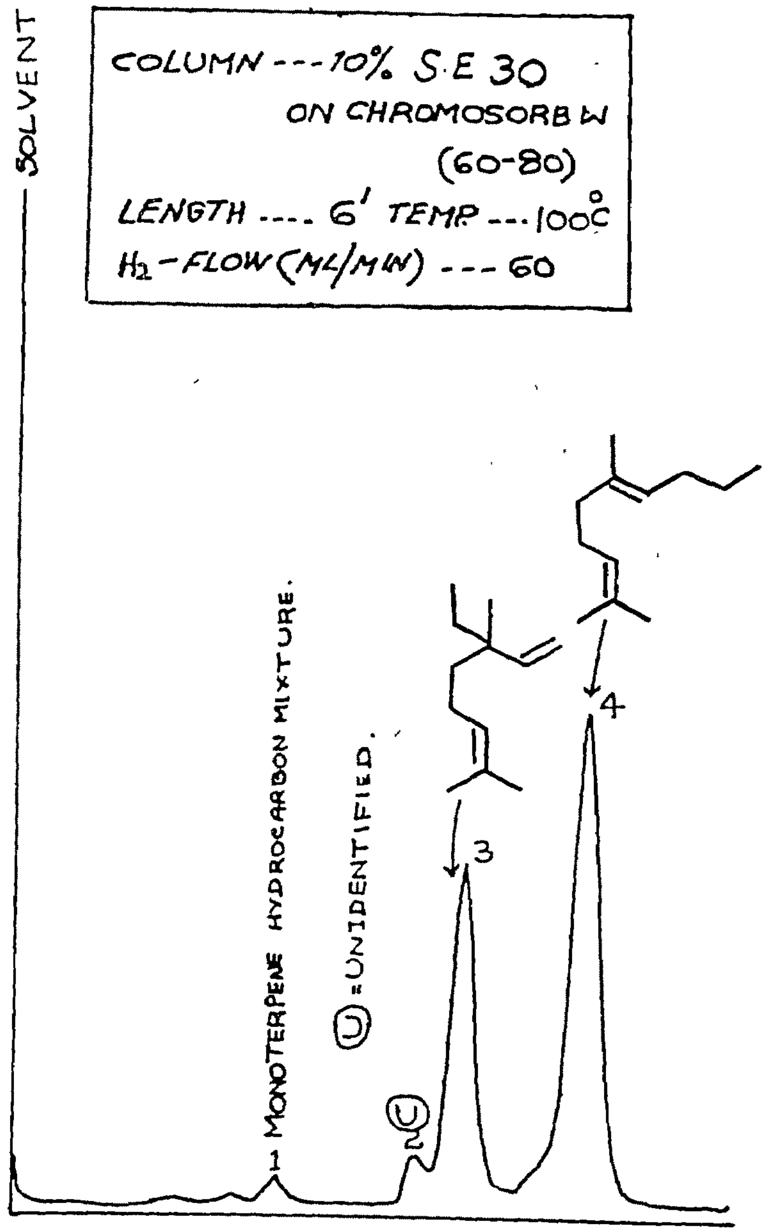


Fig.4 : GLC of reaction products from the reaction of geranyl diethyl phosphate with EtMgBr

Reaction of neryl diethyl phosphate with magnesium bromide - etherate

To the stirred solution of magnesium bromide, prepared from Mg (0.03 gm, 0.00125 mol) and dibromo ethane (0.189 gm, 0.001 mol) in solvent ether, neryl diethyl phosphate (0.29 gm, 0.001 mol) in solvent ether was added slowly at room temperature (~30°C). It was further stirred for 3 hours at room temperature (~30°C). After usual aqueous work up, it afforded a crude light yellow oil. No trace of cyclized or elimination products was observed. This reaction was not studied further.

