PREPARATION OF SOME N,N'-DIACYL-4-IMIDAZOLINES

by

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ABSTRACT

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The oxidation of benzhydrazide to benzoic acid was studied as a model reaction for the conversion of acid hydrazides to their corresponding carboxylic acids. This oxidation was carried out with iodine using both ether - aqueous sodium carbonate and ethyl acetate - aqueous sodium carbonate as media.

Four new N,N'-diacyl-4-imidazolines were prepared by catalytic hydrogenation of imidazole with platinum oxide in the presence of the corresponding acid anhydrides. The compounds prepared were N,N'-dipropionyl-4-imidazoline, N,N'-dibutyryl-4-imidazoline, N,N'-dipentanoyl-4-imidazoline and N,N'-dihexanoyl-4-imidazoline. The ultra-violet, infra-red and nuclear magnetic resonance spectra of these compounds were determined. When 1-acetylimidazole was reduced with the aforementioned catalyst in propionic anhydride, the product was not the mixed imidazoline but N,N'-dipropionyl-4-imidazoline.

Unlike the corresponding N,N'-diacylimidazolidines previously studied, the four N,N'-diacyl-4-imidazolines did not show antimycotic activity against Aspergillus flavus.

ACKNOWLEDGEMENTS

I would like to thank all the chemists at Youngstown State University, both instructors and students, for helping to make this thesis the highly rewarding learning experience it has been. I especially wish to express my sincerest graditude to my advisor, Dr. Marvin Lukin, for his ever constant encouragement and assistance throughout my work.

Finally, I would like to thank my sister-in-law, Margie Chitester, for taking the time to typè this manuscript.

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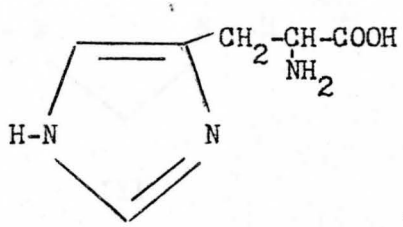
CHAPTER I

Introduction

Imidazole and its derivatives have long been known to be compounds of immense biological importance. For example, histidine (I) is an essential amino acid in man,[1] and is a constituent of most proteins.[2]

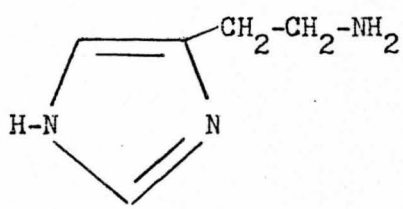


Histidine

I

It has been demonstrated, in the case of several enzymes whose active sites include histidine residues, that it is actually the imidazole ring of the histidine that is essential.[3,4]

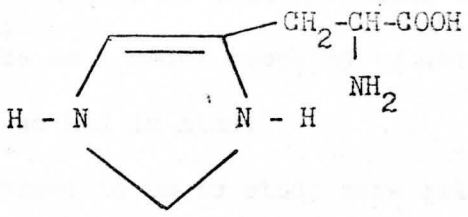
The imidazole ring has also been recognized as one of the most essential characteristics of histamine,[5] an imidazole derivative known to possess profound physiological activity.



Histamine

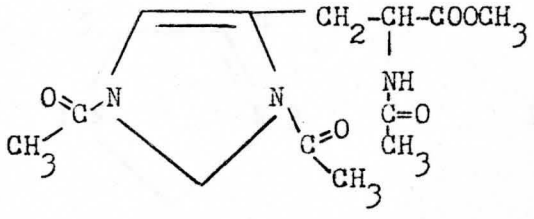
II

One of the methods frequently used by biochemists in determining the essential residues of a biologically active peptide involves modifying the structure of particular residues and determining what effect such modifications have on the peptide's activity. Our initial interest in this work was to synthesize a 4-imidazoline derivative of histidine which could eventually be incorporated into a number of biologically active peptides.



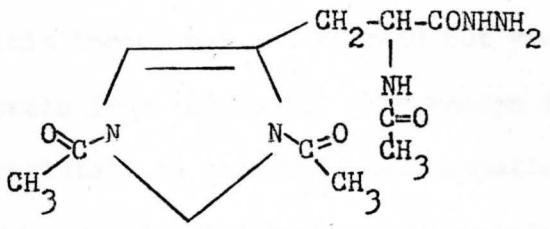
III

In an earlier work with this synthesis, Kurek[6] reduced histidine methyl ester by catalytic hydrogenation in the presence of acetic anhydride. The compound prepared had the following structure:



IV

Subsequently, the ester was subjected to hydrazinolysis to prepare the acid hydrazide:

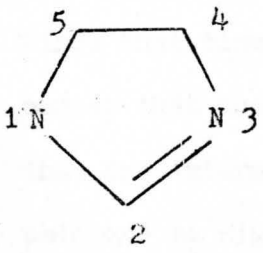


V

Our concern was with the conversion of this hydrazide to the free acid form. We used benzhydrazide as a model compound to determine the feasibility of the pathway we had in mind.

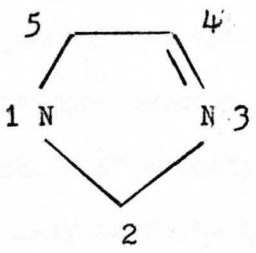
It was also of interest to us to study more extensively the hydrogenation of imidazole with platinum oxide since we anticipated this investigation to be useful in connection with the synthesis of the histidine analog.

All three forms of the theoretically predictable isomeric imidazolines can be synthesized.



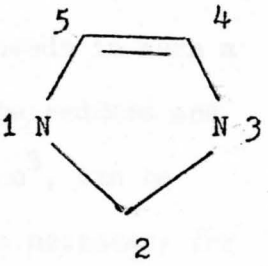
2-imidazoline

VI



3-imidazoline

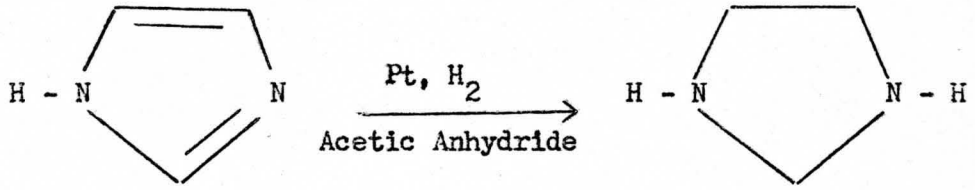
VII



4-imidazoline

VIII

The isomer formed in the catalytic reduction of imidazole with platinum oxide and, thus, the isomer of interest to us is the 4-imidazoline (VIII). This isomer is the least common of the three, and until 1961, synthesis of this isomer was not carried out via reactions involving the direct synthesis from inidazole. The reason for this is that imidazole is highly resistant to catalytic hydrogenation. However, in 1961, Bauer[7] succeeded in completely reducing imidazole to N,N'-diacetlyimidazolidine by treatment of imidazole with acetic anhydride in the presence of hydrogen and platinum oxide.



IX

Since that time it has been shown that the reduction proceeds in such a manner that the carbon to nitrogen bond is the first to be reduced and that the intermediate product, N,N'-diacetyl-4-imidazoline[8], can be obtained by simply allowing only half the hydrogen uptake necessary for the total reduction. This procedure provides a convenient route for the direct synthesis of N, N'-diacyl-4-imidazolines.

Many N-substituted fatty acid amides have been shown to possess antimycotic activity[9,10,11] (a term indicating the inhibitory condition against microbial organisms). In particular, Mod, Magne and Sumrell[10] prepared a series of N,N-diacylimidazolidines, several of which had

significant antifungal activity. Our synthesis of some N,N'-diacyl-4-imidazolines provided us an opportunity to investigate the antimycotic activity of these novel compounds and to compare their activity with that of the imidazolidines previously studied.

Statement of the Problem

This investigation involved three major objectives: 1) the development of a method for the conversion of an acid hydrazide to the free acid: 2) the synthesis of a series of N,N'-diacyl-4-imidazolines by catalytic hydrogenation of imidazole and 3) the study of the antimycotic activity of these 4-imidazolines.

CHAPTER II

Historical

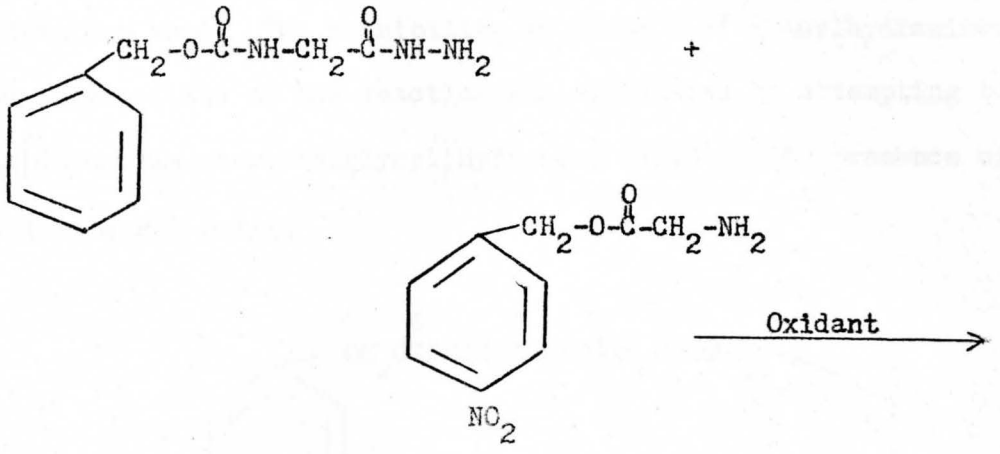
Conversion of Acid Hydrazides to Carboxylic Acids

