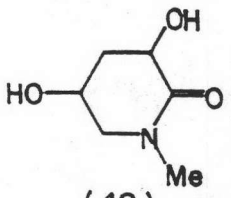
CHAPTER III

RESULTS AND DISCUSSION

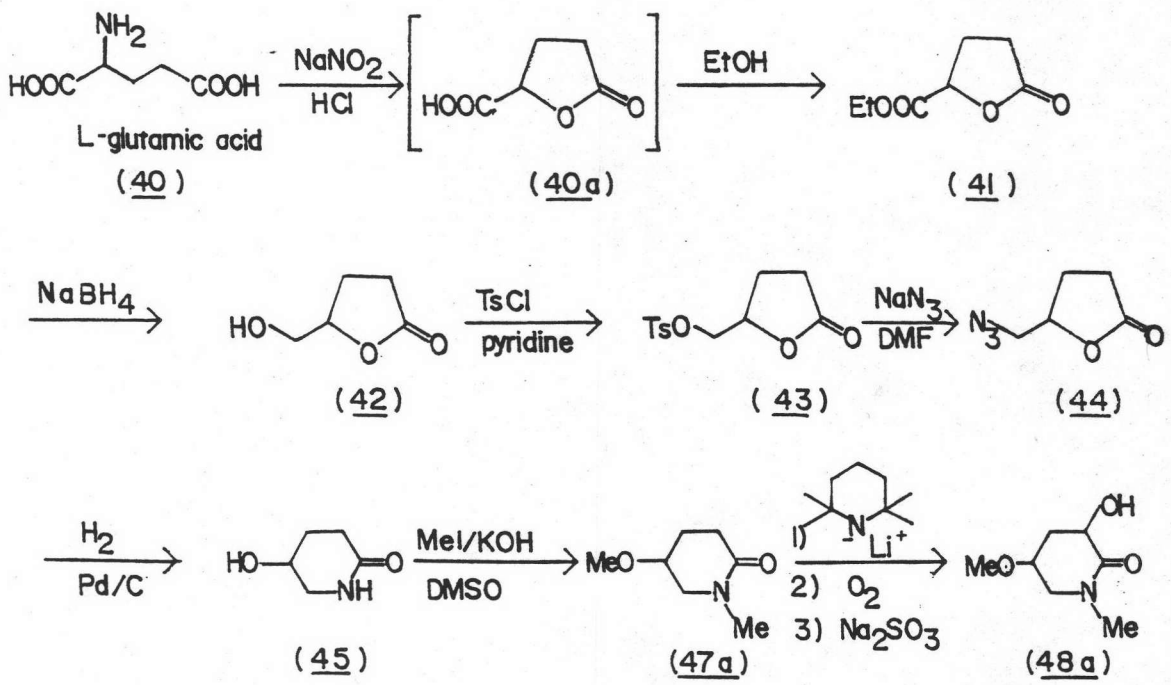
The synthesis of 3,5-dihydroxy-1-methyl-2-piperidone(48)



(48)

and/or its derivative can be carried out by various routes. In this research, 3-hydroxy-5-methoxy-1-methyl-2-piperidone(48a) was synthesized, starting from L-glutamic acid(40) via several steps as in route 1.

3.1 Route 1



3.1.1 5-Ethoxycarbonyl-2-tetrahydrofuranone(41)

5-Ethoxycarbonyl-2-tetrahydrofuranone(41) was prepared from the reaction of L-glutamic acid(40) with nitrous acid in aqueous solution. Deamination and cyclization in situ yielded a lactone ring with a carboxylic acid functional group which was esterified with ethanol by a conventional method. By this method, diethyl ester of 2-hydroxypentanedioic acid was also isolated. It may be the result of hydrolysis or transesterification of the lactonic acid.

The IR spectrum of (41) (Fig. 1) gave important absorption peak at 1790 and 1740 cm^{-1} of the C=O streching of a 5-membered ring lactone and an ester, respectively. The ^{1}H NMR data (Fig. 2) showed the signals of ethoxy group at δ1.32(t, 3H, CH_3-CH_2-O-) and 4.27(q, 2H, CH_3-CH_2-O-) ppm. while the ^{13}C NMR spectrum (Fig. 3) exhibited seven signals corresponding to seven carbon atoms in this compound including two signals of carbonyl carbons at 170.87 and 176.45 ppm.

3.1.2 5-Hydroxymethyl-2-tetrahydrofuranone(42)

The reduction of 5-ethoxycarbonyl-2-tetrahydrofuranone(41) with sodium borohydride in ethanol at room temperature afforded 5-hydroxymethyl-2-tetrahydrofuranone(42).