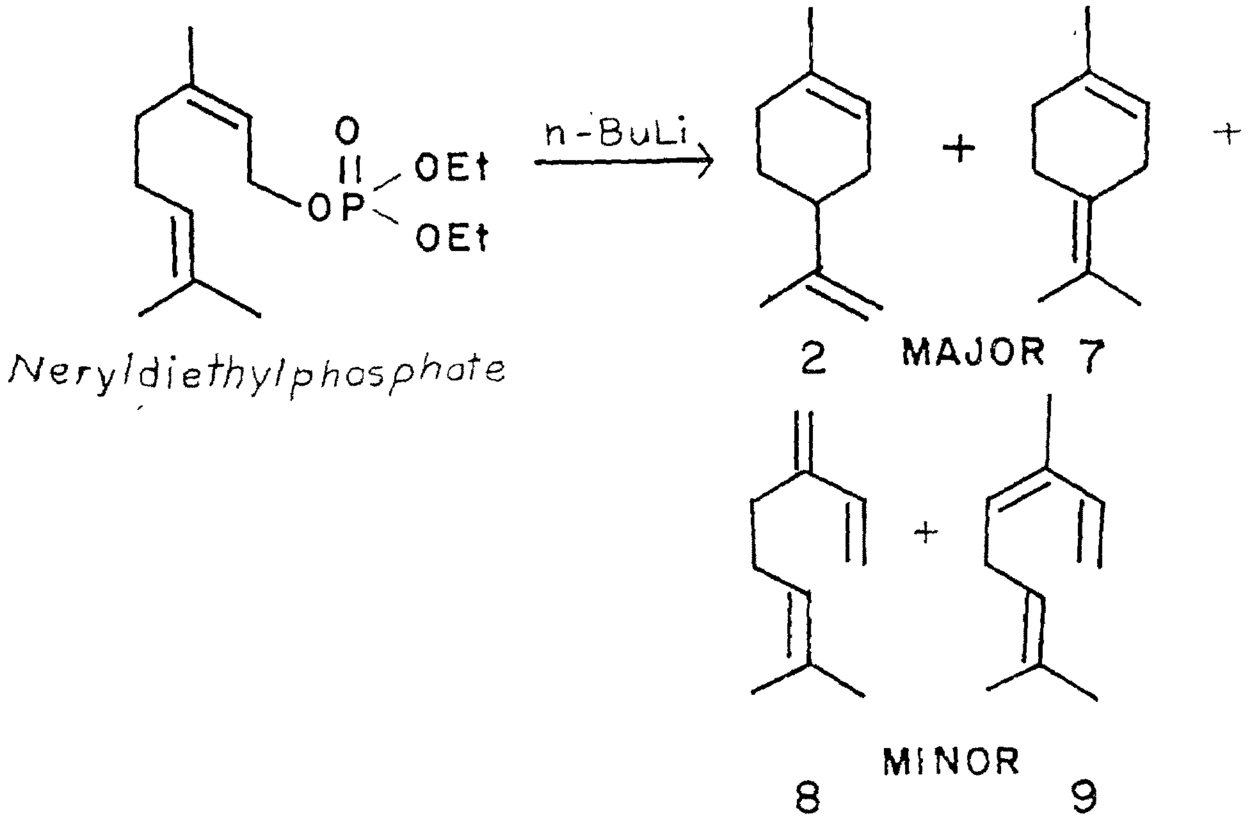
A reaction of nerol with magnesium bromide-etherate under the similar reaction conditions, did not yield any compound. Nerol was recovered.

From the above reactions, it is observed that Grignard reagent, acted both as a base and as a nucleophile, competing each other. It is known that Grignard reagent acts as base[30].

In order to show that metal ion is needed to coordinate with phosphate oxygen in ionization of C-O bond with concommitant abstraction of an allylic proton by the basic moiety of the reagent, a reaction of neryl diethyl phosphate with n-BuLi gave mainly cyclized monoterpene hydrocarbons in good yields with no nucleophilic substitution products. Literature survey showed that such a reaction of n-BuLi with allylic diethyl phosphate esters was hitherto unreported. This is here **we** are reporting for the first time the cyclization reaction of neryl diethyl phosphate with n-BuLi. Such a reaction of neryl diethyl phosphate with dialkyl aluminium halide was reported by Y. Kitagawa *et al.*[31].