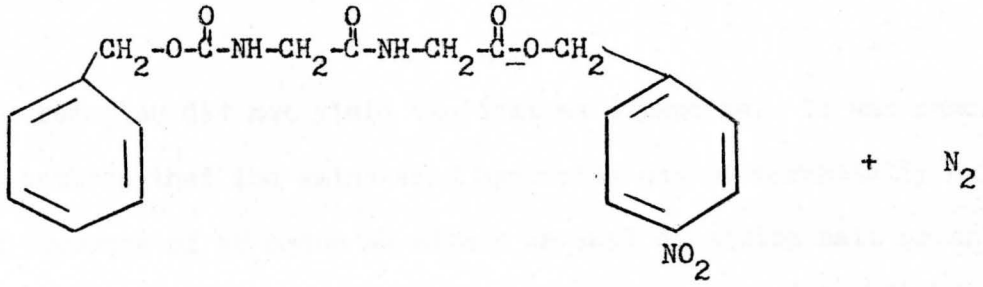
The conversion of an acid hydrazide to its free carboxylic acid can be brought about in a number of ways. As an example, hydrolytic cleavage of acid hydrazides takes place upon treatment of the hydrazide with strong acid or base and long periods of heating.[12] Another method, developed by Carpino,[13] entails the oxidation of acid hydrazides by chlorine in a highly acidic medium. This procedure leads to the formation of acid chlorides, which could then be hydrolyzed to the free acid.

$$2\ CH_3CONHNH_2 + Cl_2 \xrightarrow{[H^+]} 2\ CH_3COCl + N_2$$

Unfortunately, both of these methods require strong acid or base media, a condition to which imidazolines, due to their rather high degree of instability to hydrolysis,[14] cannot be subjected.

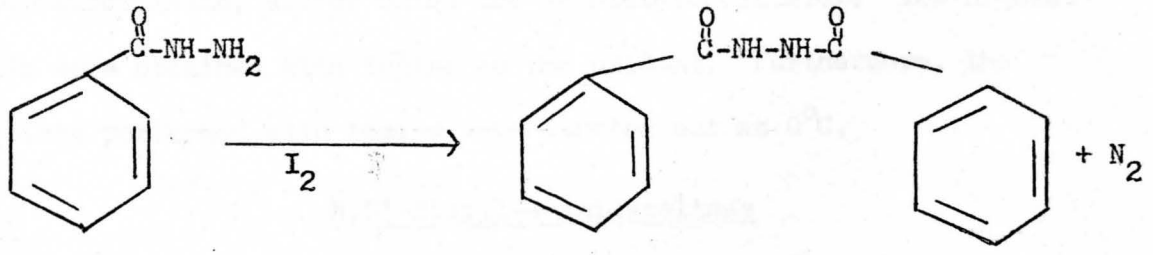
A report by Wolman, Gallup, Patchornik and Berger[15] gave support to the solution of this problem. Di- and tripeptides were synthesized by oxidizing N-protected amino acid hydrazides in the presence of amino acid esters.





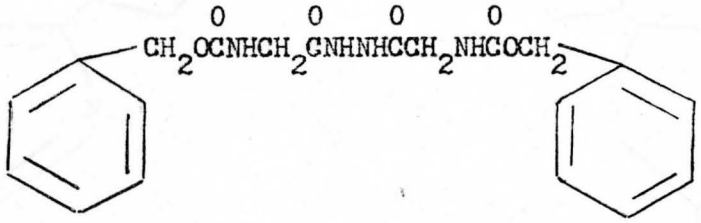
X

In an earlier work by Curtius[16], N,N'-diacylhydrazines (XI) were obtained by the oxidation of acid hydrazides by iodine.



XI

Wolman, Gallup, Patchornik and Berger[15] obtained, along with the desired peptides, N,N'-diacylhydrazines corresponding to the amino acid hydrazides used. The possibility of these N,N'-diacylhydrazines being the intermediate of the reaction was eliminated by attempting to oxidize bis[dibenzyloxycarbonylglycyl]hydrazine (XII) in the presence of ρ-nitrobenzyl ester.



XII

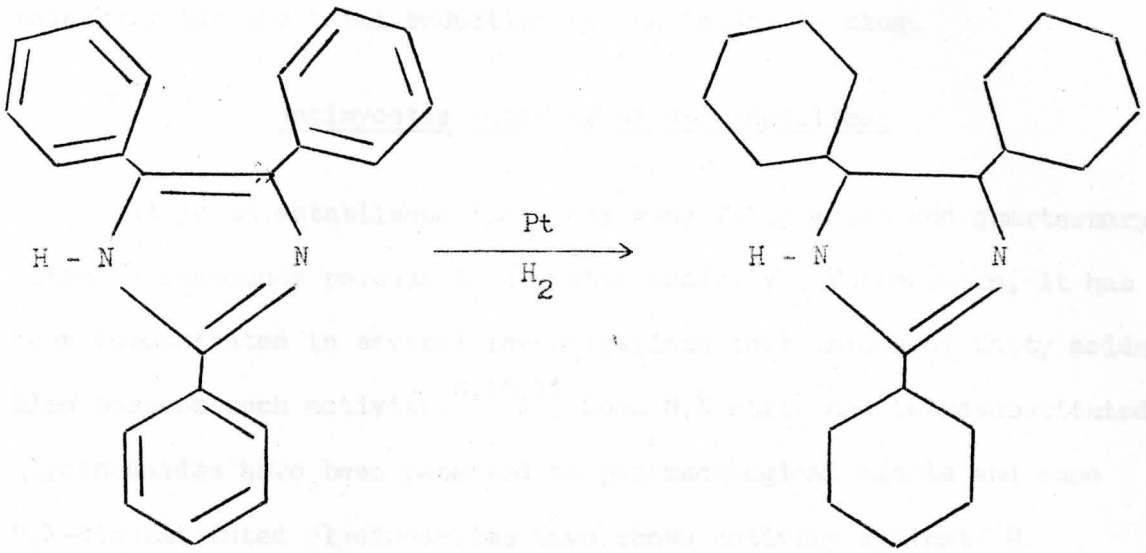
This reaction did not yield the desired dipeptide. It was concluded by the authors that the amino acid hydrazide was preferentially oxidized in the presence of an amine to either an acyl diazonium salt or an acyl diimide intermediate. The intermediate would then react with the nucleophilic amine to form the dipeptide.

Among the oxidants used, the ones which provided yields of 50% and better were iodine, hydrogen peroxide and potassium oxide, N-bromosuccinimide, silver oxide and N-chlorosuccinimide. The highest yields were obtained with iodine as the oxidant. Furthermore, the reactions performed with iodine were carried out at 0°C.

N,N'-Diacyl-4-imidazolines

Imidazoles are compounds known to have remarkable chemical stability. They are extremely resistant to treatment with strong acids and bases and, most importantly, with respect to this investigation, they are profoundly resistant to catalytic hydrogenation. Until 1961

the only reported reduction of an imidazole was that of lophine.[17] Lophine was subjected to hydrogenation with Willstatter catalyst in glacial acetic acid and 2,4,5-tricyclohexyl-2-imidazoline was obtained.



XIII

In a report by Bauer[7], the hydrogenation of imidazole in acetic anhydride, catalyzed by a suspension of platinum black made from platinum oxide, was described. The reaction, which took place at room temperature and atmospheric pressure, was allowed to proceed until no further hydrogen uptake was noted. An 80% yield of N,N'-diacetylimidazolidine was recovered.

In 1965, Vail, Barker and Moran[8] reported the synthesis of N,N-diacetyl-4-imidazoline. Imidazole, acetic anhydride and platinum oxide were placed in a stainless steel bomb which was then filled to 500 psi with hydrogen. The reaction container was shaken for seven hours at room temperature, then opened and the contents poured over ice. Both N,N'-diacetyl-4-imidazoline and N,N'-diacetylimidazolidine were obtained, the former being recovered in 55% yield.

In a similar manner, Kurek[6] prepared N,N'-diacetyl-4-imidazoline from both imidazole and 1-acetylimidazole. The reactions were carried out under a hydrogen atmosphere of 60 psi. In both cases the reactions were stopped when the hydrogen uptake reached one-half the value necessary for the total reduction of the imidazole ring.

Antimycotic Activity of 4-Imidazolines

It is an established fact that many fatty acids and quarternary nitrogen compounds possess antimycotic activity. Furthermore, it has been demonstrated in several investigations that amides of fatty acids also possess such activity.[9,10,11] Some N,N'-tri- and tetrasubstituted glycineamides have been patented as pharmacological agents and some N,N-disubstituted glycineamides have shown activity against M. tuberculosis.[9]

In a paper by Mod, Magne and Sumrell,[10] the antimycotic activity of twelve N,N'-diacylimidazolidines was reported. The synthesis of these compounds was reported in an earlier paper by the same authors.[18] The N,N'-diacylimidazolidines were prepared from amides of ethylenediamine and formaldehyde solution in acetic acid containing a catalytic amount of hydrochloric acid.

The compounds were screened against four pathogenic genera of fungi. Nine of the twelve imidazolidines displayed significant inhibition against at least one of the test organisms. Maximum activity was obtained with N,N'-dihexanoylimidazolidine. This compound inhibited all four test organisms.

CHAPTER III

Experimental

Reagents and Materials

All melting points performed in this investigation are uncorrected. A Thomas-Hoover capillary melting point apparatus was used for the determinations.

Samples of the novel compounds submitted for elemental analysis were dried in an Abderhalden drying apparatus. Infra-red spectrums taken in this investigation were performed on the Beckman IR-5. All untra-violet spectrums were obtained using a Beckman-25 uv-visible spectrophotometer.

All evaporations were carried out using a Buchler Instruments Rotary Evaporator (Model No. 18653).

All hydrogenations were carried out using a Parr Instruments Hydrogenator, at initial pressures of 60 psi.