Some important absorption bands in the IR spectrum (Fig. 4) of the hydroxy compound (42) were 3700-3100 cm^{-1} of O-H streching and 1760 cm^{-1} of C=O streching of 5-membered ring lactone. The disappearance of the signals of ethoxy group in ^{1}H NMR (Fig. 5) indicated that the ester group has been converted to the hydroxyl one, furthermore it showed a signal of hydroxyl

proton at δ3.26 ppm. which was exchanged with the deuterium of deuterium oxide. The structure of the compound (42) was confirmed by ^{13}C NMR spectrum (Fig. 6) which revealed five signals corresponded to five carbons of this compound.

3.1.3 5-Tosyloxymethyl-2-tetrahydrofuranone(43)

5-Tosyloxymethyl-2-tetrahydrofuranone(43) was prepared by tosylation of the hydroxyl lactone (42) with p-toluenesulfonyl chloride using pyridine as solvent and base.

The IR spectrum of this tosylated product (43) (Fig. 7) revealed the information which agreed with the expected structure i.e., there were the absorption bands belonging to the C=O(lactone) streching vibration at 1770 cm^{-1}, the C=C(aromatic) streching vibration at 1660 and 1445 cm^{-1}, and the O=S-O symmetric and asymmetric streching of sulfonic ester at 1365 and 1190 cm^{-1}, while the absorption band of O-H streching disappeared. The ^{1}H NMR spectrum (Fig. 8) displayed the signal of aromatic protons with 1,4-disubstitued pattern at δ7.58(dd, J = 8.30, 28.08 Hz, 4H)ppm. The ^{13}C NMR spectrum (Fig. 9) showed ten signals, four signals at δ 127.85, 130.07, 132.13, and 145.40 ppm. owing to the aromatic (1,4-disubstitued benzene) carbons, the signal at 176.12 ppm. corresponding to the remaining carbonyl carbon of the lactone ring.

3.1.4 5-Azidomethyl-2-tetrafuranone(44)

Displacement of the tosylate group of compound (43) with sodium azide in N,N-dimethylformamide by mean of the nucleophilic substitution reaction yielded azido compound (44).

The IR spectrum (Fig. 10) exhibited some important absorption bands at 2100 cm^{-1} which belonged to the $N\equiv\overset{+}{N}-$ streching vibration of azido group. The band at 1780 cm^{-1} indicated C=O streching vibration of lactone. The signals of aromatic protons in the ^{1}H NMR spectrum (Fig. 11) disappeared. The ^{13}C NMR spectrum (Fig. 12) showed five signals according to the five carbons of this compound.

3.1.5 5-Hydroxy-2-piperidone(45)

The six-membered ring lactam, 5-hydroxy-2-piperidone(45), was prepared by reductive cyclization of the azidolactone(44). The azido group of compound (44) was reduced by catalytic hydrogenation using palladium on charcoal as catalyst, then recyclized to form amide linkage (lactam ring) instead of the ester one (lactone ring).

The IR spectrum (Fig. 13) showed some important absorption peaks at 3280 and 3200 cm^{-1} of O-H streching and N-H streching, 1635, 1500, 1290 cm^{-1} according to C=O streching (amide I band), N-H bending of 2° amide (amide II band) and C-N streching coupled with N-H bending of 2° amide (amide III band), respectively. The ^{1}H NMR spectrum (Fig. 14) gave the signals at δ 4.87 and 7.16 ppm., which disappeared when shaken with deuterium oxide, attributed to the hydroxyl proton and N-amido proton, respectively. The signal at δ 4.00 ppm. with the coupling

