CHAPTER IV

RESULT AND DISCUSSION

In this research, there are two different types of compound aimed to synthesize, the cyclic acetals of pyridoxine and the valproate esters of pyridoxine.

The six-membered ring cyclic acetal of pyridoxine was prepared by the condensation of pyridoxine and 2-propylpentanal, the corresponding aldehyde of valproic acid. The aldehyde was synthesized by the reaction of ethyl ethoxyacetate with the corresponding Grignard reagent and then condensed with pyridoxine in the presence of dry hydrochloric acid as a catalyst to form a 2-propylpentanylidene derivative, isolated as a hydrochloride in an almost quantitative yield.

The seven-membered ring cyclic acetal of pyridoxine was decided to synthesize by the Diels-Alder reaction of 4-methyl-5-ethoxyoxazole and 2-(1-propylbutyl)-1,3-dioxep-5-ene. But unfortunately, the attempt to prepare this compound failed.

The mono-, di- and trivalproate esters of pyridoxine were successfully prepared by selective esterification of pyridoxine using valproyl chloride as acylating agent. Two monovalproate esters, two divalproate esters and a trivalproate ester were synthesized in the yield of 50-70 %.

Ethyl Chloroacetate.

This ester type compound was prepared by esterifying chloroacetic acid with absolute ethanol in the presence of concentrated sulfuric acid as a catalyst. (Figure 79A)

The interaction between chloroacetic acid and ethanol was a reversible process and proceeded very slowly. Without the catalyst, equilibrium was only attained after refluxing for several days. When a
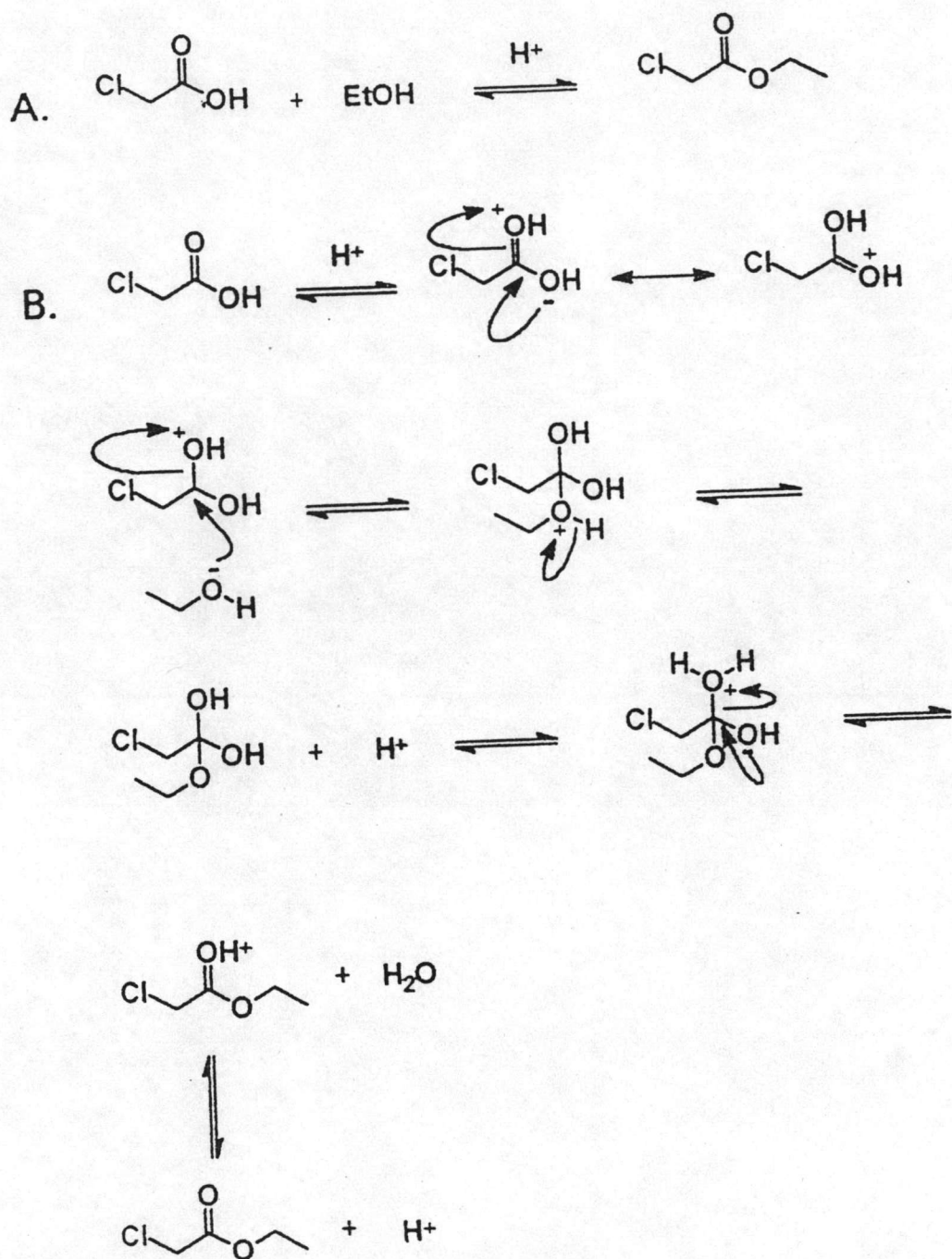
Figure 79. The mechanism of the formation of ethyl chloroacetate.

small amount of acid was used as catalyst, the same point could be reached after a few hours.

This acid-catalyzed esterification reaction proceeded *via* an acyl-oxygen fission process. This involved the cleavage of the bond between the original carbonyl-carbon atom and an oxygen of a hydroxy group in the intermediate arising from nucleophilic attack by an ethanol molecule on the protonated chloroacetic acid. (Figure 79B)

According to the law of mass action, the equilibrium may be displaced in favor of the ester by the use of an excess of one of the components, chloroacetic acid or ethanol. Because the chloroacetic acid is more expensive, a large excess of the absolute ethanol was employed to drive the reaction forward.

Ethyl chloroacetate was purified by simple distillation, its boiling point was 144.5 °c. (Lit. 144.2 °c; Weast, 1986)

The IR spectrum of ethyl chloroacetate showed a very strong C=O stretching band at 1754 cm^{-1}. It was accompanied by the stretching bands of the carbon-oxygen single bond (C(=O)-O-R) between 1313-1026 cm^{-1}. In this region, the chloroacetate carbonyl-oxygen stretching vibration appeared at 1313-1098 cm^{-1} and the carbon-oxygen stretching band of the ethyl was at 1026 cm^{-1}. The C-Cl stretching bands were at 781 cm^{-1}.

Ethyl Ethoxyacetate.

This compound represents ether type. Ethyl ethoxyacetate was obtained from the reaction of sodium ethoxide and ethyl chloroacetate. This reaction has been known as Williamson reaction. The reaction took place immediately and was recognized by the formation of white precipitate of sodium chloride which was one of the reaction's products. The reaction involved the direct nucleophilic displacement of chloride in ethyl chloroacetate by sodium ethoxide as shown in Figure 80A (Furniss et al., 1991).

This compound was purified by simple distillation. Its boiling point was about 156-157 °c (Lit 158 °c; Prager and Jacobson, 1921).

The IR spectrum of ethyl ethoxyacetate showed very strong C=O stretching band at 1755 cm^{-1}. The carbonyl-oxygen stretching vibration appeared between 1274-1203 cm^{-1} and the carbon-oxygen stretching band of the ethyl ester was at 1034 cm^{-1}. The carbon-oxygen stretching band of the ethyl ether was at 1136 cm^{-1}.

2-Propylpentanal.

This compound represents aliphatic aldehyde. To obtain 2-propylpentanal, 2 moles of Grignard reagent, propylmagnesium bromide reacted with 1 mole of ethyl ethoxyacetate. The solution of propyl magnesium bromide was easily obtained since 1-bromopropane is a reactive organic halide. Great care should be taken to assure complete dryness of solvent (diethyl ether), magnesium turnings and apparatus to exclude moisture which reacts with Grignard reagent. The resulting alkylmagnesium halide is soluble in the diethyl ether solvent as a result of coordinate of two ether molecules onto the magnesium that may be represented as shown in Figure 80B.

The true structural nature of the reactive species in solution is uncertain and for convenience the reagent may be represented as a polarized species as shown in Figure 80C.

The reaction of propylmagnesium bromide with ethyl ethoxy acetate followed a three-step mechanisms, namely nucleophilic addition, then β-elimination and then nucleophilic addition again. This reaction was quite straight-forward since propylmagnesium bromide was a strong nucleophile. The first mole of propylmagnesium bromide attacked ethyl ethoxyacetate by the way of nucleophilic addition and the elimination of ethoxy anion followed, resulted in the addition-elimination product which was most reactive than ethyl ethoxyacetate. Thus it was then attacked by the second mole of propylmagnesium bromide by the way of nucleophilic addition again as illustrated in Figure 80D (Furniss et al., 1991).