Reagents and other materials used in this are listed below.

1. 1-Acetylimidazole - Aldrich, 98%.
2. Ammonium chloride - Fisher Scientific Company analytical reagent.
3. Benzhydrazide - Eastman, reagent grade.
4. Benzene - Fisher Scientific Company, reagent grade.
5. Butyric anhydride - Aldrich, 99%.
6. Chloroform - Fisher Scientific Company, reagent grade.
7. Dimethyl acetamide - BDH laboratory reagents.
8. Dimethyl formamide - Matheson, Coleman and Bell, reagent grade.
9. Dioxane - J.T. Baker, reagent grade.
10. Ether - Fisher Scientific Company, reagent grade.
11. Ethyl acetate - Fisher Scientific Company, reagent grade.
12. 95% Ethanol - Fisher Scientific Company, reagent grade.
13. Glass wool - A.H. Thomas
14. Hexanoic anhydride - Eastman, reagent grade.
15. Heptafluorobutyric anhydride - PCR, Inc., reagent grade.

16. Hydrochloric acid (conc) - analytical reagent, 37%.
17. Imidazole - Eastman, reagent grade.
18. Iodine - J. T. Baker, analytical reagent.
19. Magnesium sulfate (anhydrous) - 'Baker Analyzed'.
20. Pyridine - J. T. Baker, reagent grade.
21. Pentanoic anhydride - Eastman, reagent grade.
22. Propionic anhydride - Eastman, reagent grade.
23. n-Propanol - J. T. Baker, reagent grade.
24. Platinum wire - Engelhart Industries.
25. Sodium carbonate - 'Baker Analyzed'.
26. Succinic anhydride - Eastman, reagent grade.
27. Thionyl chloride - Eastman, reagent grade.

Preparation of Benzoic Acid from Benzhydrazide

In a 500-ml, three-necked, round-bottom flask fitted with a reflux condenser, a mechanical stirrer and a cylindrical separatory funnel, was placed 50 ml of anhydrous ether, 5.08 g (0.04 mol) of iodine and 10 ml of a 10% aqueous solution of sodium carbonate. The flask was cooled in an ice bath. A 1.37 g (0.01 mol) sample of benzhydrazide was dissolved in 50 ml of hot 10% aqueous sodium carbonate, and about one-half of this solution was added dropwise to the reaction flask, with stirring, over a period of about twenty minutes. The remainder of the benzhydrazide solution was heated to redissolve precipitated benzhydrazide and then added dropwise to the reaction flask over about twenty minutes. After stirring for about twelve hours at 0°C, no characteristic iodine color remained. The flask contained two liquids and a small amount of white solid. The solid was removed by suction filtration. It weighed 0.021 g and melted at 232-236°C. It is believed to be N,N'-dibenzoylhydrazine which is reported to melt at 234-236°C.[19]

Ether was separated from the aqueous layer by evaporation in a steam bath. A 10% hydrochloric acid solution was added dropwise to the aqueous solution. At pH 2, a white precipitate formed which was

recovered by filtration. After recrystallization twice from hot water, a final melting point of 121-123°C was observed. The melting point of benzoic acid is reported as 122.5°C.[20] The product weighed 0.973 g after recrystallization, representing a 40% yield.

Preparation of Benzoic Acid in Ethyl Acetate

A 100-ml volume of ethyl acetate was placed in a 500-ml, three-necked, round-bottom flask fitted with a mechanical stirrer, a cylindrical funnel and a ground glass stopper. The flask was lowered into an ice bath. With the stirrer on, 20 ml of a 10% sodium carbonate solution was added. Then, 2.76 g (0.02 mol) of benzhydrazide was dissolved in hot aqueous sodium carbonate and added to the flask over a period of forty minutes. The solution had to be reheated twice to dissolve precipitated hydrazide. After stirring for approximately eighteen hours, a separatory funnel was used to separate the two layers (liquid). To the water layer was added 2 N HCl until the solution reached pH 2. Solid sodium thiosulfate was added slowly until the iodine color disappeared. The white precipitate that had formed was recovered by filtration and recrystallized in hot water. The product weighed 0.58 g and had a melting point of 121-123°C. The reported melting point of benzoic acid is 122.5°C.[20]

To the ethyl acetate layer was added sodium thiosulfate crystals to remove excess iodine. The solvent was then evaporated on a rotary evaporator. The residue remaining in the flask was recrystallized in hot water. It weighed 0.009 g and melted at 229-232°C. It is thought to be N,N'-dibenzoylhydrazine, which melts at 234-238°C.[19]

Preparation of N,N'-Dipropionyl-4-imidazoline from Imidazole

This and subsequent hydrogenations were carried out using a Parr Instruments hydrogenator.[21] The reaction vessel was a pressure-tested one liter pyrex bottle. To 75 ml (0.45 mol) of propionic anhydride in the reaction bottle was added 1.00 g of platinum oxide and the bottle was attached to the hydrogenator. The air was evacuated from the bottle by aspiration and then the hydrogen was introduced. The hydrogen was then evacuated with the aspirator. Again the hydrogen was let into the bottle and then evacuated. This was done a total of four times. Finally, the hydrogen was introduced into the bottle until the pressure reached 60 psi. The shaker was turned on and allowed to run until no further uptake of hydrogen was noted. The hydrogen uptake (0.11 mol) was much greater than would be expected for the reduction of the catalyst (0.009 mol).

A 2.04 g (0.03 mol) sample of imidazole was pulverized using a mortar and pestle. The imidazole was then dissolved in the mixture of propionic anhydride and catalyst. The bottle was placed back on the hydrogenator and filled to a final pressure of 60 psi using the procedure stated previously. The reaction was allowed to proceed until the calculated pressure drop (2.1 psi) had been reached. Since 0.03 mol of imidazole were used, 0.03 mol of hydrogen were necessary for the conversion of the imidazole to the 4-imidazoline. A 0.03 mol quantity of hydrogen corresponds to 2.1 psi. The reaction was complete in one and one-half hours. Hot ethyl acetate (200 ml) was added to the reaction mixture to dissolve the insoluble reaction product. The mixture was then filtered hot through Whatman (No. 1) filter paper to remove the catalyst.

The filtrate was stoppered and placed in the refrigerator. In a few hours white crystals had formed in the filtrate. The crystal were filtered off and weighed 1.12 g. The product melted at 203-205°C. Solvent was evaporated from the filtrate using the rotary evaporator at a temperature of 60°C and a pressure of 1 Torr. The white solid that remained was essentially insoluble in water, melted at 193-199°C and weighed 1.14 g.

Both samples were recrystallized twice from ethyl acetate and both reached a constant melting point of 203-205°C. The identical melting points were convincing evidence that they were the same compound and thus they were combined. The 1.83 g of N,N'-dipropionyl-4-imidazoline recovered after recrystallization represents a 33% yield.

A sample of the product was submitted for elemental analysis. Analysis: Calculated for $C_9H_{14}N_2O_2$: C, 59.31; H, 7.76; N, 15.37; Found: C, 59.21; H, 7.79; N, 15.48; ir (KBr pellet) 1634 cm^{-1} (C=O), 1538 cm^{-1} (CH_3) and 2941 cm^{-1} (aliphatic CH); nmr ($CDCl_3$) 1.10 (t, 3H, CH_3), 2.24 (q, 2H, CH_2), 5.45 (s, 2H, ring- CH_2) and 6.27 - 6.67 (m, 2H, HC=CH); uv max ($CHCl_3$) 282 nm (ε, 17,000).

Preparation of N,N'-Dibutyryl-4-imidazoline from Imidazole

To 75 ml (2.8 mol) of butyric anhydride was added 1.00 g of platinum oxide and the mixture was then hydrogenated by the same procedure described above. When no further drop in pressure was observed, 4.04 g (0.06 mol) of imidazole was added and the hydrogenation started again. The reaction was stopped after 16 hours, with a 0.059 mol of hydrogen being consumed. Then 500 ml of hot ethyl acetate was added to dissolve the insoluble product and the platinum was separated

by filtration. Crystals formed in the filtrate upon standing and a 2.08 g sample of product was recovered by filtration.

The ethyl acetate and butyric anhydride were evaporated, using a rotary evaporator, at a temperature of 35°C and a pressure of .5 Torr. The remaining water insoluble product weighed 2.32 g and melted at 189-194°C.

Both products were recrystallized twice from ethyl acetate and their constant melting points were identical, 191-193°C. They were combined. The N,N'-dibutyryl-4-imidazoline was obtained in 35% yield. A sample was submitted for elemental analysis. Analysis: Calculated for $C_{11}H_{18}N_2O_2$: C, 62.81; H, 8.64; N, 13.47: Found: C, 62.65; H, 8.71; N, 13.47: ir (KBr pellet) 1634 cm^{-1}(C=O), 1458 cm^{-1}(CH_3), 2933 cm^{-1} (aliphatic CH); nmr ($CDCl_3$)δ, .87(t, 3H, CH_3), 1.56-2.48 (m, 4H, $COCH_2CH_2$), 5.44(s, 2H, ring-CH_2) and 6.22-6.64(m, 2H, HC=CH); uv max ($CHCl_3$) 282 nm (ϵ, 17,000).

Preparation of N,N'-Dipentanoyl-4-imidazoline from Imidazole

A mixture of 85 ml of pentanoic anhydride and 1.00 g of platinum oxide was hydrogenated by the previously described procedure. Then 4.08 g of imidazole was introduced into the mixture. About twelve hours were necessary for the pressure drop to reach the calculated value of 4.2 psi. Not all of the hydrogen had been evacuated before disconnecting the bottle and this resulted in a mild explosion, in which about three-fourths of the bottle's contents were lost. The mixture was filtered using suction filtration. The product was separated from the platinum by dissolving it in an equal volume mixture of n-propanol and chloroform. The product was obtained by evaporating

the solvents on a rotary evaporator. After recrystallization from 95% ethanol, the product weighed 0.26 g and melted at 172.5-174.5°C.