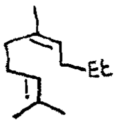
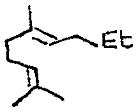
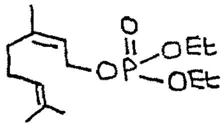
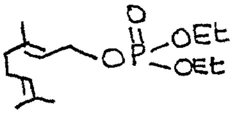
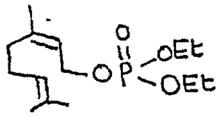
Reaction of neryl diethyl phosphate with n-BuLi

To a stirred solution of neryl diethyl phosphate (0.29 gm, 0.001 mol) in solvent ether, n-butyl lithium (prepared from reported procedure[32], 0.07 gm, 0.0012 mol) in solvent ether was injected slowly in a period of 2 minutes, in dry nitrogen atmosphere at -50°C. The reaction mixture was further stirred at this temperature for 2 hours and slowly brought to room temperature and stirred at this temperature (~30°C) for 30 minutes. After usual aqueous work up, the crude was distilled to furnish 0.1 gm of distillate (73 %) and 0.01 gm of the residue. GLC of the distillate showed the formation of mainly 4 compounds which were identified, as myrcene (8), limonene (2), terpinolene (7) and *cis*-ocimene (9) (Scheme-III) by their relative retention times and mixed GLC with authentic samples using peak augmentation technique and mixed PMR studies.



SCHEME - III

TABLE-2 : REACTION OF ORGANOMETALLIC REAGENTS WITH ALLYLIC PHOSPHATE ESTERS[a]

Sr. No.	Substrate	Reagent	Product distribution(% GLC)[b]					Yield
			Cyclic Hydro-carbon mixture				Unid-enti-fied	
1.		EtMgBr	21.81	20.98	48.97	-	8.22	73 %
2.	-do-	EtMgI	7.33	37.61	46.33	-	8.71	50 %
3.		EtMgBr	1.24	34	-	62.25	2.48	80 %
4.	-do-	EtMgI	2.1	51.7	-	43.7	2.52	80 %
5.		n-BuLi[c]	80.76	-	-	-	5.76	73 %

---

a. To the substrate in ether at room temp. (~30°C), reagent in ether was added under dry N_2 conditions and further stirred for 3 hours at room temp.

b. Column: 180 cm x 0.317 cm stainless steel column packed with 10 % SE 30 on 60-80 mesh Chromosorb W; temp. 100°C; Carrier gas: 60 ml H_2/min.

c. To the substrate in ether at (-50°C), n-Bu-Li/ether was added in dry N_2 atmosphere and stirred for 2 hrs and slowly brought to room temperature and further stirred for 30 minutes (~30°C).

C. MISCELLANEOUS STUDIES

Allylic diethyl phosphate esters, when exposed to organo-metallic bases (RMgX, RLi), were found to undergo transformations into cyclic and nucleophilic substitution products. In view of this observation, it was considered worthwhile to evaluate the importance of other bases (both organic and inorganic) and amphoteric salts, in inducing such transformations, and so the present study was undertaken. Neryl diethyl phosphate was chosen as substrate for the present study.

(a) Attempted reaction neryl diethyl phosphate with Calcium hydride

A mixture of neryl diethyl phosphate (0.5 gm, 0.0017 mol) and CaH_2 (0.140 gm, 0.0028 mol) in dry pet. ether was stirred at room temperature (~30°C) for 4 hrs under exclusion of moisture. It was further stirred under reflux for 2 hrs. No reaction took place. (TLC shows no disappearance of starting material)

(b) Attempted reaction of neryl diethyl phosphate with pyridine[33]

A mixture, of neryl diethyl phosphate (0.5 gm, 0.0017 mol) and dry pyridine (4 ml), was stirred at room temperature for 4 hrs under exclusion of the moisture. It was further stirred under reflux for 2 hrs. No reaction took place. Pyridine was distilled off and the starting material was recovered.