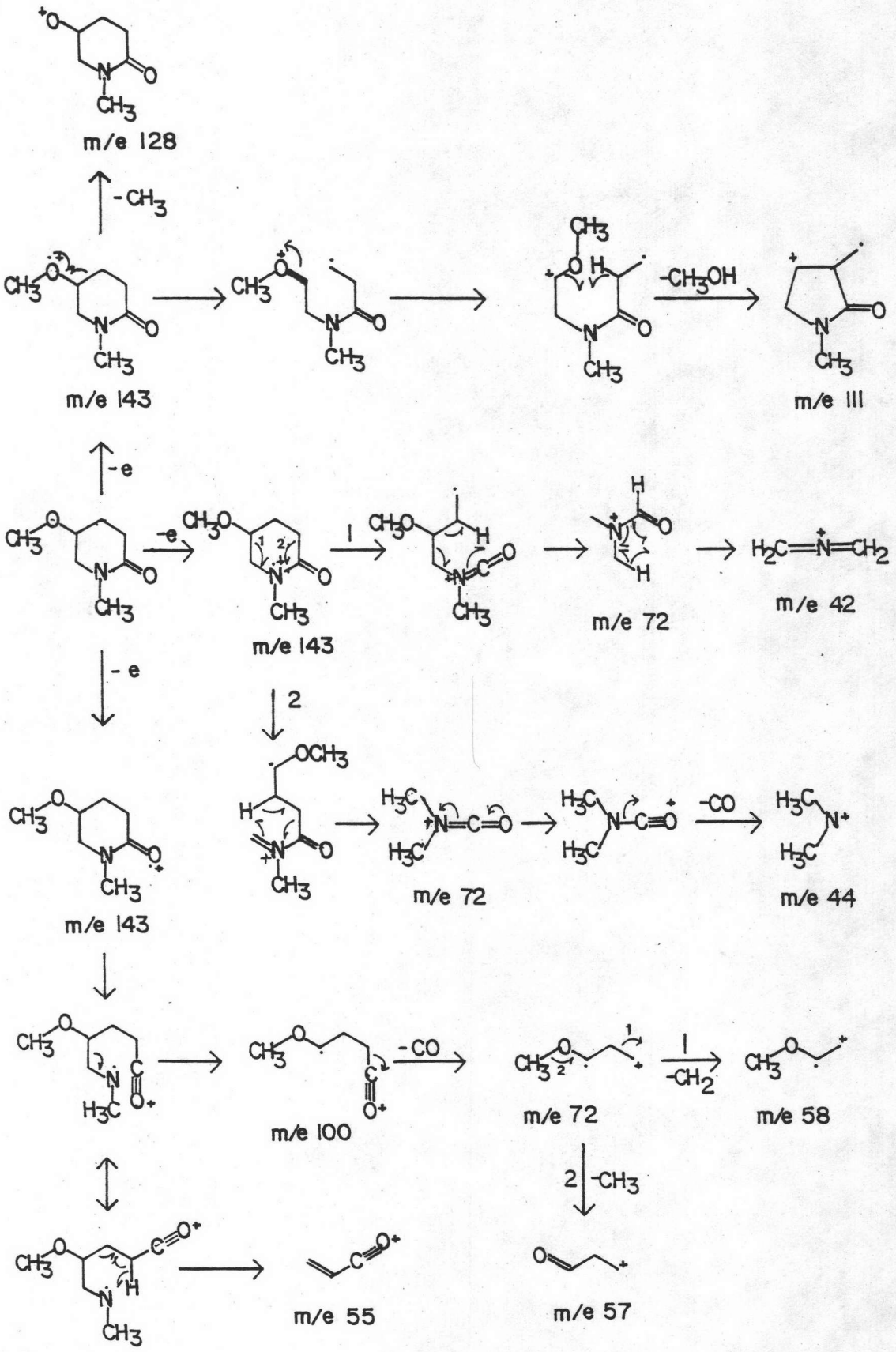
constant of ca. 4 Hz. indicated that the CH-OH proton was an axial proton, so the hydroxy functional group as ahown in fig. 14 was in the equatorial position corresponding to the conformational effect of cyclic six-membered ring and possessed the S-configuration accòrding to the starting material, L-glutamic acid. The ^{13}C NMR spectrum (Fig. 15) exhibited six signals corresponding to the number of carbons of this compound.

3.1.6 5-Methoxy-1-methyl-2-piperidone(47a)

Reacting 5-hydroxy-2-piperidone(45) with methyl iodide in the presence of base e.g., potassium hydroxide, gave 5-methoxy-1-methyl-2-piperidone(47a). Thus, 5-hydroxy-1-methyl-2-piperidone(47) could not be prepared as expected since the neucleophilic substitution took place at 5-position to form an ether linkage.

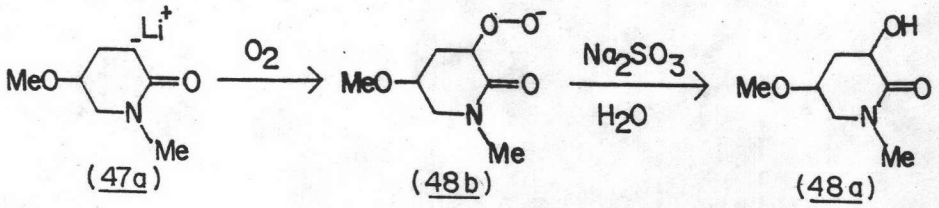
The ^{1}H NMR spectrum (Fig. 17) showed the singlet signals of methoxy protons at δ 3.38 ppm. and of N-methyl protons at δ 2.93 ppm. These were indicated by the difference of ^{13}C NMR spectrum of (47a) (Fig. 18) and (45) (Fig. 15) that there were two additional signals at δ 56.07 and 34.62 ppm. in the spectrum of (47a), belonging to the methoxy carbon and the N-methyl one, respectively. The mass spectrum (Fig. 19) displayed the molecular ion peak at m/e (%relative intensity) 143(40.6,M^+) (cald. for $C_7H_{13}NO_2$: MW. 143.19) and other fragmentation ion peaks at m/e 128(58.7, M^+-CH_3), 72(35.6), 58(100.0), 57(45.0), 55(18.9), 44(75.2), 42(36.2). The possible mass fragmentation pattern of this compound is presented in scheme 1.