A sample was submitted for elemental analysis. Analysis: Calculated for $C_{13}H_{22}N_2O_2$: C, 65.50; H, 9.32; N, 11.75: Found: C, 65.36; H, 9.55; N, 11.66: ir (KBr pellet) 1650 cm^{-1}(C=O), 1458 cm^{-1} (CH_3) and 2967 cm^{-1}(aliphatic CH); uv max ($CHCl_3$) 282 nm (ε, 18,000); nmr ($CDCl_3$)δ, .80-2.42(m, 9H, $COCH_2CH_2CH_2CH_3$), 5.40(s, 2H, ring-CH_2) and 6.20-6.72(m, 2H, HC=CH).

Preparation of N,N'-Dihexanoyl-4-imidazoline from Imidazole

A 50-ml volume of hexanoic anhydride was placed in the hydrogenation bottle along with 1.00 g of platinum oxide. Then the catalyst was reduced as in earlier experiments. After the hydrogen uptake by the catalyst and anhydride had ceased, the bottle was opened and 2.04 g of imidazole was added. After 35 hours on the hydrogenator, the mixture had consumed 0.027 mol of hydrogen. The bottle was removed from the hydrogenator and the contents filtered, to remove the catalyst and insoluble product. Then the anhydride was poured over 1 kg of ice and stirred for three days to hydrolyze it to the acid.

The insoluble product was separated from the platinum by dissolving it in chloroform and filtering. The chloroform was evaporated and the residue recrystallized from 95% ethanol. The product weighed 1.65 g (0.006 mol) and melted at 165-167°C.

The mixture of water and hexanoic acid was evaporated on the rotary evaporator. The acid distilled over at .5 Torr and 82°C. A 40-ml volume of water was added to the residue. The water insoluble product was recovered by filtration. The product weighed 0.02 g,

melted at 165-167°C and was combined with the product above. The total yield of product was 10%.

A sample was submitted for elemental analysis. Analysis: Calculated for $C_{15}H_{26}N_2O_2$: C, 67.61; H, 9.86; N, 10.62: Found: C, 67.26; H, 10.29; N, 10.20: ir (KBr pellet) 1650 cm^{-1}(C=O), 1475(CH_3) and 2941 cm^{-1}(aliphatic CH); uv max ($CHCl_3$) 282 nm (ε, 17,400); nmr ($CDCl_3$) δ, .87-2.53(m, 11H, $CH_2CH_2CH_2CH_2CH_3$), 5.48(s, 2H, ring-CH_2) and 6.20-6.71(m, 2H, HC=CH).

Preparation of N,N'-Dipropionyl-4-imidazole from 1-Acetylimidazole

This reaction was carried out in the same manner as the hydrogenations previously described. A 40-ml volume of propionic anhydride and 1.00 g of platinum oxide were hydrogenated until no further uptake of hydrogen was observed. Then 8.16 g (0.03 mol) of 1-acetylimidazole was added to the flask and the hydrogenator started again. The reaction was stopped after the pressure dropped one-half the value calculated to be necessary for total reduction of the imidazole to imidazoline. The reaction took about 48 hours.

Insoluble product and platinum were removed from the mixture by filtration. Then, the product was recovered from the platinum by extraction with chloroform. The chloroform was evaporated and 0.61 g of product was recovered. The product was recrystallized three times from 95% ethanol and reached a constant melting point of 203-205°C. A mixed melting point with an authenic sample of N,N'-dipropionyl-4-imidazoline was undepressed.

The propionic anhydride was poured over 1 kg of ice and, after melting of the ice, was stirred for two days to hydrolyze the anhydride. Then the liquids were evaporated on the rotary evaporator, with warming at a temperature of 40°C and a pressure of 14 Torr. Water was added to the residue and the water insoluble product was recovered by filtration. This product weighed 0.02 g and melted at 202-204°C.

An infra-red spectrum of the product was found to be identical with that of N,N'-dipropionyl-4-imidazoline. The pure product was obtained in 11.5% yield.

Microbiological Testing Procedures

The first of two investigations into the antimycotic activity of the 4-imidazolines was carried out using filter paper discs of 5 mm diameter which had been soaked in chloroform solutions of the compounds. The discs were prepared from Whatman (No. 1) filter paper. Small strips were cut and placed into a large test tube containing one of the solutions of the molarities listed in Table I.

Table I

Concentrations of Chloroform Solutions of 4-Imidazolines

Compound	I (Molarity)	II (Molarity)	III (Molarity)
N,N'-Dipropionyl-4-imidazoline	5.9×10^{-1}	5.9×10^{-2}	5.9×10^{-3}
N,N'-Dibutyryl-4-imidazoline	4.75×10^{-1}	4.75×10^{-2}	4.75×10^{-3}
N,N'-Dihexanoyl-4-imidazoline	3.75×10^{-1}	3.75×10^{-2}	3.75×10^{-3}

The strips were allowed to air dry. Then a sterilized paper punch was used to cut the filter paper into the discs. The discs were cut directly into sterilized petri dishes and the dishes stored.

The Aspergillus fungus used for the initial test was obtained from the laboratory air. Nutrient agar plates were exposed to the air for three days. Then, they were covered and allowed to stand for three days. At that time, the fungus colony, identified by Dr. John Yemma,[22] was isolated from the other growths by subculturing.

Innoculation of nutrient agar with the Aspergillus was followed immediately by the application of the product-containing paper discs. Three plates were used for each of the nine dilutions, and three plates were used for discs soaked in pure chloroform, making a total of thirty plates.

The cultures were checked after 48 and 72 hours. Because no signs of inhibition of growth were evident at these times, the cultures were further checked periodically for the next 96 hours. No changes were noted.

The second test was performed using solid 5 mg samples of the imidazolines. A sample of N,N'-dipentanoyl-4-imidazoline was available and used at that time for the tests, in addition to the other compounds used previously. Also, another fungus, Aspergillus flavus, had been purchased and was used here. The solid samples of the compounds were applied to the plates at the time of innoculation.

Again, growth was checked after 48 and 72 hours, with results similar to those above. Further observations during the following 96 hours revealed no changes.

Attempted Reactions

The oxidation of benzhydrazide was also attempted using water as the nucleophilic species. These reactions were attempted in three different solvents; ethyl acetate, ether and dioxane. Although none of the three attempts resulted in the formation of benzoic acid, 0.178 g (12% yield) of N,N'-dibenzoylhydrazine was isolated in the reaction in which ether was used as the solvent.

A reduction of imidazole with platinum oxide was attempted in heptafluorobutyic anhydride. No hydrogen consumption was observed. Another reduction of imidazole was attempted using succinic anhydride with dimethyl acetamide as solvent. Again, no hydrogen consumption was observed.

CHAPTER IV

Results and Discussion

Conversion of Benzoic Acid Hydrazides to Benzoic Acid

The report by Wolman, Gallup, Patchornik and Berger[15] concerning the preparation of peptides by the oxidation of N-protected amino acid hydrazides in the presence of amino acid esters, suggested to us that the conversion of acid hydrazides to free acids might be brought about in a similar manner. The authors mentioned above stated that the oxidatively activated intermediate was most likely an acyl diazonium salt or an acyl diazo compound, and it is of interest here to speculate somewhat on the possible mechanism of this reaction.

The stoichiometry of the oxidation of acid hydrazides to bis-diacylhydrazines, as put forth by Curtius,[16] is shown below:

$$2\ RCONHNH_2 + 2\ I_2 \longrightarrow RCONHNHCOR + N_2 + 4\ HI$$

If one assumes that the intermediate of this oxidation is a diazonium salt then the following mechanism could be postulated, compatible with the stoichiometry.

$$RCONHNH_2 + 2\ I_2 \longrightarrow RCON_2I + 3\ HI$$
$$RCON_2I + RCONHNH_2 \longrightarrow RCONHNHCOR + N_2 + HI$$

On the other hand, a compatible mechanism can also be proposed involving an acyl diazo intermediate:

$$RCONHNH_2 + I_2 \longrightarrow RCON{=}NH + 2\ HI$$
$$RCONHNH_2 + RCON{=}NH + I_2 \longrightarrow RCONHNHCOR + N_2 + 2\ HI$$

The oxidation of 1,2 diacylhydrazines by various oxidants, including iodine and N-bromosuccinimide, has been shown to yield isolable

disubstituted diimides.[22]

RCONHNHCOR —oxidant→ RCON=NCOR

Although this fact tends to increase the liklihood of a monoacyldiimide intermediate in the case of the oxidation of monoacylhydrazines, it is not at all a positive proof for that mechanism. Furthermore, it is conceivable that both postulated intermediates could be involved. That is, the diazo intermediate could be further oxidized to the diazonium ion which then could go on to react with the unreacted hydrazide.

The above mechanisms are merely speculations and are not meant to be taken as concretely proposed pathways for the reaction. In examining these possibilities, it was not our intention to elucidate the mechanism, but rather to arrive at some general concept of the reaction pathway in order to construct an avenue of approach to our particular problem. Actually, both of these mechanisms were acceptable for our purposes because essentially they are similar in that the intermediates serve as acylating agents and should be subject to mucleophilic substitution. Thus, we assumed that the oxidatively activated intermediate would be some sort of an electrophile and that the formation of the acid might result by supplying the proper nucleophile.