(c) Attempted reaction of neryl diethyl phosphate with DBU[34] [1,5-Diazabicyclo [5.4.0] undec-5-ene]

A stirred mixture, of neryl diethyl phosphate (0.29 gm, 0.001 mol), DBU (0.3 gm, 0.002 mol) and dry benzene (4 ml) was refluxed for 4 hrs under exclusion of moisture. No reaction was observed. (TLC shows no disappearance of starting material).

(d) Attempted reaction of neryl diethyl phosphate with benzo-thiazolium salt

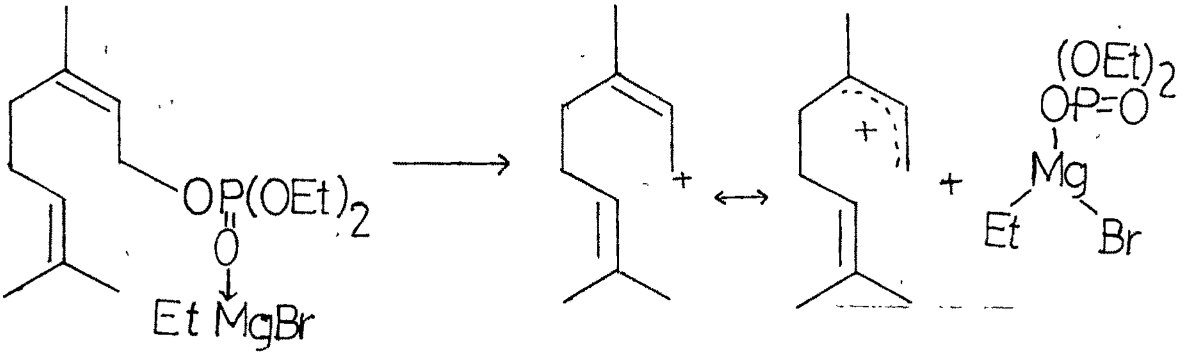
To the stirred mixture of N-Benzyl-5-(2-hydroxy ethyl)-4-methyl-1,3-thiazolium-chloride (0.092 gm, 0.00034 mol) (prepared[35] from N-Benzylchloride and 5-(2-hydroxy ethyl)-4-methyl-1,3-thiazole) in dry DMF, triethyl amine (0.076 gm, 0.00075 mol) was added dropwise in 2 minutes at room temperature (30°C).

To this stirred mixture, neryl diethyl phosphate (0.1 gm, 0.00034 mol) in dry DMF (1 ml) was added and stirred at room temperature (30°C) for 5 hrs and heated to 80°C for 6 hrs. No reaction was observed. After usual aqueous work up, starting material was recovered.

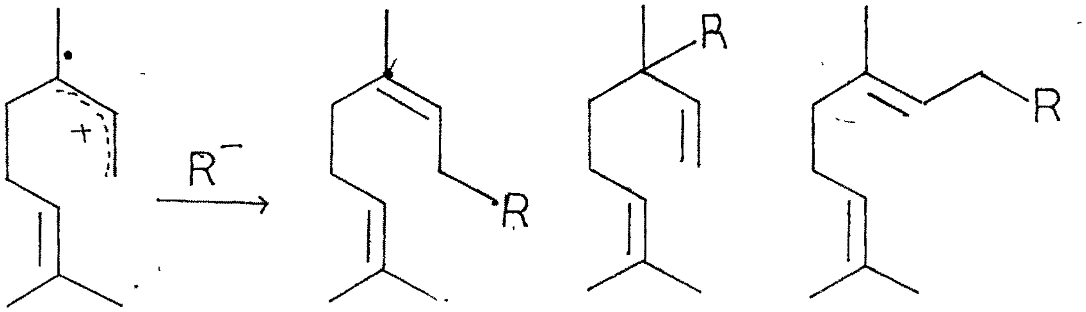
These results proved that, only organo-metallic bases can induce transformations of allylic diethyl phosphate esters thus emphasizing the importance of metal ions in the ionization of the C-O bond of these esters.