To the resulting reaction mixture, water was then added slowly to protonate the magnesium salt and followed by sufficient aqueous acid such as hydrochloric or sulfuric acid to dissolve all inorganic salts. The crude product of 1,1-dipropyl-2-ethoxyethanol was not further purified since the reaction was quite complete. The IR spectrum (Figure 28) of
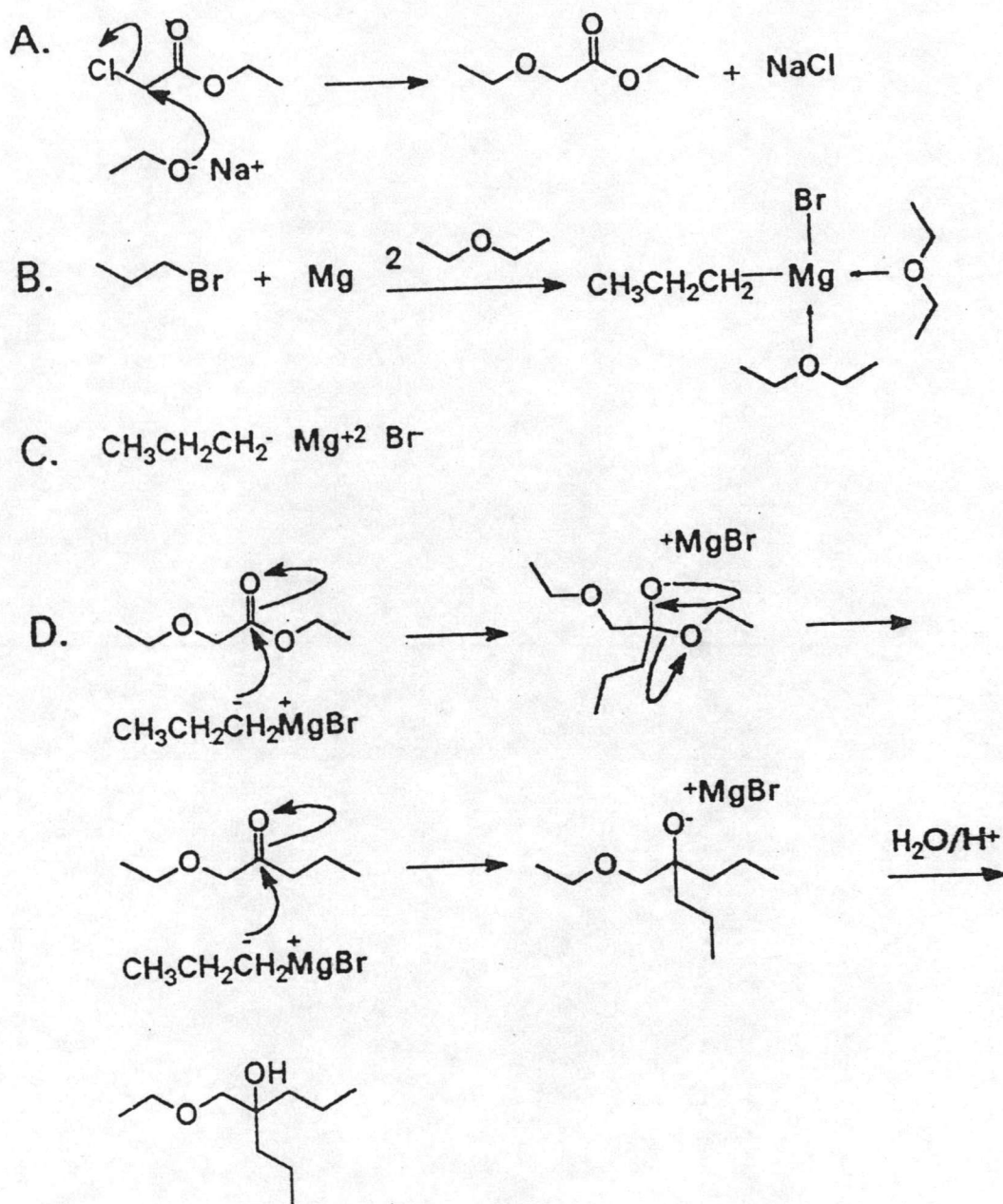
Figure 80. A. The mechanism of the formation of ethyl ethoxyacetate.
B. The formation of propylmagnesium bromide.
C. The structure representing Grignard reagent, propylmagmesium bromide.
D. The mechanism of the formation of 1,1-dipropyl-2-ethoxyethanol.

the crude product showed absorptions corresponding to its chemical structure. The O-H stretching band was between 3600-3300 cm^{-1}. The C-H stretching bands appeared between 3000-2800 cm^{-1} and were accompanied by C-H bending bands which appeared at 1462-1380 cm^{-1}. The bands between 1200-878 cm^{-1} corresponded for the C-O stretching vibration of both alcohol and ether .

1,1-Dipropyl-2-ethoxyethanol was then transformed to 2-propyl pentanal by being heated in the presence of anhydrous oxalic acid. The transformation proceeded through 2 phases. Firstly, one molecule of water was eliminated from 1,1-dipropyl-2-ethoxyethanol and was then absorbed by anhydrous oxalic acid which acted as a dehydrating agent. Secondly, oxalic acid acted as a saponifying agent which resulted in the 2-propylpentanal and diethyl oxalate as illustrated in Figure 81 (Behal and Sommelet, 1904).

2-Propylpentanal was isolated by steam distillation. The IR spectrum of 2-propylpentanal (Figure 29) showed the two characteristic carbon-aldehydic hydrogen stretching bands at 2810 and 2702 cm^{-1}. The former almost superimposed on the alkyl C-H stretching bands. Only the shoulder appearance was observed. The strong C=O stretching band of aldehyde was at 1728 cm^{-1}. The C-H bending bands appeared between 1462-1310 cm^{-1}.

5-Hydroxymethyl-8-methyl-2-(1-propylbutyl)-*4H*-dioxino[4,5-*c*] pyridine or α^4,3-O-(2-Propylpentanylidene)pyridoxine (LXIX).

Pyridoxine hydrochloride and 2-propylpentanal in the presence of dry hydrochloric acid formed a 2-propylpentanylidene derivative, isolated as a hydrochloride $C_{16}H_{25}O_3N\cdot HCl$. Elemental analysis, infrared and NMR spectroscopy indicated that the product is (2-propyl pentanylidene)pyridoxine hydrochloride.

Of the possible structures the α^4,3-O-(2-propylpentanylidene) pyridoxine (LXIX), α^4,α^5-O-(2-propylpentanylidene)pyridoxine (LXX) for the free base, the former was to be prefered since, apart from the less likely seven-membered ring in LXX, involvement of the phenolic group was shown by a negative test with the ferric chloride reagent for a phenol.
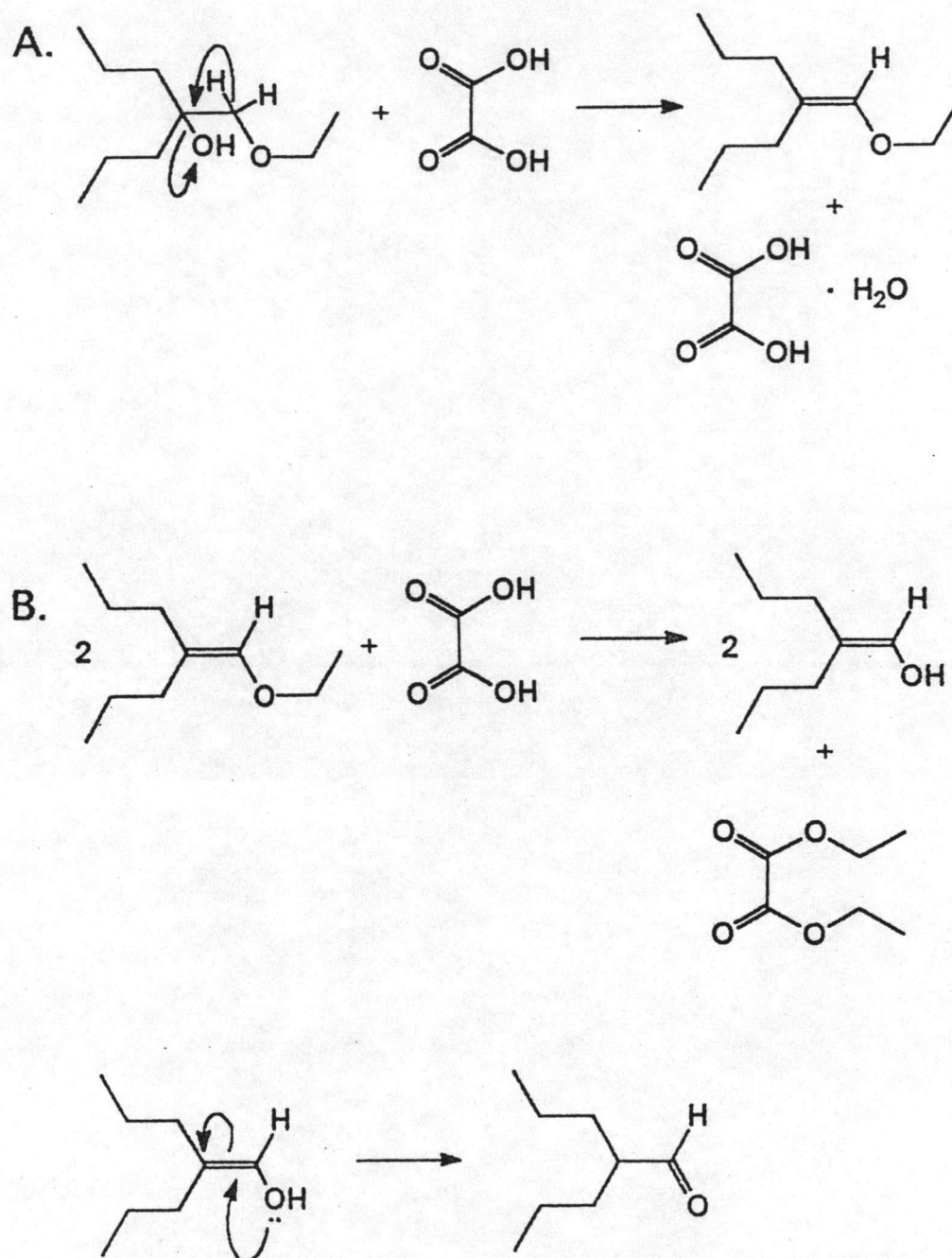
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A.

B.

Figure 81. The mechanism of the formation of 2-propylpentanal from the reaction of 1,1-dipropyl-2-ethoxyethanol and anhydrous oxalic acid.

Pure α^4,3-O-(2-propylpentanylidene)pyridoxine gave no colour reaction with ferric chloride, but the characteristic deep red colour for a phenol was shown if the compound was previously hydrolysed by mere dissolution in warm aqueous acid for a few seconds, which regenerated pyridoxine hydrochloride. In alkaline solution this compound was stable. The rate of hydrolysis had, however, not been studied quantitatively, and the phenol test was extremely sensitive.

The IR spectrum of this compound (Figure 30) showed a broad band of O-H stretchinging (3289 cm^{-1}) and N$^+$-H stretchinging (2750-2008 cm^{-1}). The bands at 3100-3000 cm^{-1}, 2958-2870 cm^{-1} and 2815 cm^{-1} were assigned to be the characteristic C-H stretchinging vibration of aromatic, aliphatic and acetal hydrogen respectively. In the 1637-1422 cm^{-1} region there were the bands, undoubtedly arising from pyridine ring vibrations. The O-H bending and the C-O stretching appeared as many strong bands between 1283-1002 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of α^4,3-O-(2-propyl pentanylidene)pyridoxine hydrochloride in CDCl$_3$ had nine signals (Figure 31). The three-proton peak at 2.72 ppm and the one-proton peak at 8.36 ppm were obviously due to the 8-methyl and the C$_6$-proton. The two-proton peak at 5.05 ppm was due to the protons of 5-methylene side chain.

Two C$_4$-protons were nonequivalent because of the presence of 2-(1-propylbutyl) group. The ^{1}H-NMR spectrum showed the C$_4$-protons as two doublet in the aliphatic region at 4.74 and 4.68 ppm, distinguished by geminal coupling constant of 14.95 Hz. The roof effect was seen since the shift difference between them was small.