Scheme 1 The possible mass fragmentation pattern of 5-methoxy-1--methyl-2-piperidone(47a)



3.1.7 3-Hydroxy-5-methoxy-1-methyl-2-piperidone(48a)

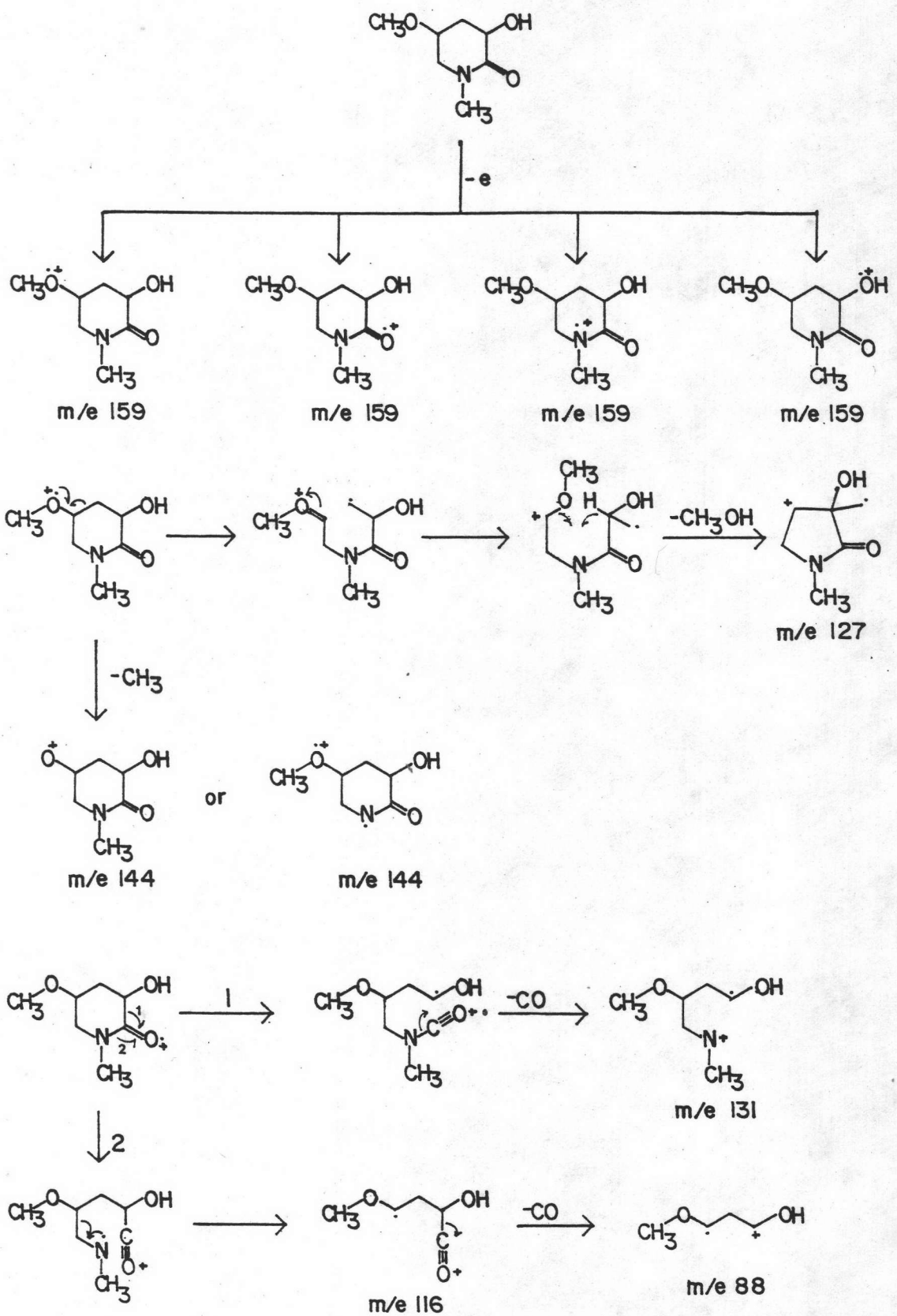
The carbanion of 5-methoxy-1-methyl-2-piperidone(47a) was generated in dry tetrahydrofuran solution using lithium 2,2,6,6-tetramethylpiperidide as the base. This anion (47b) reacted with molecular oxygen, a weak electrophile, at 0°C to form peroxide anion (48b) which was reduced to α-hydroxy derivative, 3-hydroxy-5-methoxy-1-methyl-2-piperidone(48a), by aqueous sodium sulfite solution.