Discussion :

The overall picture can be visualised in terms of the several opportunities available to the reactive complex formed by phosphate oxygen with Mg of Grignard reagent, by the rate determining heterolysis of a coordination complex.

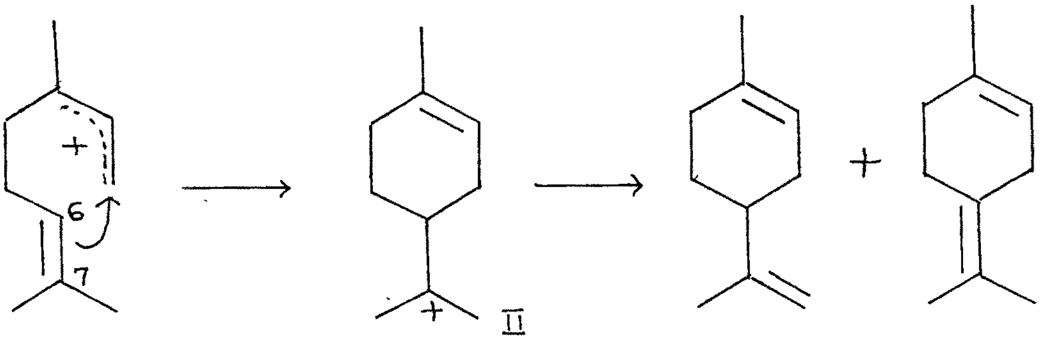


Thus, the alkyl group of the complex anion being visualized as source of $R^{(-)}$ and on the assumption that it is possible, in part, for the cis-trans-interconversion barrier in the carbonium ion to be overcome, the following routes for the open-chain products become possible.



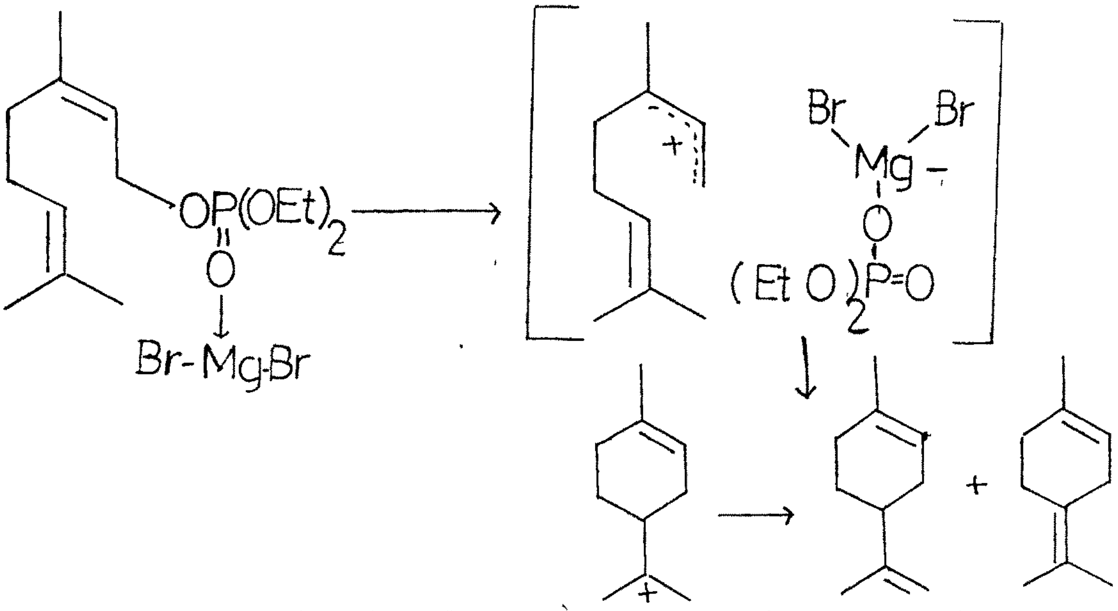
Significantly, there is no experimental evidence of loss of proton from either cis- or trans- allylic carbonium

In addition to these processes, the original cis-allylic carbonium ion might rearrange by intramolecular nucleophilic attack at the primary position by the conformationally well-placed 6,7-π-bond, to the tertiary carbonium ion as a new reactive entity (II), which can afford to form cyclic hydrocarbons in fast consummation steps.