The acetal proton was coupled with the β-methine hydrogens that occurred as a doublet at δ 5.09 ppm, distinguished by J = 3.96 Hz. The fifteen-proton of 2-(1-propylbutyl) side chain appeared as complex multiplets between 0.80-2.00 ppm. The signals of methyl protons appeared at 0.91-0.95 ppm (6H, m). The signals of methylene protons appeared at 1.34-1.59 ppm (6H, m and 2H, m). The signal of methine proton appeared at 1.85-1.93 ppm (1H, m).

The ^{13}C-NMR spectrum of this compound (Figure 33) showed obvious 3 main groups of peaks, the aromatic carbons, the acetal carbon

and finally, the aliphatic carbons. The group of peaks that characterized the aromatic carbons appeared at 150.08, 141.14, 136.45, 135.70 and 129.81 ppm were clearly assignable to five carbon atoms of the pyridine ring. The peak appearing at 103.39 ppm was assigned to the acetal carbon since it attached to two oxygen atom. Two peaks resonated at 64.18 and 57.97 ppm were assigned to carbons which attached to one oxygen atom that are C_4-carbon and 5-methylene carbon respectively. The group of peaks that characterized the alkyl 2-(1-propylbutyl) chain appeared in 6 signals. The peaks at 41.33 ppm was assigned to the methine carbon of this alkyl chain. Four methylene carbons appeared at 30.84, 30.69, 20.26 and 20.23 ppm. The methyl carbons appeared at 14.32 ppm. Finally, a single resonance at 13.78 ppm was clearly assignable to the 8-methyl carbon atom.

The EIMS spectrum of the free base is shown in Figure 34. The peak at m/e 279 represented the molecular ion. Elimination of 2-propylpentanal from the molecular ion yielded an orthoquinoid ion m/e 151. Elimination of carbon monoxide from fragment 151 and ring expansion would lead to a fragment of m/e 123 that would be expected also to lose one hydroxyl group led to the base peak at m/e 106. The fragment at m/e 123 also lost one hydrogen atom followed by carbon monoxide to give the fragments at m/e 122 and 94 respectively. The fragments at m/e 99 was 1-propylbutyl carbonium ion that would be decomposed by loss of even-electron neutral molecule to give the fragments at m/e 57, 55, 43 and 41. The pattern of fragmentation is shown in Figure 82.

5-Hydroxymethyl-2,2,8-trimethyl-*4H*-dioxino[4,5-*c*]pyridine or α^4,3-O-Isopropylidenepyridoxine.

This compound was obtained in quantitative yield by saturating with hydrogen chloride on acetone suspension of pyridoxine hydrochloride. As described for α^4,3-O-(2-propylpentanylidene) pyridoxine. Of the two possible formulae the six-membered ring and the seven-membered ring cyclic ketal, the former seemed the more probable. This was supported by the compound gave a negative ferric chloride test, when freshly dissolved.

The IR spectrum of its hydrochloride salt (Figure 35) showed a similar pattern of bands compared with α^4,3-O-(2-propylpentanylidene)
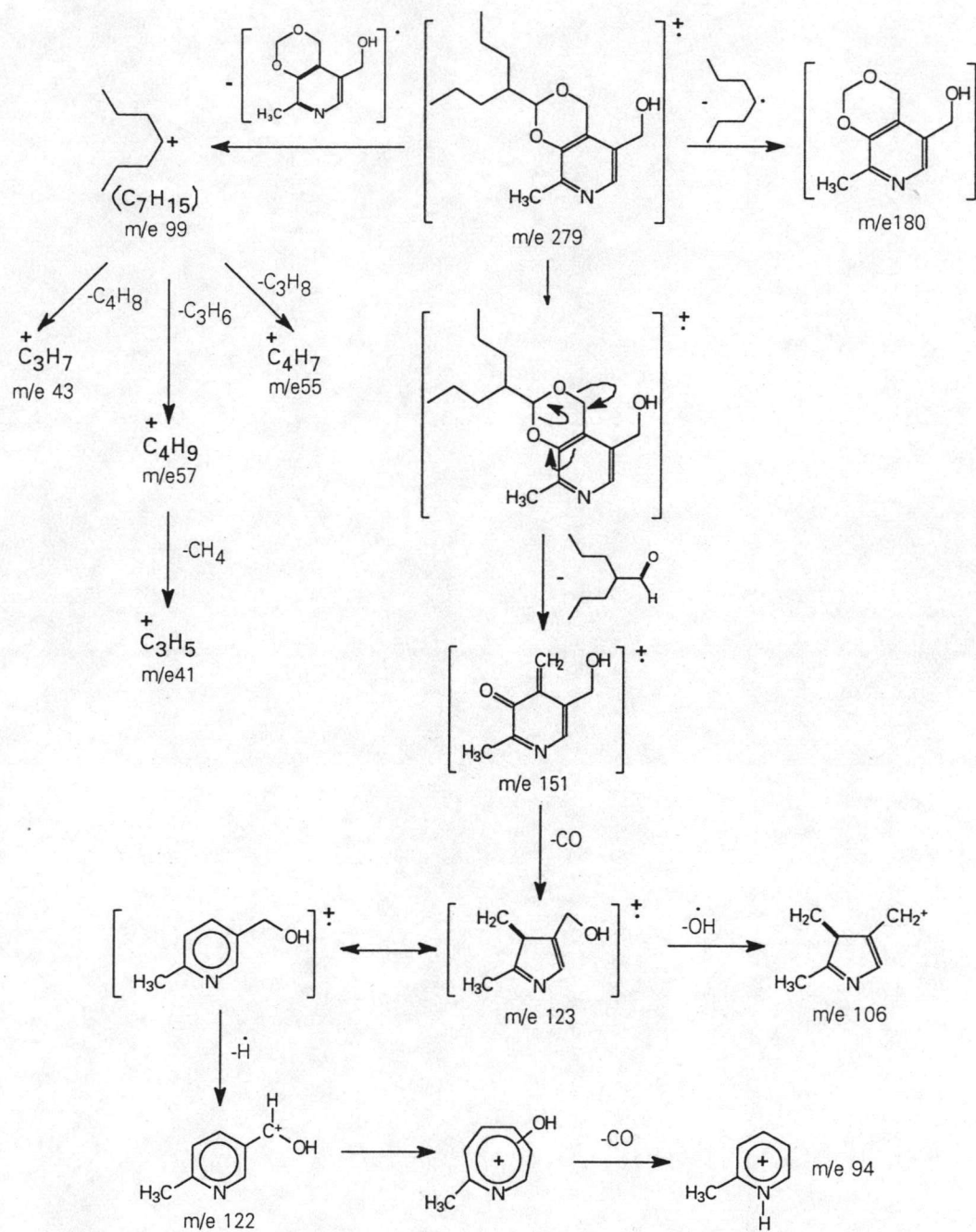
Figure 82. Mass fragmentation of α^4,3-O-(2-propylpentanylidene) pyridoxine.

pyridoxine hydrochloride. It had O-H stretching at 3325 cm^{-1}, aromatic C-H stretching at 3089 cm^{-1}, aliphatic C-H stretching in the 2985-2861 cm^{-1} region, N^+-H stretching at 2494 cm^{-1}. Pyridine ring vibrations occurred in the 1622-1444 cm^{-1} region. The O-H bending and the C-O stretching appeared as many bands between 1298-1051 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of α^4,3-O-isopropylidene pyridoxine hydrochloride in D_2O had five signals (Figure 36). The spectrum showed a sharp singlet at 1.58 ppm for six protons of two 2-methyl group side chains which were magnetically equivalent. The three-proton peak at 2.54 ppm and the one-proton peak at 8.09 ppm were obviously due to the 8-methyl and the C_6-proton. The two-proton peaks at 4.65 and 5.07 ppm were due to the protons of C_4-protons and 5-methylene side chain.

The ^{13}C-NMR spectrum of this compound (Figure 37) showed obvious 3 main groups of peak. First, the group of five peaks that characterized the aromatic carbons appeared in the 150.87-131.54 ppm were clearly assignable to five carbon atoms of the pyridine ring. Second, the peak of ketal carbon which appeared at 105.29 ppm. Third, the aliphatic carbon peaks. Two peaks resonated at 61.21 and 60.16 ppm were assigned to C_4-carbon and C_5-methylene carbon which attached to one oxygen atom. Two peaks resonated at 26.29 and 16.19 ppm were assigned to the 2-methyl and the 8-methyl carbon.

The preparation of α^4,3-O-isopropylidenepyridoxine is the simultaneous protection of phenolic and alcoholic carboxyl groups by cyclic ketal formation. It leaves the 5-hydroxymethyl group free for further reaction such as esterification.

5-(2-Propylpentanoyl)oxymethyl-2,2,8-trimethyl-*4H*-dioxino[4,5-*c*] pyridine or α^4,3-O-Isopropylidenepyridoxine-5-valproate.

This compound was obtained by esterified α^4,3-O-(2-isopropylidenepyridoxine with valproyl chloride in the presence of pyridine to yield the corresponding ester.

Acylation of pyridoxine was performed in the presence of an organic base pyridine. The base served two purposed. It neutralized the protons generated in the reaction and prevented the development of high

acid concentration. Pyridine also became directly involved in the reaction as a nucleophilic catalyst. Pyridine was more nuclophilic than an α^4,3-O-isopropylidenepyridoxine toward the carbonyl center of an valproyl chloride. The resulted product, an valproylpyridinium ion, was more reactive toward an α^4,3-O-isopropylidenepyridoxine than the original valproyl chloride. The condition required for nucleophilic catalysts therefore exist, and acylation of the α^4,3-O-isopropylidenepyridoxine by valproyl chloride was faster in the presence of pyridine than in its absence as illustrated in Figure 83 (Carey and Sundberg, 1993).

Its IR spectrum (Figure 38) showed no hydroxyl frequency but a single carbonyl absorption at 1736 cm^{-1}. Moreover, the high frequency of the C-H stretching band between 2958-2712 cm^{-1} were detectable. These implied the introduction of the valproyl part to the molecule of α^4,3-O-isopropylidenepyridoxine.

The ^{1}H-NMR spectrum of a solution of α^4,3-O-isopropylidene pyridoxine-5-valproate hydrochloride in CDCl$_3$ is showed in Figure 39. Its showed not only five sharp singlets at 8.20 ppm (1H), 5.07 ppm(2H), 5.02 ppm (2H), 2.80 ppm (3H) and 1.63 ppm (6H) for fourteen protons of isopropylidenepyridoxine part, but also a pattern of fifteen-proton signals of valproyl part appeared as complex multiplets at 0.88-0.91 ppm (6H,CH_3), 1.21-1.29 ppm (4H,CH_2), 1.42-1.49 ppm (2H,CH_2), 1.55-1.61 ppm (2H, CH_2) and 2.42-2.47 ppm (1H,CH).