The initial attempt to oxidize benzhydrazide to benzoic acid was carried out in dimethyl formamide. Since the highest yields of peptides were obtained by Wolman, Gallup, Patchornik and Berger[15] when iodine was used as the oxidant, and since the reactions with this oxidant were all carried out a 0°C, iodine was selected as the oxidant in this work. Water was first thought to be a strong enough nucleophile for the reaction and was used for that purpose in this reaction. The choice of dimethyl formamide as solvent was based on the facts that it is miscible

in water and that iodine can be dissolved in it. A suitable volume ratio of dimethyl formamide and water had to be and was determined in order to prevent precipitation of the iodine upon addition of water. The benzhydrazide solution was added to an excess of iodine in an attempt to avoid the possibility of the hydrazide reacting with itself to form N,N'-dibenzoylhydrazine.

After stirring for 24 hours, removing excess iodine with sodium thiosulfate and evaporating the solvent, a yellow precipitate was obtained. Although most of this residue was redissolved in water, 0.24 g of water-insoluble product was recovered. This product was not identified. It was neither benzoic acid nor N,N'-dibenzoylhydrazine, since it began to melt at 190°C, which is about 70°C higher than the reported melting point of benzoic acid[20] and about 50°C lower than the reported melting point of N,N'-dibenzoylhydrazine.[19] A second product was recovered when the aqueous solution just mentioned was acidified with HCl. This product had a very sharp melting point (117-118°C) and was thought to be elemental sulfur produced by the acidification of the solution which contained excess sodium thiosulfate.

$$S_2O_3^{-2} + 2H^+ \longrightarrow H_2S_2O_3 \longrightarrow H_2O + SO_2 + S$$

This product was found to be insoluble in NaOH. This observation along with the fact that titration with sodium thiosulfate indicated no reduction of the iodine, led us to conclude that no benzoic acid had been formed.

It was then decided to attempt the reaction in a different solvent. Tetrahydrofuran was found to be unsuitable because iodine is not sufficiently soluble in mixtures of water and tetrahydrofuran. A two phase reaction using water and ether was tried. A 6.5% yield of N,N'-dibenzoylhydrazine was recovered from this reaction.

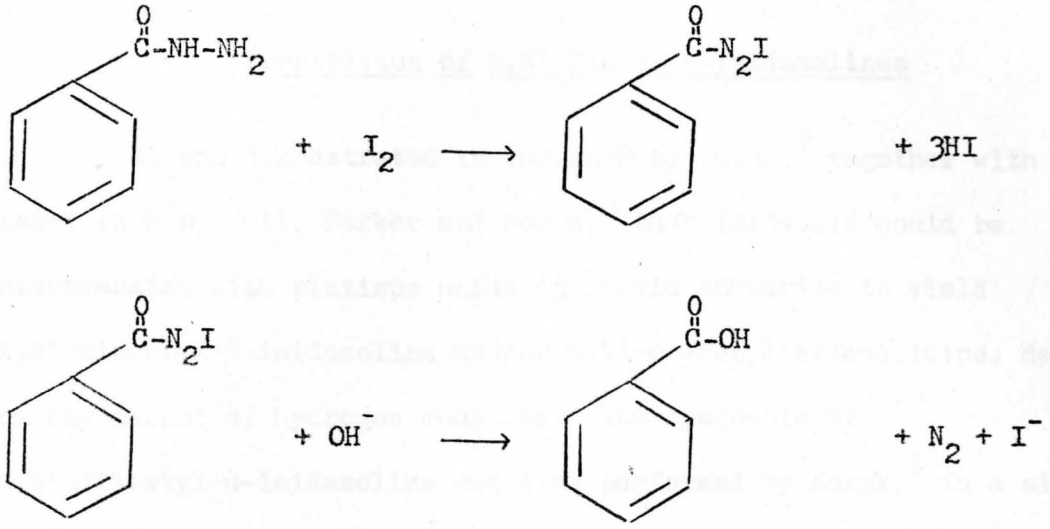
Ethyl acetate was then used as solvent in hopes that it might prove to be a better solvent for the desired reaction. Results similar to those obtained with dimethyl formamide were observed.

After these unsuccessful attempts, it was suggested, while assuming the general characteristics of the reaction to be accurate, that perhaps the water was not a strong enough nucleophile. This prompted us to try the oxidation using an aqueous solution of base in place of pure water. The first reaction was done using ether as the iodine solvent and an aqueous solution of sodium carbonate as the base. A 40% yield of benzoic acid (0.973 g) was recovered, after recrystallization. It is of interest to note that only 0.02 g of N,N'-dibenzoylhydrazine was obtained in this reaction as opposed to 0.17 g recovered in the reaction with ether and water. Of course, the quantities of the reactants were the same in both cases.

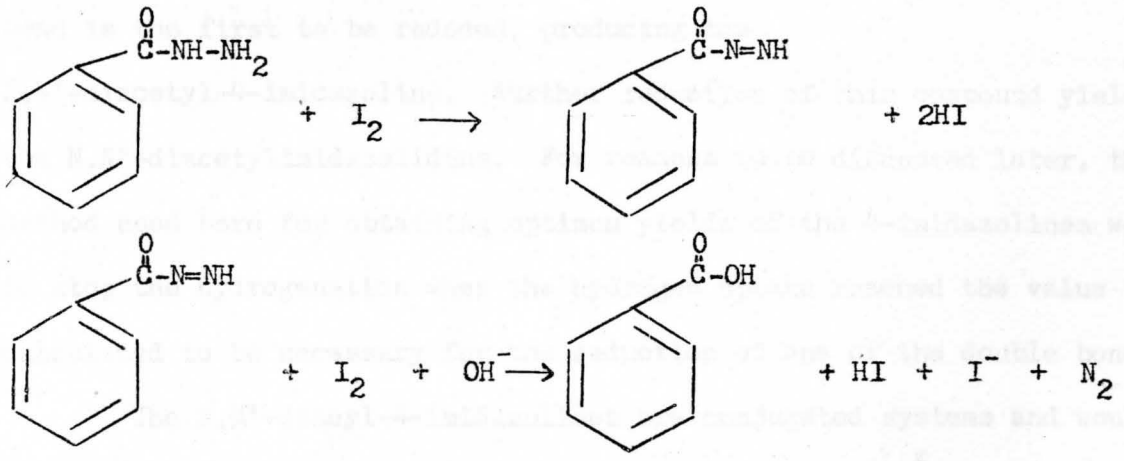
A similar reaction was carried out using ethyl acetate and aqueous sodium carbonate as solvents. A 28% yield of benzoic acid (0.58 g) was recovered. In addition, 0.009 g of N,N'-dibenzoylhydrazine was recovered from the ethyl acetate layer.

The success of the two reactions just described appears to be due to the presence of the base. This is consistent with our initial assumption that the oxidation of the hydrazide to the acid would be a nucleophilic substitution. Water, apparently, is not sufficiently nucleophilic to attack the activated hydrazide. If we again assume the intermediate of the reaction to be either an acyl diazonium salt or an acyl diazo compound, as suggested by Wolman, Gallup, Patchornik and Berger,[15] then we can speculate on the mechanism of the oxidation of the acid hydrazide to the acid.

Firstly, assuming an acyl diazonium salt intermediate, the following mechanism can be proposed:



Secondly, the mechanism below can be written by assumimg the formation of an acyl diazo intermediate:



Both mechanisms are compatible with the results of this investigation. Of course, a more complete understanding of the mechanism would be beneficial to anyone who wished to employ this synthetic method. However, the results obtained in this investigation were sufficient for our problem. The reaction should be satisfactory for the conversion of the hydrazide of the imidazoline derivative of histidine to the acid.

The reaction does take place under mild conditions and thus, the most essential demand in the development of this method has been met.

Preparation of N,N'-Diacyl-4-imidazolines

It was demonstrated in the work by Bauer,[7] together with the later work by Vail, Barker and Moran,[8] that imidazole could be hydrogenated with platinum oxide in acetic anhydride to yield N,N'-diacetyl-4-imidazoline and/or N,N'-diacetylimidazolidine, depending on the amount of hydrogen consumed. The synthesis of N,N'-diacetyl-4-imidazoline was also performed by Kurek,[6] in a similar manner.

As mentioned above, the product obtained depends on how far the reaction is allowed to proceed. Apparently, the carbon to nitrogen bond is the first to be reduced, producing the N,N'-diacetyl-4-imidazoline. Further reduction of this compound yields the N,N'-diacetylimidazolidine. For reasons to be discussed later, the method used here for obtaining optimum yields of the 4-imidazolines was to stop the hydrogenation when the hydrogen uptake reached the value calculated to be necessary for the reduction of one of the double bonds.

The N,N'-diacyl-4-imidazolines are conjugated systems and would be expected to absorb ultra-violet light. Indeed, Kurek[6] found N,N'-diacetyl-4-imidazoline to have a molar absorptivity of 1.93×10^4 with a maximum absorbance at 277 nm. On the other hand, N,N'-diacylimidazolidines do not have this conjugated system and, therefore, would not be expected to show absorbance in the near ultra-violet region. The lack of absorbance by N,N'-diacetylimidazolidine near 280 nm has been shown experimentally.[24] Imidazole, also, absorbs

very little in the near ultra-violet region. The molar absorptivities of the N,N'-diacyl-4-imidazolines prepared in this work are summarized below.

Table II

Molar Absorptivities of N,N'-Diacyl-4-imidazolines, Max =282 nm

Compound	Concentration	Molar Absorptivity
N,N'-dipropionyl-4-imidazoline	5.8×10^{-5} M	1.70×10^{4}
N,N'-dibutyryl-4-imidazoline	4.75×10^{-5} M	1.82×10^{4}
N,N'-dipentanoyl-4-imidazoline	5.36×10^{-5} M	1.80×10^{4}
N,N'-dihexanoyl-4-imidazoline	3.75×10^{-5} M	1.74×10^{4}

The first compound in this series to be synthesized was N,N'-dibutyryl-4-imidazoline. Imidazole was reduced with platinum oxide in butanoic anhydride. Total time for the reduction was ten hours, with the calculated pressure drop taking place. The procedure used for the isolation of the product was quite involved, using several solvents in attempts to extract the product, and the low yield of 5.6% is probably due to this cumbersome technique.