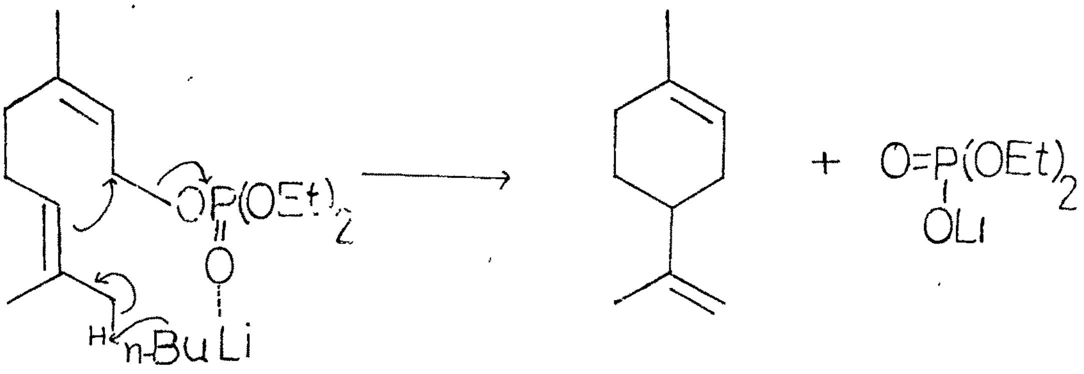
These olefines might further isomerise to form α-terpinene and p-cymene under these reaction conditions. The major decomposition route of II, however, is one of proton removal as shown by the presence of limonene and other cyclized products, a result with sharp contrast with the complete absence of deprotonation as a mode of reaction of the allylic ion precursor. One is, therefore, led to believe that electrophilic magnesium centre is all important in coordinating to the ester's oxygen atom to provide heterolytically reactive complex.

If this were so it was thought magnesium bromide itself presumably better as an electrophile than alkyl magnesium compound should be able to cause decomposition of neryl diethyl phosphate ester in the following way.



An experiment with ethreal magnesium bromide and neryl diethyl phosphate showed this not to be true.

In order to explain the results obtained in reactions with EtMgBr and $MgBr_2$, with neryl diethyl phosphate, a concerted mechanism is proposed, which is further supported by an experiment, carried out with n-BuLi.



It is proposed that phosphate oxygen forms a complex with Mg of the Grignard reagent and the $Et^{(-)}$, abstracts an allylic proton, thereby facilitating the attack of 6,7-double bond, in a synchronons manner, leading to the formation of limonene. In case of geranyl diethyl phosphate, absence of cyclization or elimination products also support the concerted approach. The formation of nucleophilic substitution products in case of geranyl and neryl diethyl phosphate esters can also be explained by invoking the S_N-2 attack of $R^{(-)}$, in a concerted mechanism, at C_1 or C_3, thus substituting the phosphate group by an alkyl group.

The concerted approach is well supported by the retention of cis- or trans- geometry of double bonds, in the products.

The formation of cyclized products with n-BuLi, supports, the role of alkyl group of organo metallic reagent, as a base , leading to a concerted mechanism.

Thus, the role of metal ions in catalysing the reaction of allylic phosphate esters was unequivocally established by these studies.

EXPERIMENTAL

All m.ps and b.ps are uncorrected. For general remarks see Chapter-II.