The ^{13}C-NMR of this compound showed 4 main groups of peaks, the carbonyl carbon which appeared at 175.56 ppm, five aromatic carbons which appeared in the 148.66-129.40 ppm region, the ketal carbon which appeared at 102.48 ppm, and finally the aliphatic carbon which appeared in the 58.90-13.86 region. The peaks at 58.90 and 58.41 ppm were due to the resonance of the C$_4$-carbon and C$_5$-methylene carbon which attached to one oxygen atom. The peak at 44.94 ppm was assigned to the methine carbon of the valproyl part. The four methylene carbons of valproyl part appeared at 34.34-24.50 ppm. The 2-methyl carbons (2 atoms) and the 8-methyl carbon resonated at 20.59 ppm and 13.91 ppm respectively. Finally, a single resonance at 13.86 ppm was clearly assignable to the methyl carbon of the valproyl part.
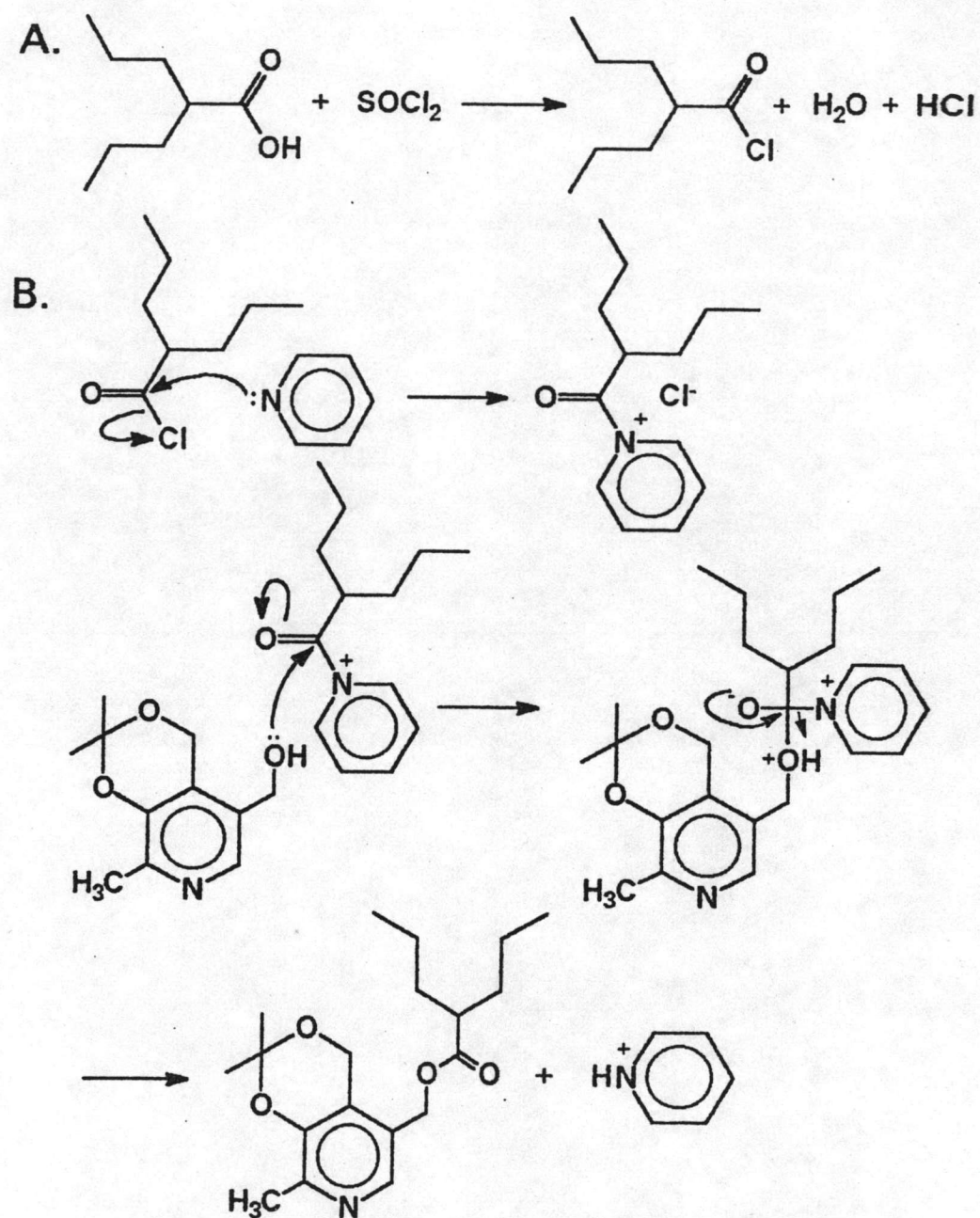
Figure 83. The mechanism of the formation of α^4,3-O-isopropylidene pyridoxine-5-valproate.

3-Hydroxy-4-hydroxymethyl-2-methyl-5-(2-propylpentanoyl)oxy methylpyridine or Pyridoxine-5-valproate (LXXI).

This monovalproate ester of pyridoxine was obtained by selective hydrolysis of the isopropylidene group of α^4,3-O-isopropylidene pyridoxine-5-valproate with dilute formic acid. The product was isolated as the free base, $C_{16}H_{25}O_4N$ which was confirmed by elemental analysis.

The IR spectrum (Figure 42) of this compound had been considered as the indication of the dipolar nature of this compounds in crystalline state. In the case of crystalline pyridoxine-5-valproate, however, no free O-H frequency was observed as the result of the one intramolecular O-H frequency at 3180-3140 cm^{-1}, and an intermolecular O$\cdots$H$\cdots$N frequency at 2608 cm^{-1}. On this basis the proposed structure of crystalline pyridoxine-5-valproate indicated by Figure 84 seem to be reasonable. The three weak sharp absorption bands between 2100-2028 cm^{-1} had further supported this proposed structure since the bands frequently are observed at this region for amino acid, amino acid hydrochloride and amine hydrochloride. Moreover, the spectrum showed an intense bands of C-H stretching between 2959-2871 cm^{-1} and a characteristic strong band of carbonyl absorption at 1729 cm^{-1}. The pyridine ring vibrations occurred in the 1622-1429 cm^{-1} region. The C-O stretching appeared as many bands between 1289-1036 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of pyridoxine-5-valproate in CDCl$_3$ (Figure 43) had nine signals. A group of four sharp singlet between 7.97-2.73 ppm was obviously due to eight protons of pyridoxine part. The other five signals appeared as complex multiplets between 2.46-0.87 ppm were due to fifteen protons of the valproyl part. The signals of methyl protons which were coupled of the β-methylene appeared as a triplet between 0.87-0.90 ppm (6H). The signals of methylene protons appeared between 1.20-1.61 ppm (8H, complex). The signal of methine proton appeared between 2.40-2.46 ppm (1H, m).

The ^{13}C-NMR showed 3 main groups of peaks, the carbonyl carbon which appeared at 175.63 ppm, five aromatic carbons which appeared in the 154.83-129.34 ppm region and finally the aliphatic carbons which appeared in the 60.12-13.88 ppm region. The peaks at 60.12 and 59.63 ppm were due to the resonance of the 4-methylene and 5-methylene carbons which attached to one oxygen atom. The peak at
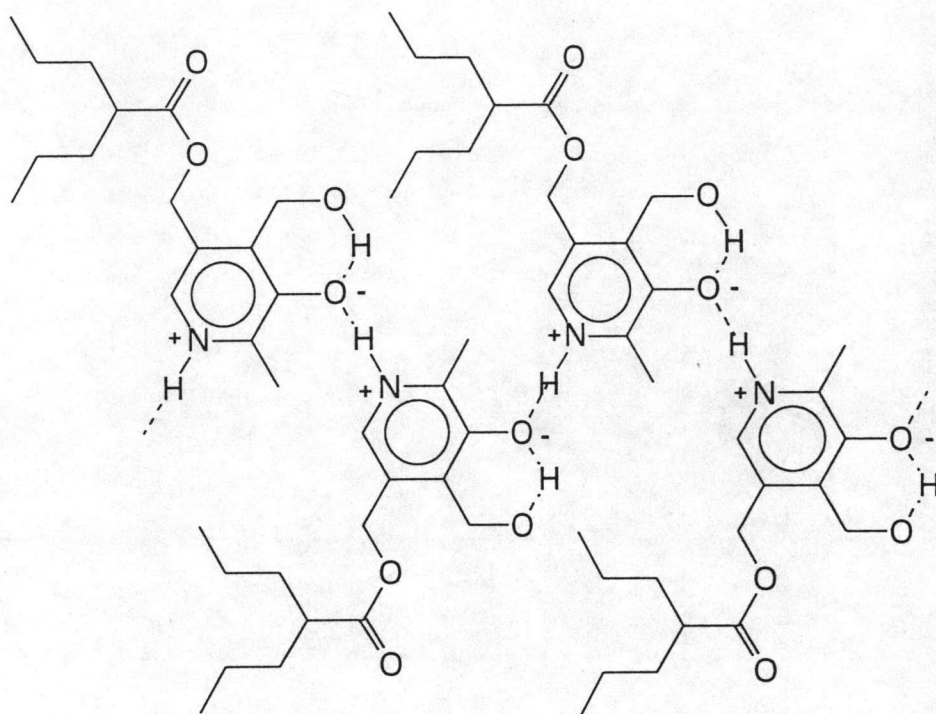
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Figure 84. Proposed structure of crystalline pyridoxine-5-valproate (free base).

44.96 ppm was assigned to the methine carbon of the valproyl part. The four methylene carbons of valproyl part appeared at 34.31-20.56 ppm. The 2-methyl carbon resonated at 14.31ppm. Finally, a single resonance at 13.86 ppm was clearly assignable to the methyl carbons of the valproyl part which was slightly upfield from an aromatic methyl group.

The EIMS spectrum of this compound is shown in Figure 47. The peak at m/e 295 represented the molecular ion. The formation of four peaks at m/e 94, 106, 122 and 123 can be also rationalized as arising from the orthoquinoid ion, m/e 151. Acylium ion resulted from C-O bond cleavage next to C=O gave a peak at m/e 127. Decarboxylation of the acylium ion gave 1-propylbutyl carbonium ion, m/e 99 that would be decomposed by loss of even-electron neutral molecule to give the fragments at m/e 57, 55, 43 and 41. The peaks at m/e 102 resulted from explusion of propene from the undetectable ion of valproic acid which would be formed by loss of m/e 151 from the molecular ion. Loss of C_2H_5 from m/e 102 led to the peak at m/e 73. The speculative pattern of fragmentation is shown in Figure 85.

4-Hydroxymethyl-5-hydroxymethyl-2-methyl-3-(2-propylpentanoyl) oxypyridine or Pyridoxine-3-valproate (LXXII).

This compound was obtained by the reaction of pyridoxine with valproyl chloride. Since the phenolic hydroxyl in pyridoxine is more acidic than the other two hydroxyl group, monoesterification of pyridoxine with 1 mole of valproyl chloride would be expected to give pyridoxine-3-valproate.