The reaction was then attempted in dioxane. It was noted that the product in the first reaction was insoluble in cold dioxane and it was thought that the product would be obtained as a precipitate. The insolubility would also increase the yield of the desired product. However, no hydrogen uptake was noted after several hours on the hydrogenator. Furthermore, an ultra-violet spectrum of the reaction

mixture showed no significant absorbance. It was concluded from these observations that none of the desired product had formed.

The hydrogenation was then attempted again in 150 ml of butanoic anhydride with 1.00 g of platinum oxide and 4.04 g of imidazole. The platinum was separated from the reaction mixture by adding hot ethyl acetate to dissolve the insoluble product and then filtering. When the filtrate was cooled, crystals formed. A 2.08 g yield of product was recovered. This represents a 35% yield. The product melted at 190-193°C. A subsequent hydrogenation was performed and a 29% yield was obtained.

As stated previously, the N,N'-diacyl-4-imidazolines were expected to and did significantly absorb ultra-violet light. The ultra-violet spectrum for this compound, N,N'-dibutyryl-4-imidazoline, is shown in Figure I. The spectrum shows the maximum absorbance to be at 282 nm. This, of course, could not serve as positive proof that the product was the 4-imidazoline. It did, however, eliminate the possibility of the product being the imidazolidine. Also, our product melted at 190-193°C, whereas the imidazolidine is reported to melt at 84-86°C.[18]

Confirmation of the products identity was obtained using four analytical techniques. The results of the elemental analysis (see experimental) were well within the theoretical values.

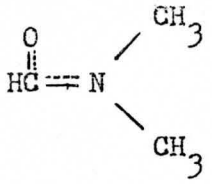
An infra-red spectrum was taken of the product using a KBr pellet and the Beckman IR-5. A tertiary amide carbonyl was expected between 1669-1629 cm^{-1},[25] and was found on the products spectrum at 1634 cm^{-1}. Furthermore, three types of C-H stretching were anticipated for this compound and located on the spectrum at points well within

experimental error. A large peak at 1458 cm^{-1} was assigned to CH_3 bending.[26] A peak corresponding to out of plane CH deformation of the cis-disubstituted ethylene group was expected at 680 cm^{-1} but was not located on the compounds spectrum at that position. However, the spectrum did show a medium peak at 746 cm^{-1}. Bellamy[27] states that the substitution of electronegative atoms causes upward frequency shifts. It is possible that the band at 746 cm^{-1} corresponds to the out of plane CH deformation of the cis-disubstituted ethylene group.

An NMR spectrum of the compound was also taken. A triplet at .87 ppm was attributed to the methyl protons and a triplet at 2.12 ppm was assigned to the protons of the CH_2 adjacent to the carbonyl. The protons of the CH_2 adjacent to the CH_3 resulted in a multiplet at 1.55-2.05 ppm. A singlet at 5.43 ppm was assigned to the ring-CH_2. Intergration of the spectrum showed the CH_3 triplet to represent three times the number of protons as the ring-CH_2 singlet. The peak assigned to the protons of the CH_2 adjacent to the carbonyl was also consistent in that it represented twice the number of protons as the ring-CH_2 singlet. Two doublets, at 6.22 ppm and 6.64 ppm were assigned to the HC=CH protons. This was done for two reasons. Firstly, the peaks are located in the area of the spectrum where one would expect olefinic protons to be. Secondly, it was necessary to add the areas under these two peaks in order to arrive at a value which would be consistent with the remainder of the spectrum.

Although no final conclusions were made as to the interpretation of these doublets, it is thought that restricted rotation about the amide bond may be responsible, in part, for this phenomenon. It is well established that this restricted rotation, owing to the partial double

bond character of the C-N bond, results in two N-methyl peaks in the spectrum of N,N'-dimethylformamide.[28]



N,N'-dipropionyl-4-imidazoline was then prepared in a similar manner and the compound identified using the four analytical methods employed previously. The molar absorbtivity of this compound was calculated to be 1.70×10^4, with a maximum absorbance at 282 nm. The infra-red spectrum for the new compound showed the tertiary amide carbonyl peak at 1634 cm^{-1}. The relative height of the aliphatic C-H stretching peak was less than that observed in the spectrum of the first compound, but relatively larger than that observed in the spectrum of N,N'-diacetyl-4-imidazoline. This is in agreement with our expectations because the dipropionyl compound would have two fewer CH_2 groups than the dibutyryl compound and two more than the diacetyl compound. A large peak at 1484 cm^{-1} appears to correspond to that at 1538 cm^{-1} on the dibutyryl spectrum and is attributed to CH_3 bending. The most significant differences were the relative heights of the aliphatic C-H stretching peaks and the peaks found in the finger print region (1429-909 cm^{-1}) of the spectrum. A peak at 744 cm^{-1} was assigned to out of plane C-H deformation of the cis-disubstituted ethylene group.

The NMR spectrum of this compound showed a multiplet at 6.27-6.67 ppm which was assigned to the HC=CH protons. The ring CH_2 protons gave a singlet at 5.45 ppm. A triplet at 1.09 ppm was assigned to the methyl protons and a quartet at 2.20 ppm corresponds to the protons of the CH_2 adjacent to the CH_3 group.

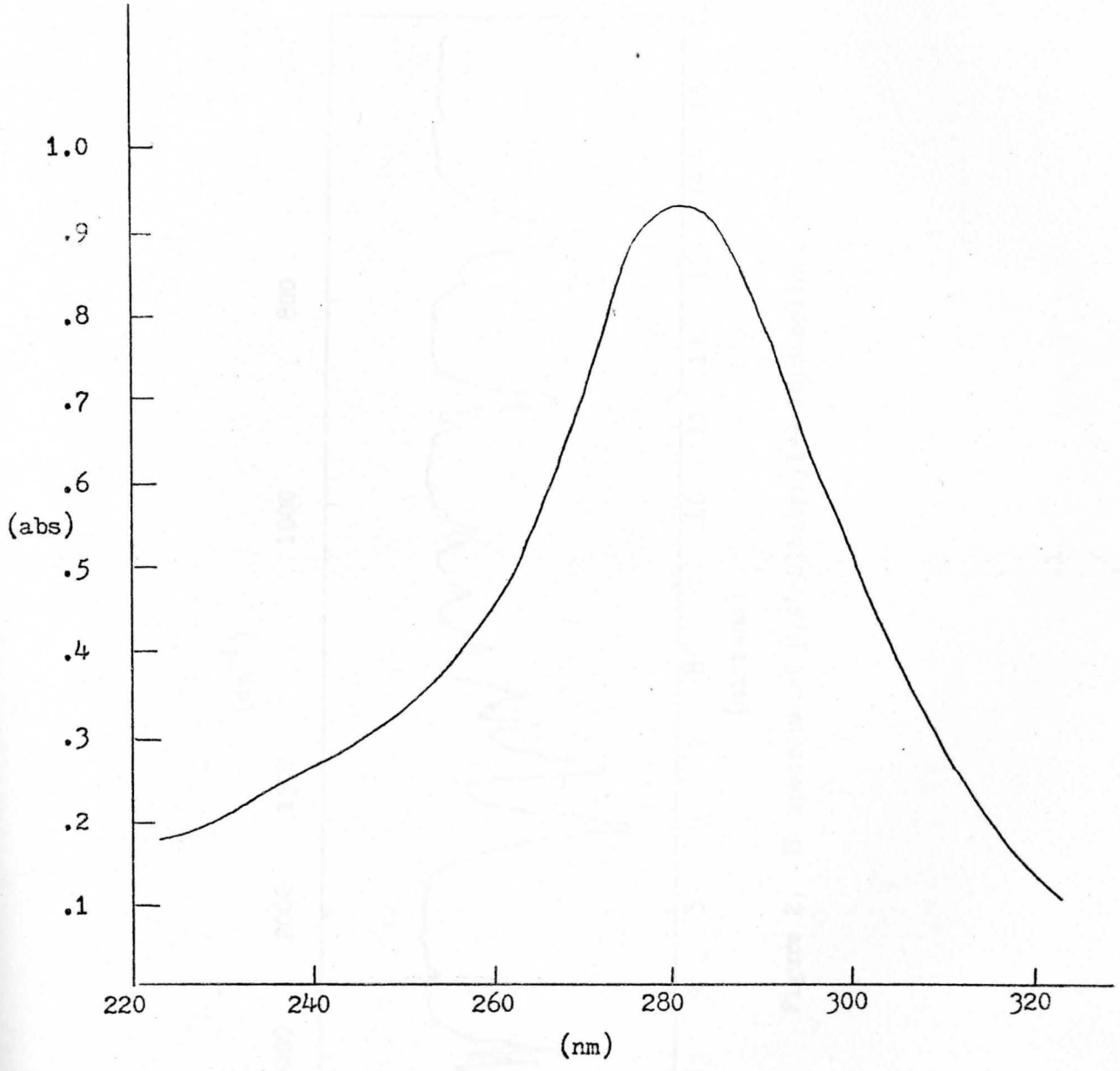


Figure 1. UV Spectrum of N,N'-dibutyryl-4-imidazoline.