Preparation of 5 % MgO/Al_2O_3:

Magnesium (1.5 gm, 0.0617 mol) and aluminium (25.15 gm, 0.93 mol) pieces were placed in a 1 litre 3 neck r.b.f. fitted with a reflux condenser (in turn attached to a anhy. $CaCl_2$ guard tube), and addition funnel. Isopropanol (distilled, 30 ml) and $HgCl_2$ (0.5 gm) were added to the above mixture and heated the contents on a waterbath for 5 minutes followed by the addition of CCl_4 (2 ml). After heating on a water bath (10 min), a vigorous reaction took place. When it subsided (30 min) the remaining isopropanol (270 ml) was added slowly in a period of 30 mins. The reaction contents were refluxed on water bath for 10 hrs during the period, a homogeneous solution was formed. This hot solution was quickly filtered. Excess isopropanol was distilled off. The crude after cooling to 5°C, poured into DM water (2 lit), where a white solid precipitated out. It was digested on water bath (90°C) for 4 hrs and kept at room temperature for overnight (12 hrs). It was filtered through suction. The solid was washed with hot water (100 ml x 2). The solid was dried in oven (120°/4 hrs). It was grinded and sieved through 100 mesh sieve and activated at 250°/24 hrs. Activity was determined and found to be grade-I.

Preparation of 10% MgO/Al_2O_3:

10% MgO/Al_2O_3 was prepared in a similar manner described as above. Mg (1.5 gm, 0.0617 mol) and aluminium (11.91 gm, 0.4411 mol), $HgCl_2$ (0.25 gm), Isopropanol (150 ml), CCl_4 (2 ml).

Activity was found to be grade-I.

Preparation of 10% $MgCl_2/Al_2O_3$:

To the mechanically stirred solution of $MgCl_2 \cdot 4H_2O$ (1.5 gm) in distilled water (25 ml), alumina (15 gm, prepared from the hydrolysis of aluminium isopropoxide) was slowly added. This heterogeneous mixture was stirred mechanically for 3 hrs at room temperature (30°C). Water was removed using rotatory evaporator under vacuum thereby got magnesium chloride impregnated alumina. It was activated at 250°/24 hrs. Activity was found to be grade-I.

Cyclization reaction of farnesyl diethyl phosphate in 5% MgO/Al_2O_3:

In a [illegible] neck r.b.f. fitted with reflux condenser [illegible] was attached to anhy. $CaCl_2$ guard tube) and addition funnel, [illegible] MgO/Al_2O_3 (5 gm and dry pet. ether (6[illegible], 10 ml) were taken. To this stirred suspension, farnesyl diethyl phosphate (2($\underline{Z}$)- and 2($\underline{E}$)- mixture, 0.5 gm) in dry pet. ether (2 ml) was added dropwise. The resulting mixture was refluxed under stirring for 4 hrs and kept at room temperature (25°C) for 12 hrs. The contents were transfered into a vertical chromatographic column and eluted with dichloromethane (10 ml x 10). Solvent was removed by careful distillation, which yielded a crude 0.24 gm. It was distilled (140-150°/ 1.5 mm) to furnish 0.17 gm of distillate and 0.02 gm of residue.

Reaction of neryl diethyl phosphate with EtMgBr:

Into a 3 neck r.b.f. (50 ml), equipped with addition funnel (attached to a rubber reptum), reflux condenser, neryl diethyl phosphate (2.320 gm, 0.008 mol) in solvent ether (3 ml) was added under dry nitrogen atmosphere. To the stirred solution at room temperature (30°C), EtMgBr (freshly prepared, estimated, 90 % yield, 1.064 gm, 0.008 mol) in solvent ether was added slowly in a period of 30 minutes and the resulting reaction mixture was stirred further for 3 hrs at room temperature, during which period reaction was completed. The reaction mixture was poured into a cooled aq. solution of NH_4Cl (10 %) and extracted with solvent ether (15 ml x 3). The combined ethereal extract was washed successively with water till the washings are neutral. It was washed finally with brine solution and dried over anhy. Na_2SO_4. Solvent was removed by distillation which afforded a crude light yellow oil. It was distilled (120-140°(bath)/30 **mm**) to furnish 0.95 gm of distillate and 0.18 gm of residue.

Reaction of geranyl diethyl phosphate with EtMgBr:

A reaction of geranyl diethyl phosphate (8 gm, 0.027 mol) with EtMgBr (4.392 gm, 0.033 mol) in solvent ether was carried out under the similar reaction conditions, as that used for neryl diethyl phosphate. After the work up, the crude product so obtained was distilled (120-140°(bath)/ 30 **mm**) to furnish 3.6 gm of distillate and 0.3 gm of residue.