Valproyl chloride was dissolved in tetrahydrofuran and added in portions (followed by vigorous stirring) to a cooled aqueous solution of pyridoxine and sodium carbonate. Sodium carbonate serves not only to neutralize the hydrogen chloride that would otherwise be liberated, but also to catalyse the phenol hydroxyl to react with valproyl chloride.

The product was isolated as a hydrochloride, $C_{16}H_{25}O_4N \cdot HCl$. Elemental analysis and infrared spectrum (Figure 48) indicated that the product is pyridoxine valproate hydrochloride. The failure to give the positive ferric chloride test indicated that this compound would be pyridoxine-3-valproate.
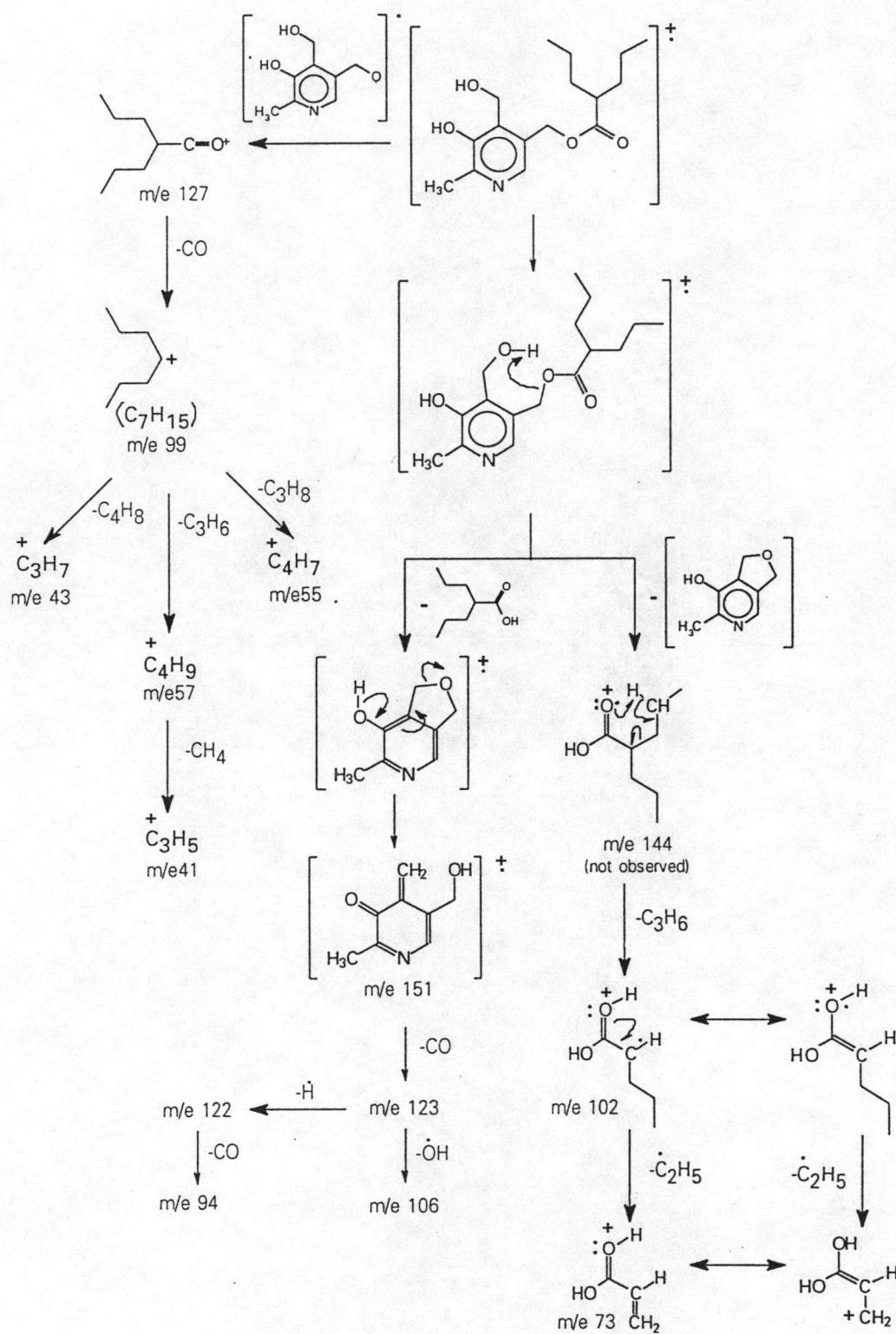
Figure 85. Mass fragmentation of pyridoxine-5-valproate.

Except for the location of the chemical shift, the NMR spectra of its hydrochloride salt in D_2O showed a similar pattern to those of pyridoxine-5-valproate. The retention of a pattern of four sharp singlets in the ^{1}H-NMR (Figure 43) was also evident from the chemical shift of eight protons of pyridoxine which were at 8.36, 5.48, 5.01 and 2.79 ppm. The presence of valproyl side chain at position 3 was showed by the characteristic splitting pattern for fifteen protons of this substituent as complex multiplets between 2.60-0.87 ppm. The ^{13}C-NMR spectrum is shown in Figure 52.

Moreover, the mass spectrum of pyridoxine-3-valproate (Figure53) and pyridoxine-5-valproate (Figure 47) showed a large degree of similarity. This can be rationalized by the formation of an identical ion at m/e 151, an acylium ion at m/e 127 and an undetectable ion of valproic acid at m/e 144 from which all further fragmentation occurred as illustrated in Figure 86.

4-Hydroxymethyl-2-methyl-3-[(2-propylpentanoyl)oxy]-5-[(2-propyl pentanoyl)oxymethyl]pyridine or Pyridoxine-3,5-divalproate (LXXIV).

This compound was obtained by monoesterification of pyridoxine-5-valproate with 1 mole of valproyl chloride in the presence of pyridine. It is almost certain that the phenolic hydroxyl is esterified first in this case hence the expected pyridoxine-3,5-valproate is obtained.

Since the compound did not appear to form the solid precipitates of the hydrochloride salt, it was isolated as the free base, $C_{24}H_{39}O_5N$. Elemental analysis and two distinguishing v C=O stretchinging at 1737 cm^{-1} in infrared spectrum (Figure 35) indicated that the product is pyridoxine divalproate. The compound gave a negative ferric chloride test. This finding confirmed that it must be the 3,5-divalproate derivative.

The ^{1}H-NMR spectrum of a solution of pyridoxine-3,5-divalproate in CDCl$_3$ is shown in Figure 57. In addition to four sharp singlet peaks of eight protons of pyridoxine betweem 8.12-2.53 ppm, there was another characteristic pattern of a thirty-proton signals of two valproyl parts between 2.46-0.82 ppm.
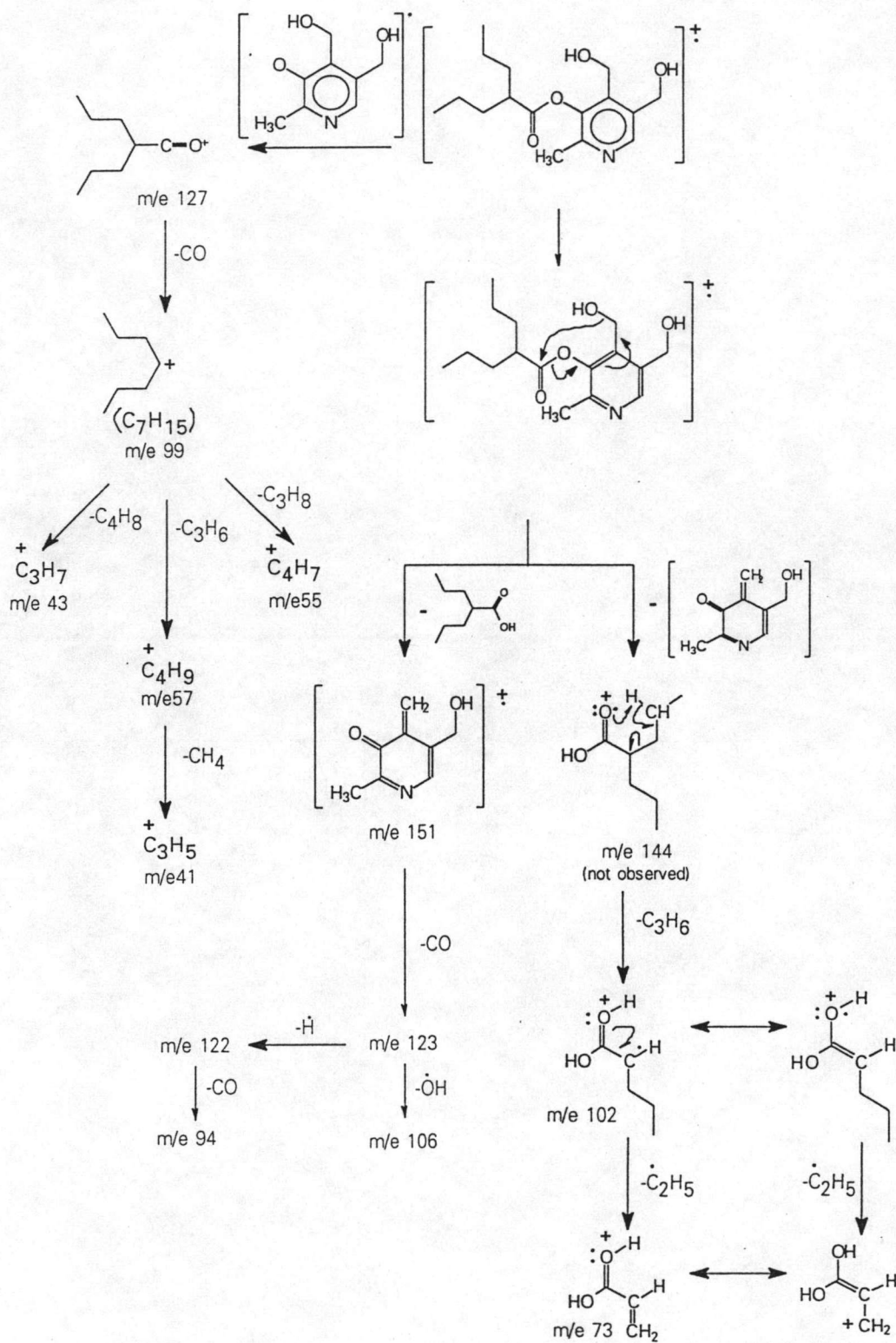
Figure 86. Mass fragmentation of pyridoxine-3-valproate.