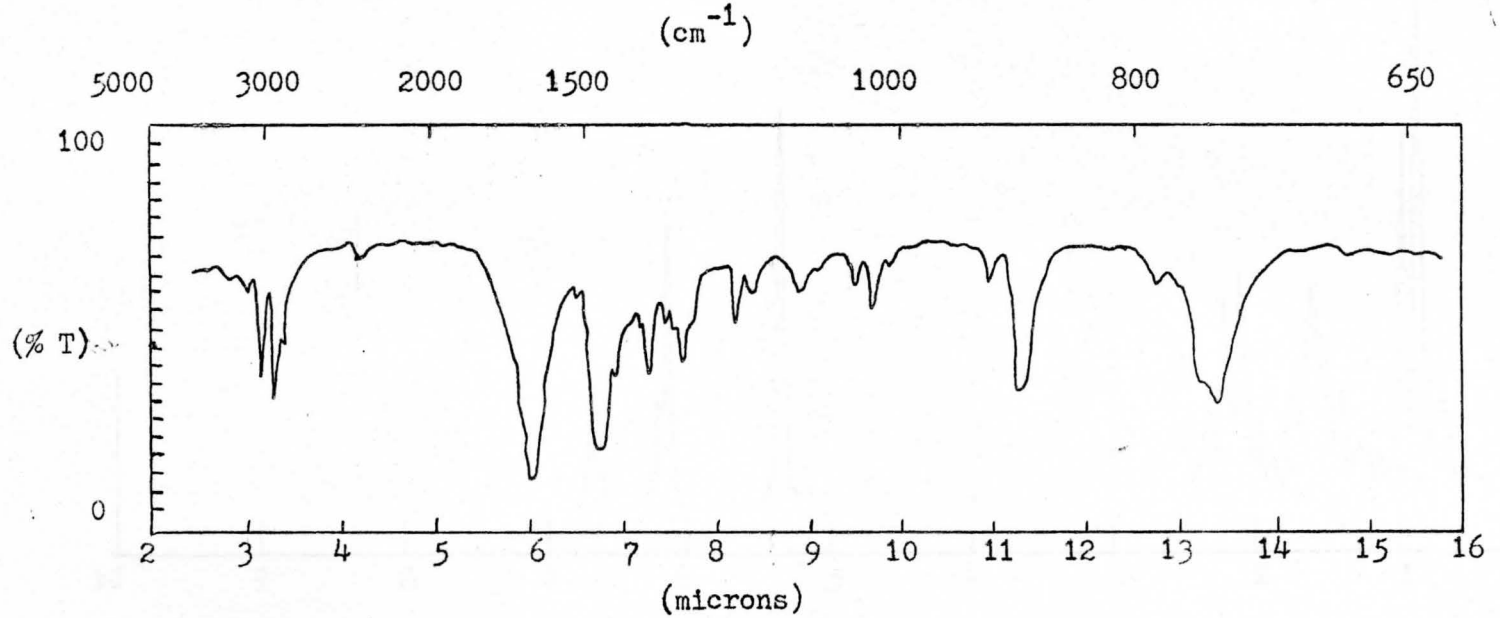


Figure 2. IR Spectrum of N,N'-Dibutyryl-4-imidazoline.

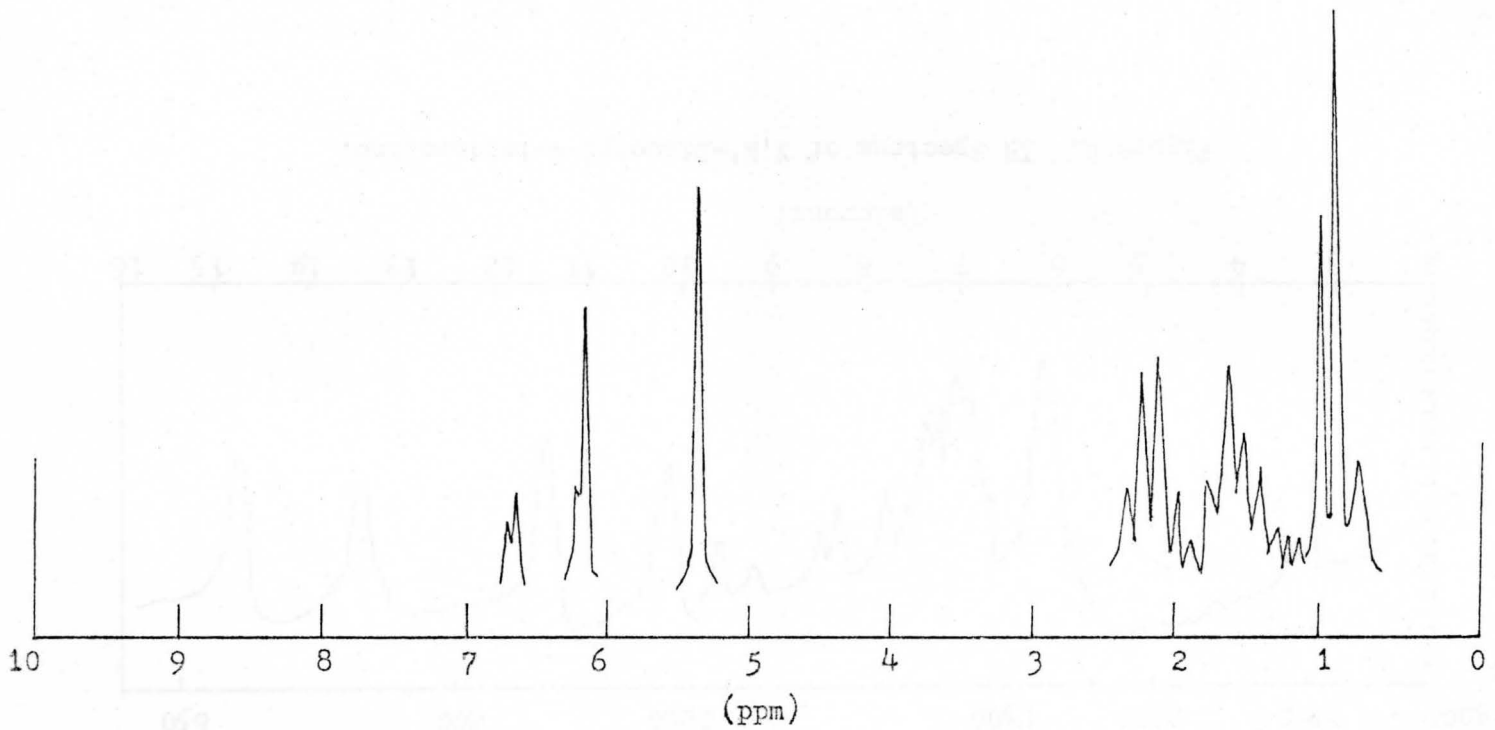


Figure 3. NMR Spectrum of N,N'-Dibutyryl-4-imidazoline.

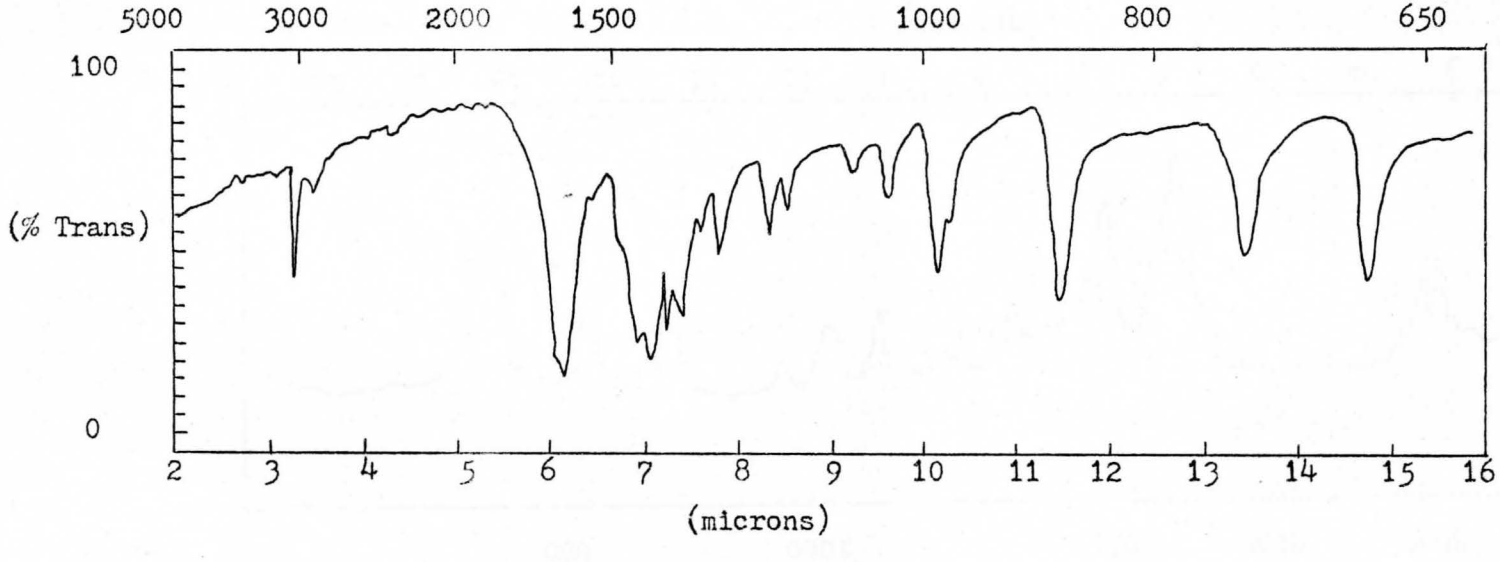


Figure 4. IR Spectrum of N,N'-Diacetyl-4-imidazoline.