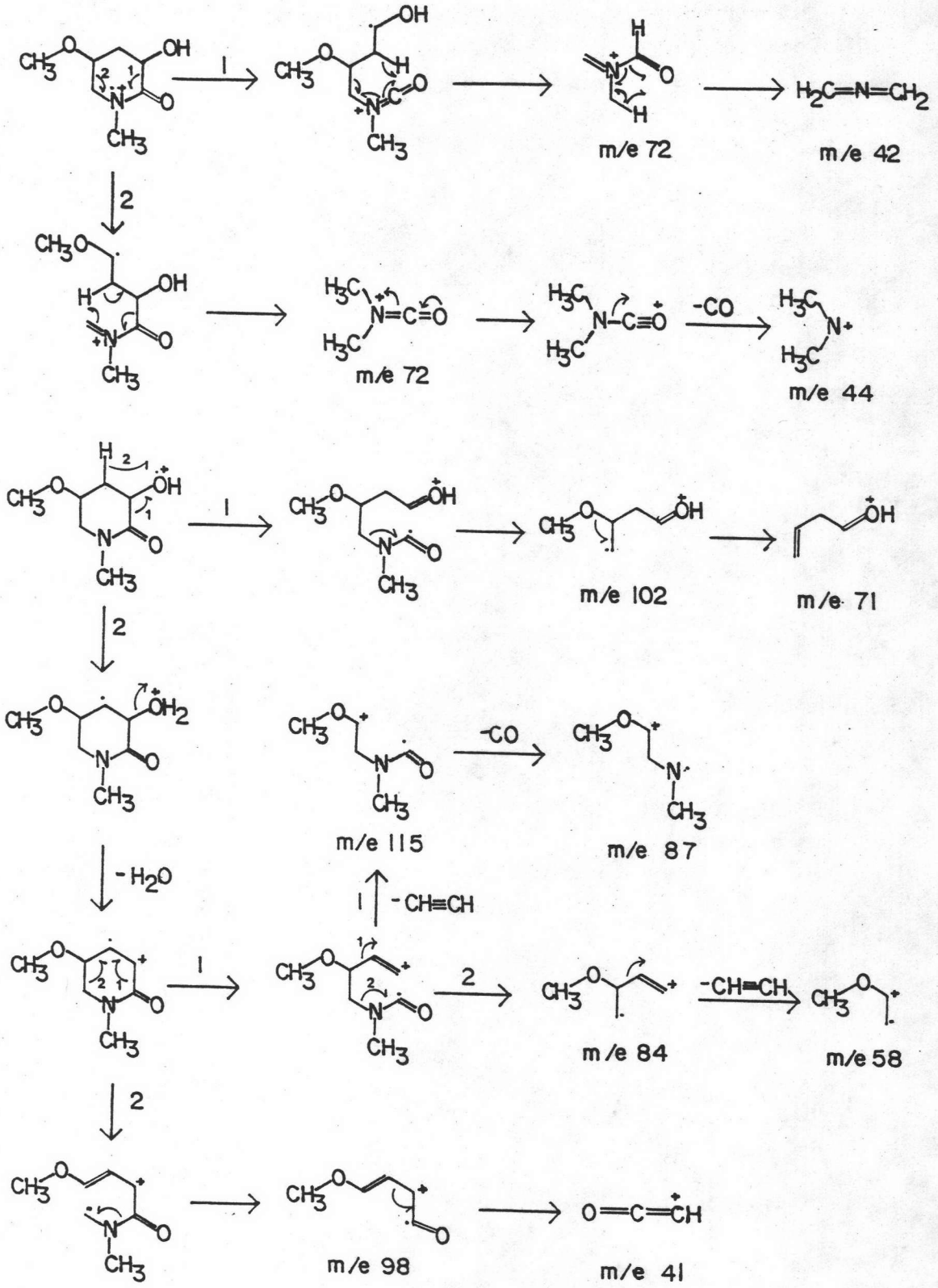
(47a) (48b) (48a)

The ^{1}H NMR spectrum (Fig. 21) showed a broad signal, which disappeared upon shaking with deuterium oxide, at δ 4.50 ppm. indicating the hydroxyl proton. The ^{13}C NMR spectrum (Fig. 22) exhibited thirteen signals, excepting the signal at δ 172.11 ppm., other twelve signals could be devided into two similar patterns, indicating that the synthesized compound (48a) was a mixture of cis- and trans- isomers whose carbonyl carbon accidentally overlapped each other at δ 172.11 ppm. The mass spectrum (Fig. 23) gave the molecular ion peak at m/e (%relative intensity) 159(49.7, M^{+}) (cald. for $C_7H_{13}NO_3$: MW. 159.19), and base peak at 44 (100.0, $(CH_3)_2N^{+}$). The possible mass fragmentation pattern of this compound is written in scheme 2.