The ^{13}C-NMR (Figure 58) showed 3 main groups of peaks, two carbonyl carbons which appeared at 179.16 and 176.12 ppm, five aromatic carbons which appeared in the 154.83-129.34 ppm region and finally the aliphatic carbons which appeared in the 60.94-13.76 region. The peaks at 60.94 and 57.64 ppm were due to the resonance of the 4-methylene and 5-methylene carbon which attached to one oxygen atom. The 2-methyl carbon resonated at 19.45 ppm. The other eight signals which devided into 4 pairs of peaks (44.15 and 44.95 ppm, 34.47 and 34.38 ppm, 20.57 and 20.39 ppm , 13.89 and 13.76 ppm) were due to the resonance of the methine, methylene and methyl carbons of two valproyl parts.

The EIMS spectrum of this compound is shown in Figure 59. The peak at m/e 422 represented the molecular ion (Calculated molecular weight is 421.58) which lost the propene molecule from the valproyl part *via* the McLafferty rearrangement to give the ion at m/e 379.

Because of the interplay of several functional groups present in the molecule, the complex degradation procedures such as rearrangement can be occured and lead to a lot of fragments in the spectrum which can not be proposed what part of molecule is present in them. The peak at m/e 102 resulted from either the m/e 144 ion of valproic acid or the fragment at m/e 379. Loss of C_2H_5 from m/e 102 led to the base peak at m/e 73 as illustrated in Figure 87.

2-Methyl-3-[(2-propylpentanoyl)oxy]-4-[(2-propylpentanoyl)oxy methyl]-5-hydroxymethylpyridine or Pyridoxine-3,4-divalproate (LXXIII).

Reaction of pyridoxine hydrochloride with 2 moles of valproyl chloride in a mixture of chloroform and pyridine at room temperature afforded a compound, isolated as a hydrochloride, m.p. 118-120 °c, which had an elemental composition and infrared spectrum (Figure 60) corresponding to pyridoxine divalproate hydrochloride, $C_{24}H_{39}O_5N\cdot HCl$ and gave a negative ferric chloride test. Of the three possible isomer of pyridoxine divalproate, the 4,5-divalproate was ruled out since the presence of 3-hydroxyl (phenolic) in this compound must be detectable by the ferric chloride test. This finding showed that it may be either the 3,4- or 3,5-divalproate. Further, the possibility of the latter could be
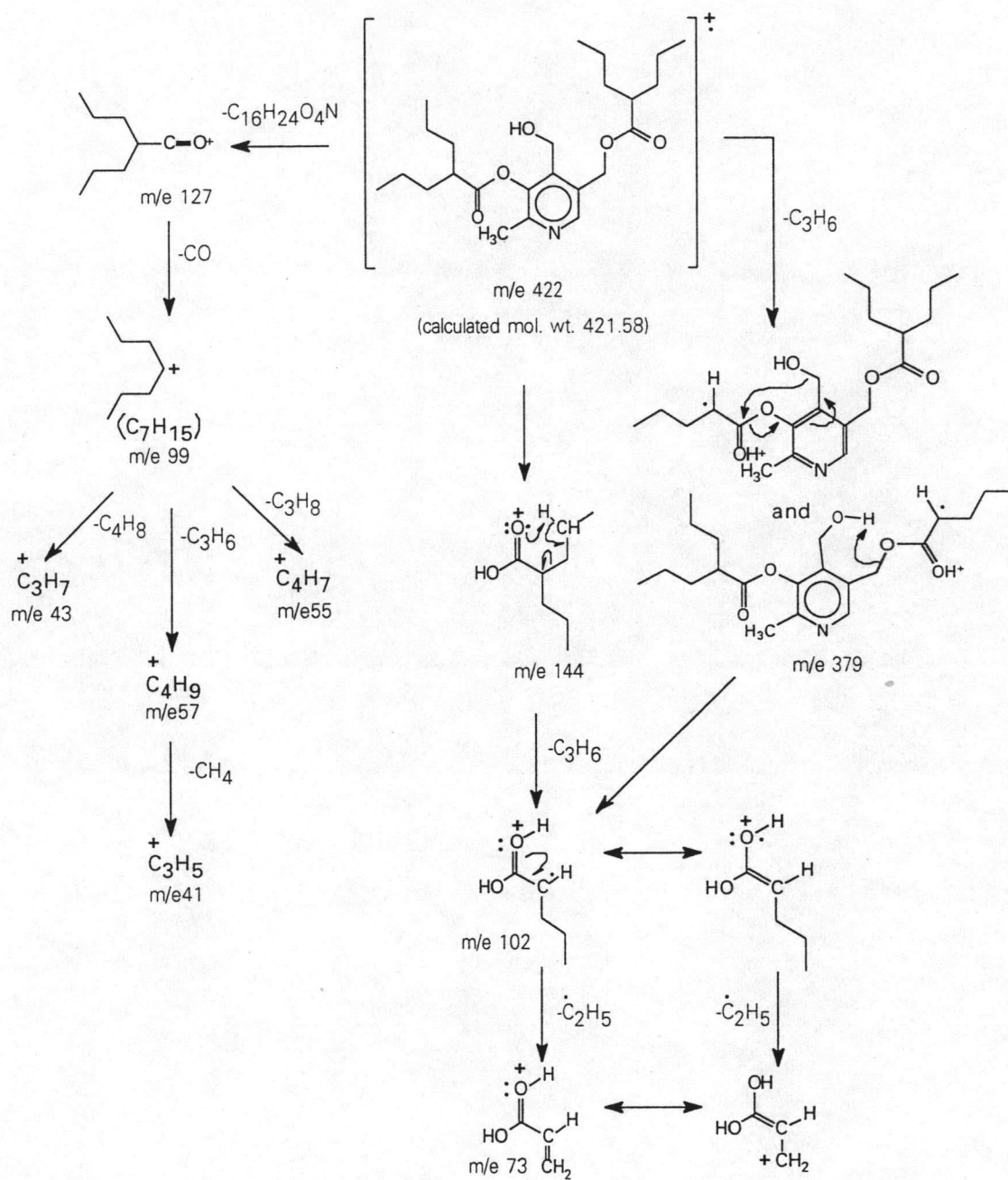
Figure 87. Mass fragmentation of pyridoxine-3,5-divalproate.

ruled out when this divalproate was not identical with the known 3,5-divalproate. It may thus be concluded that this divalproate derivative of pyridoxine is the 3,4-divalproate.

The result of this experiment showed that on mild acylation of pyridoxine with valproyl chloride, the 3-phenolic hydroxyl group and the hydroxyl of the 4-hydroxy methyl group were preferably attacked. Since the 3-phenolic hydroxyl in pyridoxine is more acidic than the other two hydroxyl groups, the acylation of the 3-phenolic hydroxyl may be easier. The hydroxyl group of the 4 hydroxymethyl may be also reactive because its para-location to the nitrogen in the pyridine ring (Uchibachi, 1961) and the hydrogen bond between its hydrogen atom and the 3-phenolic oxygen atom activate its nucleophilicity. Such difference in reactivity of the hydroxyl groups may be offered one of the possible interpretations for the selective esterification.

Except for the location of the chemical shift, the NMR spectra of its hydrochloride salt in $CDCl_3$ (Figure 61 and 63) showed a similar pattern to those of pyridoxine-3,5-divalproate (Figure 55 and 58).

The EIMS spectrum of the free base is shown in Figure 64. It differed markly from that of pyridoxine -3,5-divalproate. The molecular ion peak was undetectable (because the calculated molecular weight is 421.58, the molecular ion peak should appear at m/e 422). An M-1 peak could be seen at m/e 421. There were the formation of an orthoquinoid ion at m/e 151, and acylium ion at m/e 127 and an undetectable ion of valproic acid at m/e 144 from which all further fragmentation occurred as illustrated in Figure 88.

2-Methyl-3-[(2-propylpentanoyl)oxy]-4,5-di[(2-propylpentanoyl)oxy methyl]pyridine or Pyridoxine Trivalproate (LXXV).

This compound was obtained by the reaction of pyridoxine hydrochloride with 3 moles of valproyl chloride in a mixture of chloroform and pyridine at room temprerature. Since the compound did not appear to form the solid precipitates of the hydrochloride salt, it was isolated as the free base which had an elememtal composition corresponding to pyridoxine trivalproate.
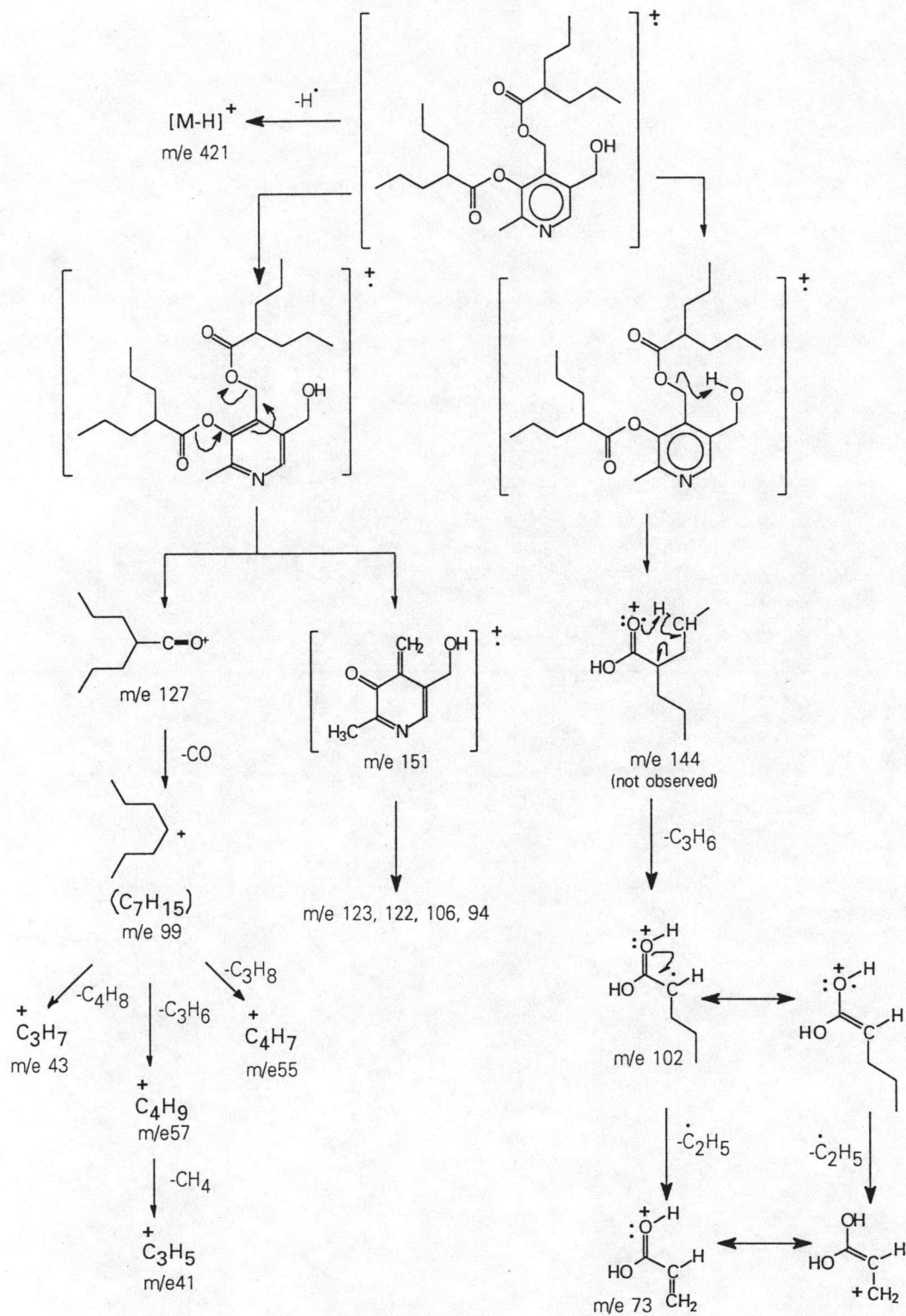
Figure 88. Mass fragmentation of pyridoxine-3,4-divalproate.