Reaction of neryl diethyl phosphate with magnesium bromide etherate:

Magnesium (0.03 gm, 0.00125 mol) and dry ether (2 ml) were introduced into 3 neck r.b.f. fitted with thermowell, reflux condenser. To the stirred Mg turnings, dibromoethane (0.189 gm, 0.001 mol) in solvent ether was added dropwise at room temperature (30°C) under dry nitrogen atmosphere. After complete addition, it was stirred for 3 hrs at room temperature to form magnesium bromide etherate solution. To this stirred solution neryl diethyl phosphate (0.29 gm, 0.001 mol) in solvent ether was added slowly at room temp.

(30°). The reaction mixture was poured into cooled aq. NH_4Cl solution and extracted with solvent ether (10 ml x 3). The combined ether extract was washed with water till the water washings are neutral to pH. It was dried over anhy. Na_2SO_4 and the solvent was removed by distillation, which afforded 0.1 gm of crude residue.

Reaction of neryl diethyl phosphate with n-BuLi:

Neryl diethyl phosphate (0.29 gm, 0.001 mol) in CH_2Cl_2 (3 ml) was added into a 3 neck r.b.f., which is equipped with addition funnel and thermowell. To this stirred solution, under cooling (-40°C), n-BuLi in ether was added dropwise in five minutes under dry nitrogen atmosphere. It was further stirred for 3 hrs at this temperature. It was brought slowly to room temperature (30°) and stirred for 30 mins. The reaction contents were poured into cold water (10 ml) and extracted with solvent ether (10 ml x 3). The combined ether extract was washed with water till the water washings are neutral to pH and finally washed with brine solution. It was dried over sodium sulphate (anhy.) and the solvent was removed by distillation, which afforded a light yellow crude oil 0.12 gm. It was distilled, bath temp. (120-140°) /40 mm to afford 0.1 gm of distillate and 0.01 gm of residue.

GAS CHROMATOGRAM

Separation of reaction products from the reaction of neryl diethyl phosphate with EtMgBr

Column : 20 % SE 30 on Chromosorb W (45-60), 360 cm X 0.95 cm

Column temperature : 100°C

Flow rate : 100 ml H_2/min

Amount injected : 500 mg

Amount/injection : 35 μl

Fractions collected	Wt. (mg)	Remarks	Identification
1	-	-	-
2	-	-	-
3	35	mixture of three cyclic hydrocarbons	compounds 1, 2 and 3.
4	10	pure compd.	unidentified
5	80	pure compd.	compound 4.
6	120	pure compd.	compound 5.

3-Ethyl-3,7-dimethyl-octa-1,6-diene (4)

It was isolated by prep. GLC and distilled.

B.P. : 120-130° (bath)/30 mm

IR (Neat) : 1640, 1410, 1000, 905, 830 cm^{-1}

PMR : $\underline{H_3}C-CH_2$(3H, t, J=7Hz, 0.8 ppm); $\underline{H_3}C-C$ (3H, s, 0.92 ppm); two $\underline{H_3}C-C=C$ (6H; 3H, s, 1.56 ppm, 3H, s, 1.64 ppm); $\underline{H_2}C=C$ and $\underline{HC}=C$ (3H, m, 4.72-5.2 ppm); $\underline{HC}=CH_2$ (1H, d, d, 5.64 ppm).

cis-3,7-dimethyl-1-ethyl-octa-2,6-diene (5)

B.P. : 120-130° (bath)/30 mm

IR (Neat) : 1650, 1070, 830 cm^{-1}

PMR : $\underline{H_3}C-CH_2$ (3H, t, J=7Hz, 0.89 ppm); $3\underline{H_3}C-C=C$ (9H; 3H, s, 1.58 ppm, 6H, s, 1.66 ppm); $2\underline{HC}=C$ (2H, t, J=7Hz, 5.08 ppm).

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