Scheme 2 The possible mass fragmentation pattern of 3-hydroxy-5--methoxy-1-methyl-2-piperidone(48a)



Scheme 2 (continue)



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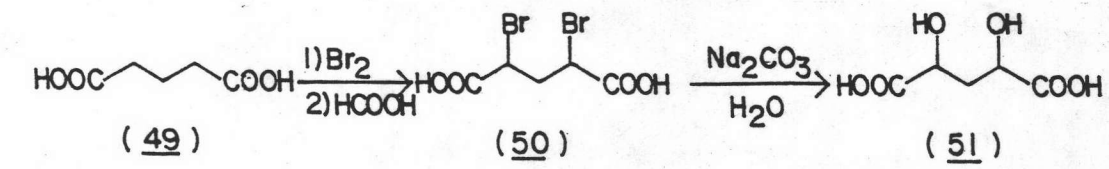
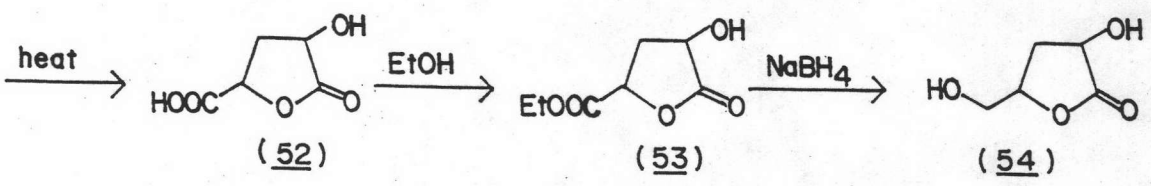
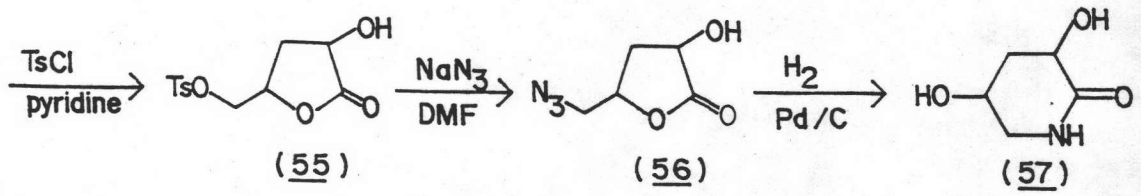
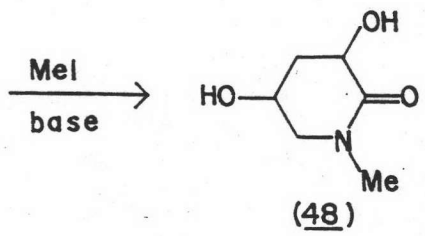
In addition to the previous routes described above, several attempts had been made to synthesize odoram or its derivatives, for example route 2, starting from pentanedioic acid.

3.2 Route 2









3.2.1 2,4-Dibromopentanedioic acid(50)

2,4-dibromopentanedioic acid (50) was prepared by α-bromination of pentanedioic acid with liquid bromine in the presence of thionyl chloride according to the procedure described by Ingold[29]. The reaction yielded two isomers, meso- and dl-, of the brominated product. These two isomers were isolated upon their different solubility in chloroform.

The IR spectrum of (50) (Fig. 24) (meso-isomer) showed a broad absorption band at 3300-2500 cm^{-1} of the O-H streching vibration and 1730 cm^{-1} of the C=O streching vibration which attributed to carboxylic acid functional group. The ^{1}H NMR spectrum (Fig. 25) exhibited a broad signal which disappeared upon shaking with deuterium oxide at δ 12.40 ppm. indicating the acidic proton of carboxylic acid. The structure was also confirmed by ^{13}C NMR spectrum (Fig. 26).

3.2.2 2,4-dihydroxypentanedioic acid(51)

Two methods of the preparation of 2,4-dihydroxy-pentanedioic acid(51) from the bromo acid (50) were carried out. The first method, the bromo compound (50) was first refluxed with aqueous solution of sodium carbonate and then acidified. A mixture of the hydroxy product and inorganic salt was obtained by evaporating the solvent. Since the solubility of the components in the mixture was so similar that it was very difficult to seperate from each other, the yield was low. The second method, which gave much better yield, the bromo compound (50) was treated with silver oxide. The precipitation of the silver salt from the reaction mixture facilitated the isolation of the

product (51).