Although the IR spectrum (Figure 65) showed two band of carbonyl stretching at 1761 and 1739 cm^{-1}, The disappearance of the O-H stretching band together with the NMR spectra and MS spectrum were consistent with the assigned structure.

The ^{1}H-NMR spectrum of a solution of pyridoxine trivalproate in $CDCl_3$ is shown in Figure 46. In addition to a pattern of eight-proton signals of pyridoxine between 8.39-2.36 ppm, there was another pattern of forty-five-proton signals of three valproyl parts between 2.36-0.79 ppm. The pattern of splitting of each signals into two equal peaks indicated that this compound presents in two conformations, in an equal quantity. The actual conformations of this compound in solution have not been proposed. It is likely that they involve the way three valproyl parts are oriented in space around the pyridine ring as illustrated in Figure 89.

The ^{13}C-NMR showed 3 main groups of peaks. Three signals of carbonyl carbons appeared at 175.79, 175.63 and 173.45 ppm. Five signals of aromatic carbons appeared in the 152.45-144.55 ppm region. Finally, thirteen signals of aliphatic carbons appeared in the 60.78-13.78 ppm region. The peaks at 60.78 and 56.67 ppm were due to the resonance of the 4-methylene and 5-methylene carbons which attached to one oxygen atom. The 2-methyl carbon resonateed at 19.82 ppm. The other ten signals were due to the resonance of the methine, methylene and methyl carbons of three valproyl parts.

The EIMS spectrum of this compound is shown in Figure 70. The peak at m/e 548 represented the molecular ion (calculated molecular weight is 547.78) which underwent fragmentation to give the fragment of pyridoxine divalproate at m/e 422, valproic acid (not observed), acylium ion at m/e 127 and orthoquinoid ion at m/e 151. The further fragmentation of these fragments showed the corresponding pattern in the spectrum. The proposed pattern of fragmentation is shown in Figure 90.

Ethyl Alaninate Hydrochloride.

The alanine was converted to the corresponding ethyl ester hydrochloride by the addition of thionyl chloride to its suspension in cold
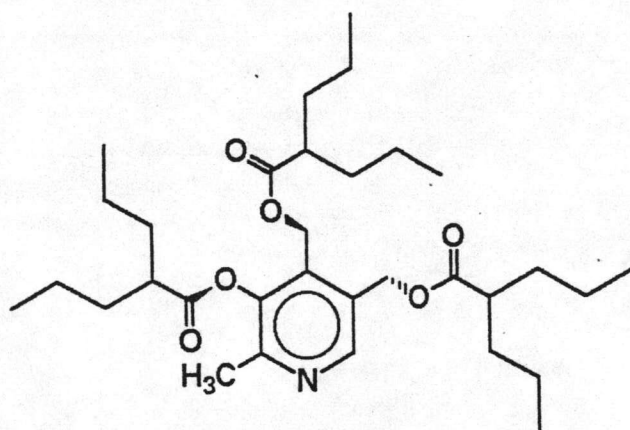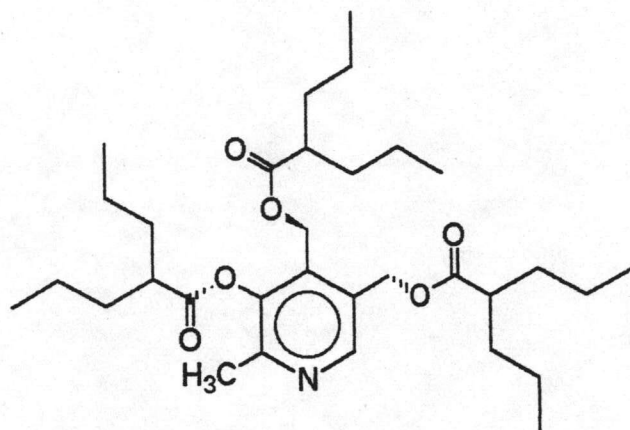
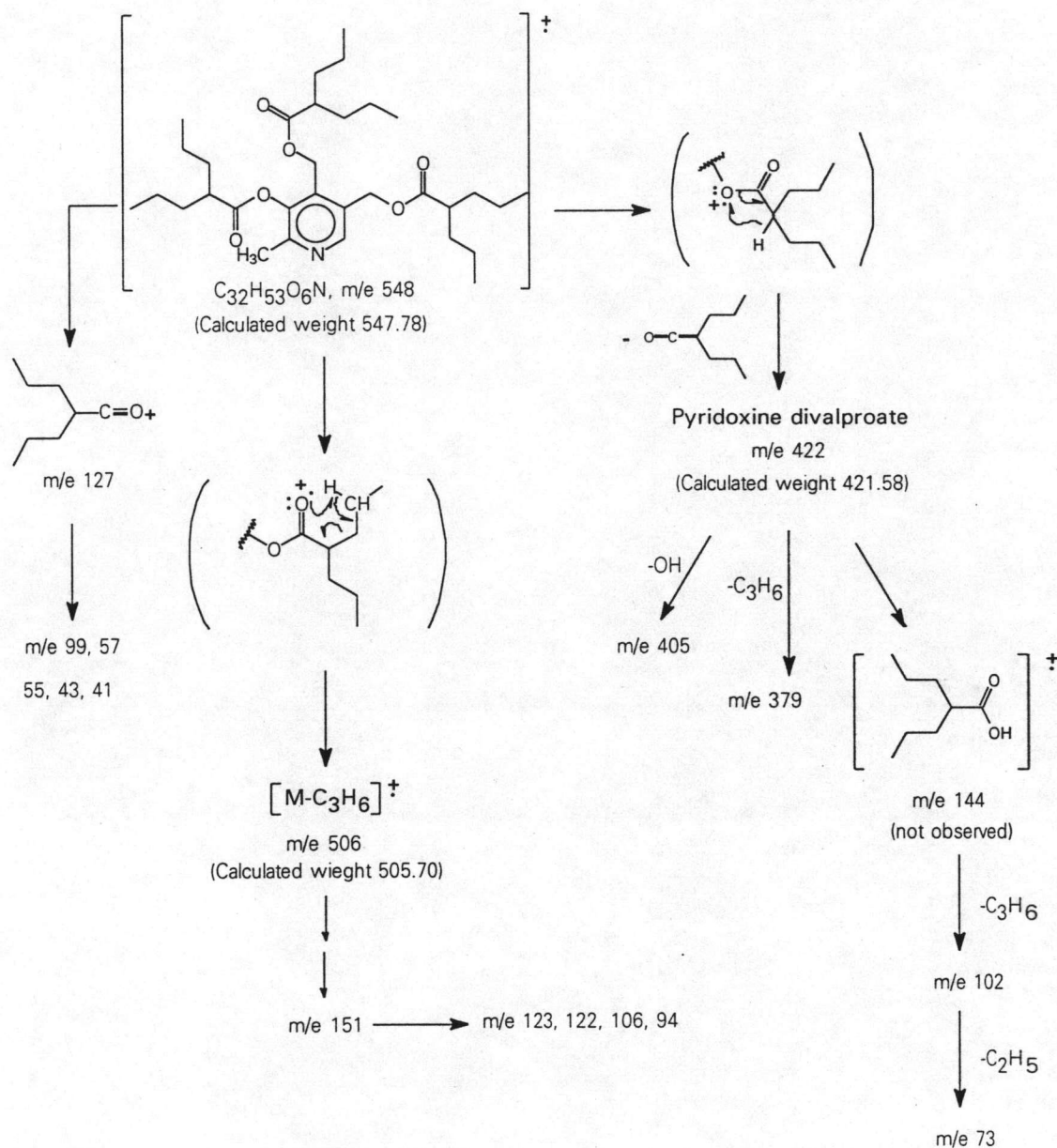Figure 89. Proposed conformations of pyridoxine trivalproate.

Figure 90. Mass fragmentation of pyridoxine trivalproate.

ethanol. The yield was quantitative and no futher purification was required.

The IR spectrum (nujol) showed a broad strong NH_3^+ stretchinging band in the 3449-2600 cm^{-1} region. Two peaks between 1609-1513 cm^{-1} were due to the asymmetrical and symmetrical NH_3^+ bending respectively. The C=O stretching vibration appeared at 1744 cm^{-1}. The C-O stretching band was at 1246-1211 cm^{-1}. (The bands at 3000-2860, 1470, 1383 cm^{-1} were Nujol's)

Ethyl-*N*-formylalaninate.

This compound was obtained by formylation an ethyl-*N*-formylalaninate hydrochloride using formamide as a formylating agent. The reaction equation is illustrated in Figure 91A.

The compound was purified by distillation under diminished pressure. It boiling point at 0.8 mmHg was about 100 °c.

The IR spectrum (Figure 72) showed N-H stretching band at 3284 cm^{-1} and two C=O stretching band at 1736-1667 cm^{-1}. The band at 1736 cm^{-1} was due to the C=O ester stretching vibration. The C=O absorption of amide occurred at lower frequency at 1667 cm^{-1}. The compound displayed N-H bending vibration at 1532 cm^{-1}. The C-N stretchinging band occurred at 1380 cm^{-1}. The C-O stretchinging band occurred in the 1206-1138 cm^{-1}. A broad, medium band in the 800-666 cm^{-1} region in the spectrum resulted from out-of-plane N-H wagging.

4-Methyl-5-ethoxyoxazole.

The preparation of this substituted oxazole was *via* cyclization of ethyl-*N*-formylalaninate, on treatment with a cyclodehydrating agent, phosphorus pentoxide.

This reaction has been known as Robinson-Gabriel oxazole synthesis. Mechanistic pathways for the reaction are shown in Figure 91B (Turchi and Dewar, 1975).

The product was purified by distillation. The phosphoric acid formed was wiped out before distillation by neutralizing with potassium
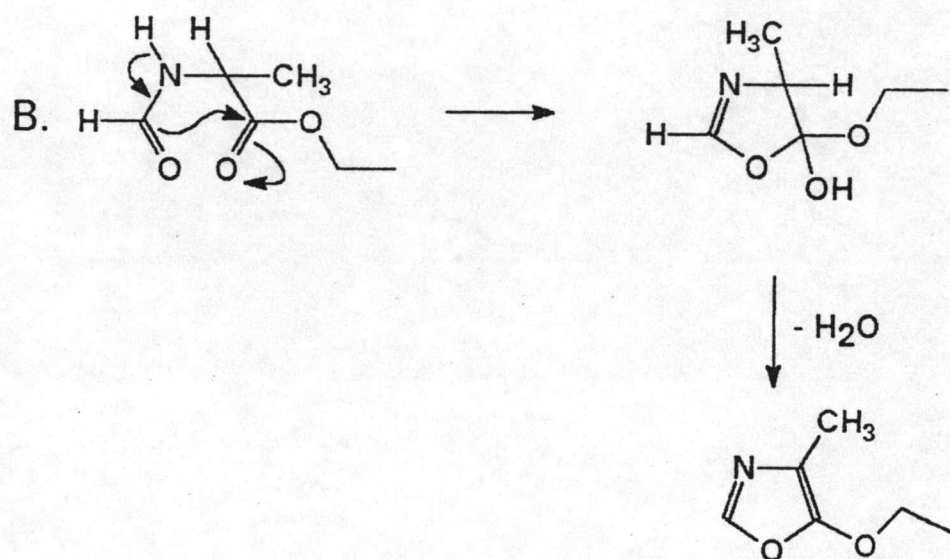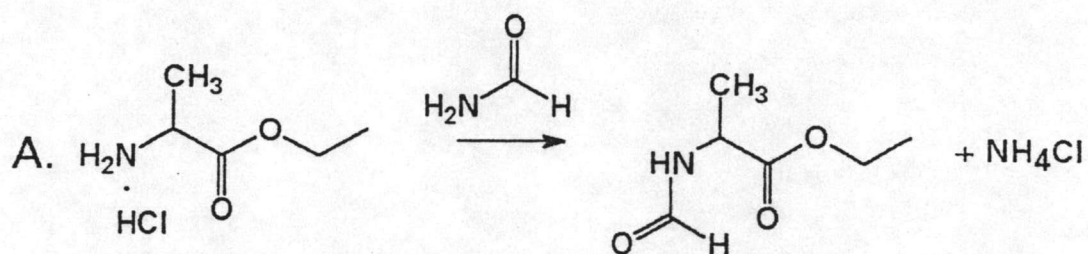
Figure 91. A. The formation of ethyl-*N*-formylalaninate.
B. The mechanism of the formation of 4-methyl-5-ethoxy oxazole from ethyl-*N*-formylalaninate.

hydroxide and then washing with water. Distillation of the residual under diminished pressure gave 4-methyl-5-ethoxyoxazole.

The IR spectrum of this compound is shown in Figure 73. The band at 3134 cm^{-1} and 2982-2930 cm^{-1} had been assigned to be the C-H stretchinging vibration characteristic of aromatic and aliphatic hydrogen respectively. The IR stretchinging modes of oxazole ring were at 1670-1406 cm^{-1}. The C-O stretching appeared as many strong bands between 1221-1020 cm^{-1}.

The ^{1}H-NMR spectrum of a solution of 4-methyl-5-ethoxyoxazole in CDCl$_3$ is shown in Figure 74. The one-proton singlet peak at 7.38 ppm was obviously due to the aromatic C$_5$-proton of oxazole ring. The three-proton singlet peak at 2.05 ppm was due to the 4-methyl. The signal of the methylene protons of 5-ethoxy side chain appeared as a quartet peak at 4.16 ppm. Finally, the signal of the methyl protons of 5-ethoxy side chain which were coupled of the β-methylene appeared as a triplet peak at 1.36 ppm.

2-(1-Propylbutyl)-1,3-dioxep-5-ene.

This cyclic actal was prepared by reacting 2-propylpentanal with cis-2-butene-1,4-diol in the presence of p-toluenesulfonic acid with azeotropic removal of water in a Dean-Stark trap.

The reaction mixture could not be seperated by fractional distillation under diminished pressure. The acid catalyst was neutralized prior to distillation by washing the reaction mixture with sodium bicarbonate solution and water successively. Finally, it was dried over anhydrous sodium sulfate. Distillation did not give 2-(1-propylbutyl)-1,3-dioxep-5-ene after removal of benzene. But a large amount of viscous, nonvolatile residue was obtained. The residue was not further investigated but was presumed to be the polymeric derivatives of the reactants.

2-(1-Propylbutyl)-1,3-dioxep-5-ene was purified by column chromatographic technique. The mobile phase used was chloroform and the stationary phase was silica gel.

The IR spectrum of the compound (Figure 75) showed not only the unsaturated C-H stretching at 3031 cm^{-1} but also the saturated C-H stretching between 2958-2871 cm^{-1}. The band at 1650 cm^{-1} was due to C=C stretching absorption. The bands between 1121-996 cm^{-1} characterized the C-O stretching vibration of acetal.

The ^{1}H-NMR spectrum of a solution of 2-(1-propylbutyl)-1,3-dioxep-5-ene in CDCl$_3$ (Figure 76) had eight signals. The two-protons peak at 5.71 ppm was due to two alkene protons which were coupled of two nonequivalent β-methylene protons as a triplet. The acetal proton coupled of the β-methine hydrogen that occurred as a doublet at 4.54 ppm. Two two-proton peaks between 4.43-4.38 ppm were assigned to four protons of two methylene groups which attached to one oxygen atom and double bond. These occurred as the complex multiplets due to the coupling with alkenic proton, long-range coupling and geminal coupling (two protons of each of methylene groups was nonequivalent because of the presence of 2-(1-propylbutyl) group. Fifteen protons of 2-(1-propylbutyl) side chain appeared as complex multiplets between 1.67-0.88 ppm.

The ^{13}C-NMR spectrum of this compound is shown in Figure 78. The unsaturated carbons peak appeared at 129.45 ppm. The peak appearing at 106.97 ppm was assigned to the acetal carbon since it attached to two oxygen atoms. The peak resonated at 65.98 ppm was assigned to the carbons which attached to one oxygen atom. The group of peaks that characterized the 2-(1-propylbutyl) group appeared in 4 signals. The peak at 41.10 ppm was assigned to the methine carbon of this alkyl chain. Four methylene carbons appeared at 31.38-19.80 ppm. The methyl carbons appeared at 14.37 ppm.

An Attempt to Synthesize 9-Hydroxy-8-methyl-3-(1-propylbutyl)-4H,9H-dioxepino[5,6-c]pyridine or α^4,α^5-O-(2-Propylpentanylidene) pyridoxine (LXX).

The Diels-Alder reaction is one of the most useful synthetic conversions in organic chemistry. It is an example of a [4+2] cycloaddition reaction between a conjugated diene and a dienophile, which leads to the formation of six-membered cyclic ring. The reaction proceeds well if the dienophile bears electron-attracting group and the diene, electron-donating group.

Since the Diels-Alder is reversible, attempts then were made to convert the readily available 2-(1-propylbutyl)-1,3-dioxep-5-ene and 4-methyl-5-ethoxyoxazole to α^4,α^5-O-(2-propylpentanylidene)pyridoxine using the lowest possible temperature.

4-Methyl-5-ethoxyoxazole and 2-(1-propylbutyl)-1,3-dioxep-5-ene are allowed to react at room temperature for upto 240 hours. It would appear that the conversion to α^4,α^5-O-(2-propylpentanylidene)pyridoxine was certainly undetectable by TLC. The reaction mixtures seemed to contain 4-methyl-5-ethoxyoxazole, 2-(1-propylbutyl)-1,3-dioxep-5-ene and the compounds that believed to be the degraded products of 4-methyl-5-ethoxyoxazole. To confirm whether the required product is fomed or not, treatment of the mixture with hot ethanolic hydrogen chloride for several hours was decided to cause the loss of the 2-propylpentanylidene group from the molecule of α^4,α^5-O-(2-propylpentanylidene)pyridoxine (if any) and resulted in pyridoxine. TLC chromatography of the mixture did not show the presence of only trace amounts of pyridoxine.

Since 4-methyl-5-ethoxyoxazole was known to react with a number of dienophile, such as maleic anhydride or diethyl maleate even if at room temperature, the low reactivity of 2-(1-propylbutyl)-1,3-dioxep-5-ene seemed to be the reason of the failure of the reaction.

On literature survey, 2-butene-1,4-diol and its derivatives are comparatively unreactive dienophiles and therefore required much more vigorous conditions for the Diels-Alder reaction, the temperature between 115-180 °c and reaction time of upto 20 hours are used. An excess of the dienophile is used as solvent in many procedures.

This suggested the elevation of the temperature used. Temperatures in the range of 80-180 °c were employed with heating peroids of 120 hours. In all cases complex and often dark coloured reaction mixture were obtained. In order to determine if α^4,α^5-O-(2-propylpentanylidene)pyridoxine had been formed during the reaction, an attempt to separate the constituents of the mixtures failed, the mixture was submitted to hydrolyse and the pyridoxine formed was determined by TLC. As the previous procedure, there was no pyridoxine found.

We next turned our attention to the usage of the catalyst. The synthesis of α^4,α^5-O-(2-propylpentanylidene)pyridoxine was complete by two-stage mechanism, the reversible Diels-Alder condensation, which led to unstable adduct, and isomerization of the adduct into α^4,α^5-O-(2-propylpentanylidene)pyridoxine (Figure 12). If the opening of the adduct was catalyzed, it would possibly lead to the formation of α^4,α^5-O-(2-propylpentanylidene)pyridoxine. An attempt to synthesize α^4,α^5-O-(2-propylpentanylidene)pyridoxine by treatment a mixture of 4-methyl-5-ethoxyoxazole and 2-(1-propylbutyl)-1,3-dioxep-5-ene with trichloroacetic acid or hydrogen chloride were completely unsucessful at room temperature for 120 hours or 180 °c for 48 hours.

Therefore, it is most probable that the failure of attempt to prepare α^4,α^5-O-(2-propylpentanylidene)pyridoxine by means of the reaction between 4-methyl-5-ethoxyoxazole and 2-(1-propylbutyl)-1,3-dioxep-5-ene is due to the low reactivity of 2-(1-propylbutyl)-1,3-dioxep-5-ene. This is possible by the nature of low reactivity of this 1,4-butenediol-based dienophile, participate with the steric hindrance of it long chain alkyl side chain.