The IR spectrum (Fig. 27) gave characteristic bands at 3510 cm^{-1} belonging to the O-H streching vibration of the hydroxyl group overlapping with a broad band around 3300-2500 cm^{-1} of the O-H streching vibration of carboxylic acid. The ^{1}H NMR and ^{13}C NMR spectra (Fig. 28 and 29) were similar to the spectra of the dibromo-dicarboxylic acid (50).

3.2.3 5-Carboxy-3-hydroxy-2-tetrahydrofuranone(52)

The dehydration and cyclization of the hydroxy acid (51) by direct heating yielded the lactonic acid (52). In small scale preparation, the reaction went smoothly to give high yield of the expected product (52). Upon scale-up, another product, amorphous, colourless solid, insoluble in organic solvent, was obtained. This substance should be the polyester, resulting from the intermolecular reaction between the hydroxy group and the carboxylic acid. Refluxing the hydroxy acid (51) in a high boiling point solvent, for example, toluene, in the presence of catalytic amount of hydrochloric acid also afforded the lactonic acid (52).

The IR spectrum (Fig. 30) revealed the important absorption bands of O-H streching vibration of hydroxy group at 3460 cm^{-1} overlapping with the O-H streching vibration of carboxylic acid around 3200-2500 cm^{-1}, and two C=O streching vibration bands at 1800 and 1742 cm^{-1} contributed with the 5-membered ring lactone and carboxylic acid, respectively. The ^{1}H NMR spectrum (Fig. 31) showed complex signals according to the coupling of the protons in the lactone ring. The ^{13}C NMR

spectrum (Fig. 32) exhibited five signals, including two signals of carbonyl carbons at δ 168.89 and 174.36 ppm., attributed to the carbon atoms of this compound.

3.2.4 5-Methoxycarbonyl-3-hydroxy-2-tetrahydrofuranone(53)

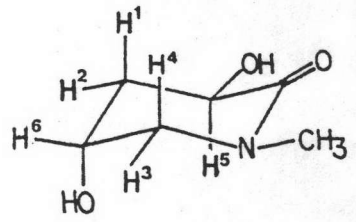
The esterification, methylation and/or ethylation, of the lactonic acid (52) to produce the lactonic ester (53) could not be carried out by a convenient method, or even in the mild condition as diazomethane at -78°C. The esterification of the lactonic acid (52) caused the lactone ring to be broken, and yielded diester of 2,4-dihydroxypentanedioic acid (53a).

The IR spectrum of the product (53a) (Fig. 33) gave the important absorption bands at 3400-3200 cm^{-1} of O-H streching vibration and at 1760-1720 cm^{-1} of C=O streching vibration of ester functional group. The ^{1}H NMR (Fig. 34) and ^{13}C NMR (Fig. 35) exhibited simple pattern signals according to the symmetry of the molecular structure of the esterified product (53a).

So the synthesis of odoram or its derivatives could not be achieved by this route.

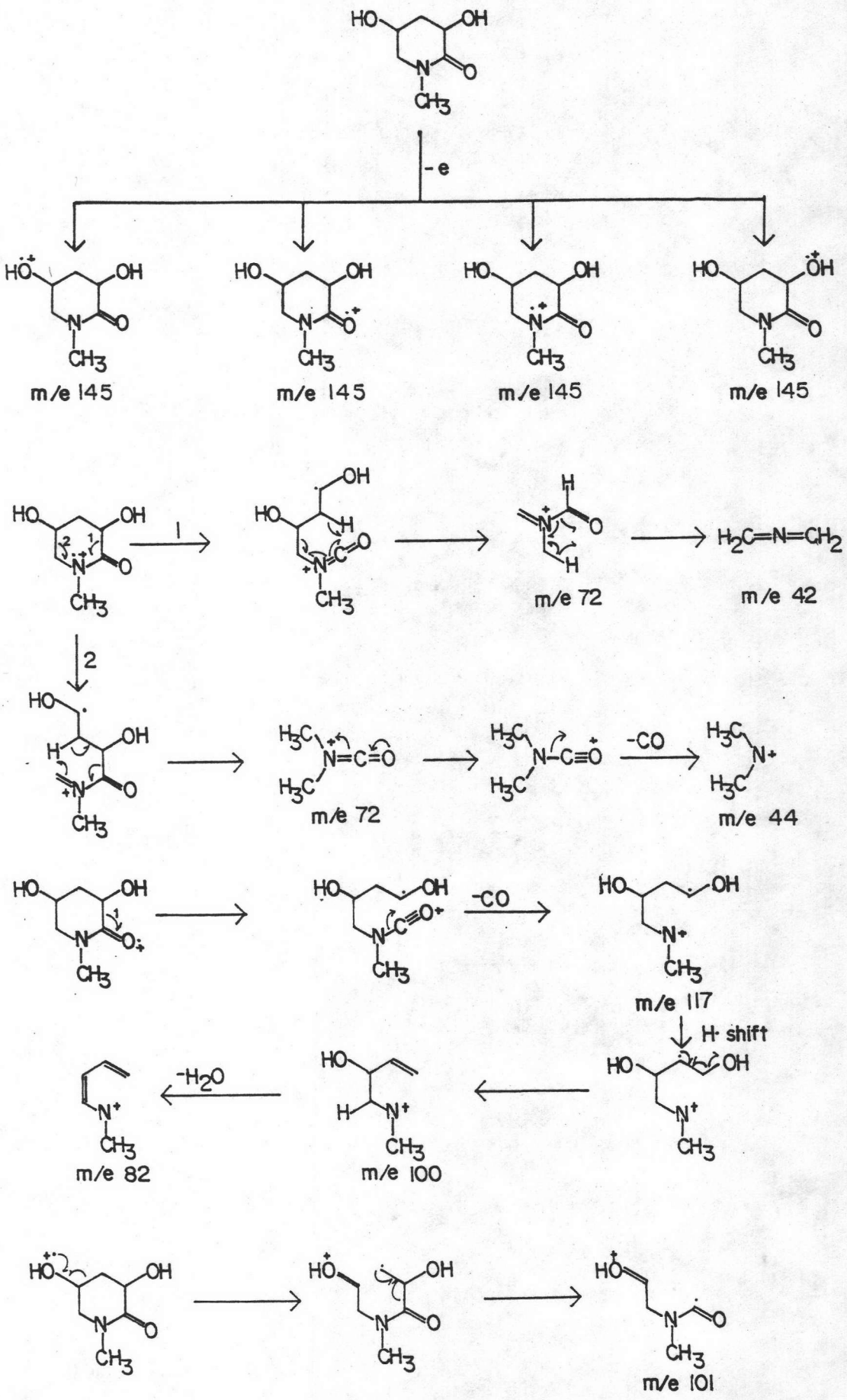
3.3 The structure of odoram

Odoram is shown to be a six-membered ring lactam with an N-methyl group and two hydroxy groups[9]. The ^{1}H NMR spectrum (Fig. 37, 38) could be assigned as follows:



The multiplet at δ 4.63 ppm. was assigned to H-6. The absence of any large axial-axial coupling constant indicated that H-6 should be in the equatorial position. The doublet of doublet at 4.19 ppm. with coupling constants of 10.9 Hz (J_{51}) and 7.6 Hz (J_{52}) was assigned to H-5. On the basis of these strong coupling constants H-5 should be in axial position. The doublet of doublet at 3.95 ppm. with coupling constants of 12.9 Hz (J_{43}) and 4.6 Hz (J_{46}) was assigned to H-4. The doublet of, doublet of doublet at 3.18 ppm. with coupling constants of 12.9 Hz (J_{34}), 2.0 Hz (J_{32}), and 2.0 Hz (J_{36}) belonged to H-3. A singlet at 3.04 ppm. was assigned to the N-methyl group. The doublet of, doublet of, doublet of doublet at 2.50 ppm. with coupling constants of 14.4 Hz (J_{21}), 7.6 Hz (J_{25}), 2.0 Hz (J_{23}), and 2.0 Hz (J_{26}) was assigned to H-2 and the doublet of, doublet of doublet at 2.21 ppm. with coupling constants of 14.4 Hz (J_{12}), 10.9 Hz (J_{15}), and 4.6 Hz (J_{16}) was assigned to H-1. According to the above assignement, the configuration of C-3 was $R_{(equatorial)}$- and of C-5 was $R_{(axial)}$-configuration. The possible mass fragmentation pattern of odoram is presented in scheme 3.

Scheme 3 The possible mass fragmentation pattern of odoram



3.4 Methylation of odoram

Odoram was methylated in order to compare with the structure of the synthesized 3-hydroxy-5-methoxy-1-methyl-2-piperidone(48a). The methylation of odoram was carried out using methyl iodide and a strong base, t-butoxide, in dry t-butanol. The physical properties of the methylated odoram, a white precipitate product, was different from those of the compound (48a), a colorless liquid.

The ^{1}H NMR spectrum of the methylated odoram (Fig. 42), showed a signal of the methoxy protons at δ 3.40 ppm.and a signal of N-methyl protons at δ 3.11 ppm., similar to those of the ^{1}H NMR spectrum of compound (48a) (Fig. 21).

The physical properties and spectroscopic data of (48a) and methylated odoram indicated that the compound (48a) was not indentical with methylated odoram due to the difference in the configuration at C-5. The methoxy group of the synthetic one from L-glutamic acid was in equatorial position, S-configuration, while that of methylated odoram was in axial position, R-configuration. So the confirmation of the structure of odoram could not be achieved by the synthesis of 3-hydroxy-5-methoxy-1-methyl-2-piperidone.