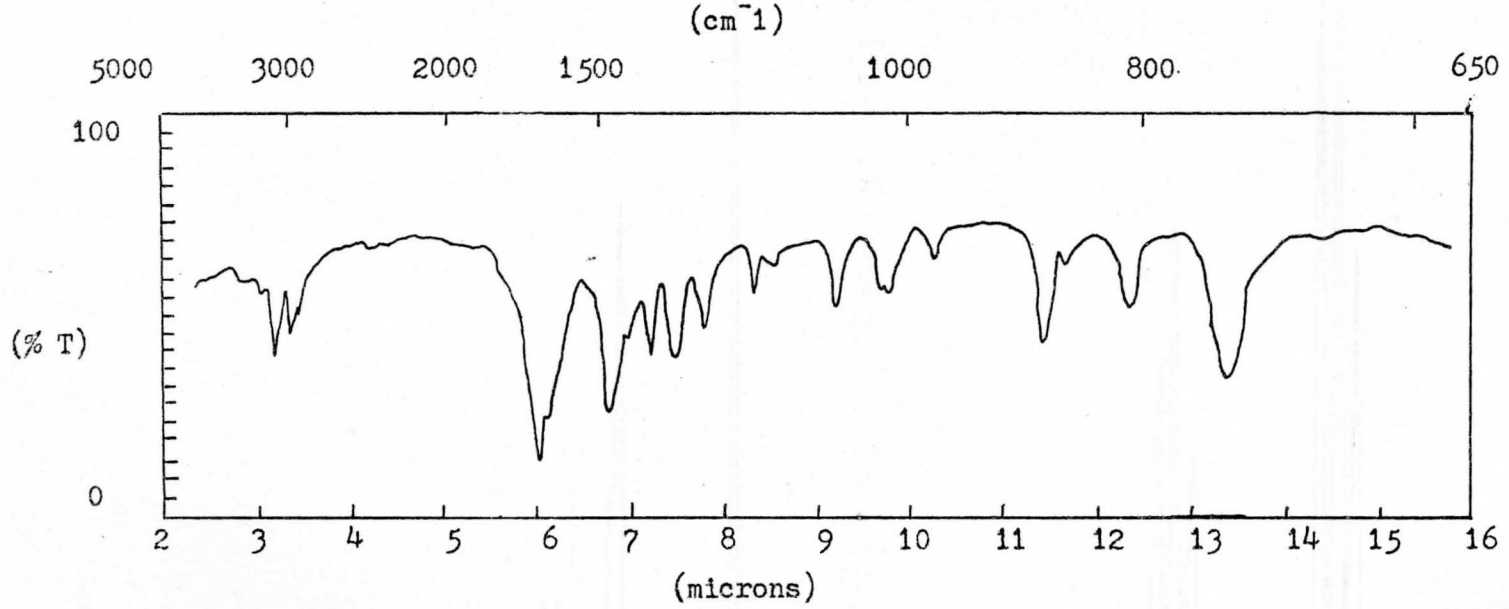


Figure 5. IR Spectrum of N,N'-Dipropionyl-4-imidazoline.

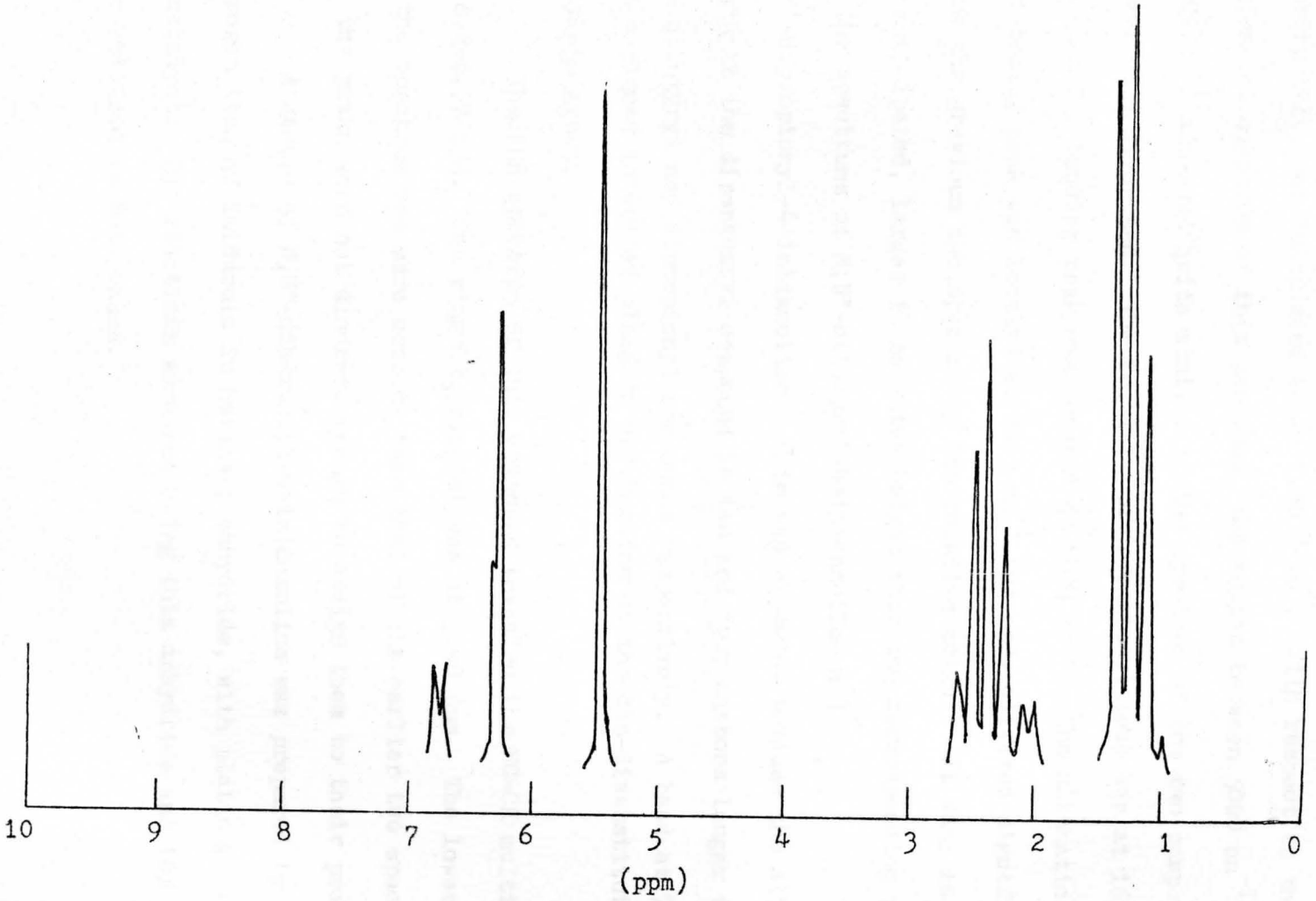


Figure 5. NMR Spectrum of N,N'-Dipropionyl-4-imidazoline.

N,N'-dipentanoyl-4-imidazoline was prepared and identified in a similar manner to that of the first two compounds. The UV spectrum of the compound showed maximum absorbance at 282 nm and the molar absorptivity was calculated to be 1.80×10^4. With respect to the infra-red spectrum of this compound, the region between 5000 cm^{-1} and 1429 cm^{-1} appeared quite similar to the spectrum of the two compounds previously discussed. The tertiary amide carbonyl peak was at 1652 cm^{-1} and the CH_3 bending peak was located at 1473 cm^{-1}. The aliphatic C-H stretching peak was located at 2967 cm^{-1} and only differed significantly from the previous two spectra by its relative height. It was, as would be anticipated, larger in relative height than the corresponding peaks in the spectrums of N,N'-dibutyryl-4-imidazoline and N,N'-dipropionyl-4-imidazoline. This was expected because the aliphatic chain in the dipentanoyl compound is two and four carbons longer than the dibutyryl and dipropionyl compounds respectively. A band at 745 cm^{-1} was assigned to out of plane CH deformation of the cis-disubstituted ethylene group.

The NMR spectrum of this compound revealed the HC=CH multiplet at 6.2-6.72 ppm. The ring CH_2 singlet was at 5.40 ppm. The lower end of the spectrum was more complex than that of the earlier two spectra and the peaks were not discrete enough to assign them to their protons.

A sample of N,N'-dihexanoyl-4-imidazoline was prepared by hydrogenation of imidazole in hexanoic anhydride, with platinum oxide as catalyst. Two reactions were run using this anhydride and 10% yields were obtained in both cases.

The identification of this compound was based on the results of the four analytical methods used previously. The molar absorptivity was calculated to be 1.74×10^4, with a maximum absorption at 282 nm.

The infra-red spectrum showed a tertiary amide peak at 1650 cm^{-1}. The spectrum differed from the previous spectrums in both the finger print region and the relative height of the aliphatic CH peak.

A singlet, corresponding to the ring-CH_2 protons, was located on the NMR spectrum at 5.42 ppm. The multiplet of the HC=CH protons was at 6.26-6.72 ppm. As in the case of N,N'-dipentanoyl-4-imidazoline, the peaks due to the protons of the aliphatic chain were indistinguishable, but this area of the spectrum did agree with our expectations.

The 4-imidazolines prepared in this work were quite similar to each other in their solubilities in various solvents. All four compounds were insoluble in hot and cold water, cold ethanol and cold ethyl acetate and only sparingly soluble in their corresponding anhydrides. They were, however, soluble in chloroform.

The melting points of these compounds are 70-100C° higher than the corresponding imidazolidines.[18]

Although the kinetics of the hydrogenation were not known, it was assumed that at some point during the reaction, before the calculated hydrogen uptake necessary for the complete conversion to imidazoline was reached, the imidazoline that had formed would begin further reduction to the imidazolidine. In hopes of obtaining maximum yield of the imidazoline, we attempted to follow the reaction by measuring the absorbance of the reaction mixture. It was our intention to stop the reaction at the point where the absorbance of the reaction

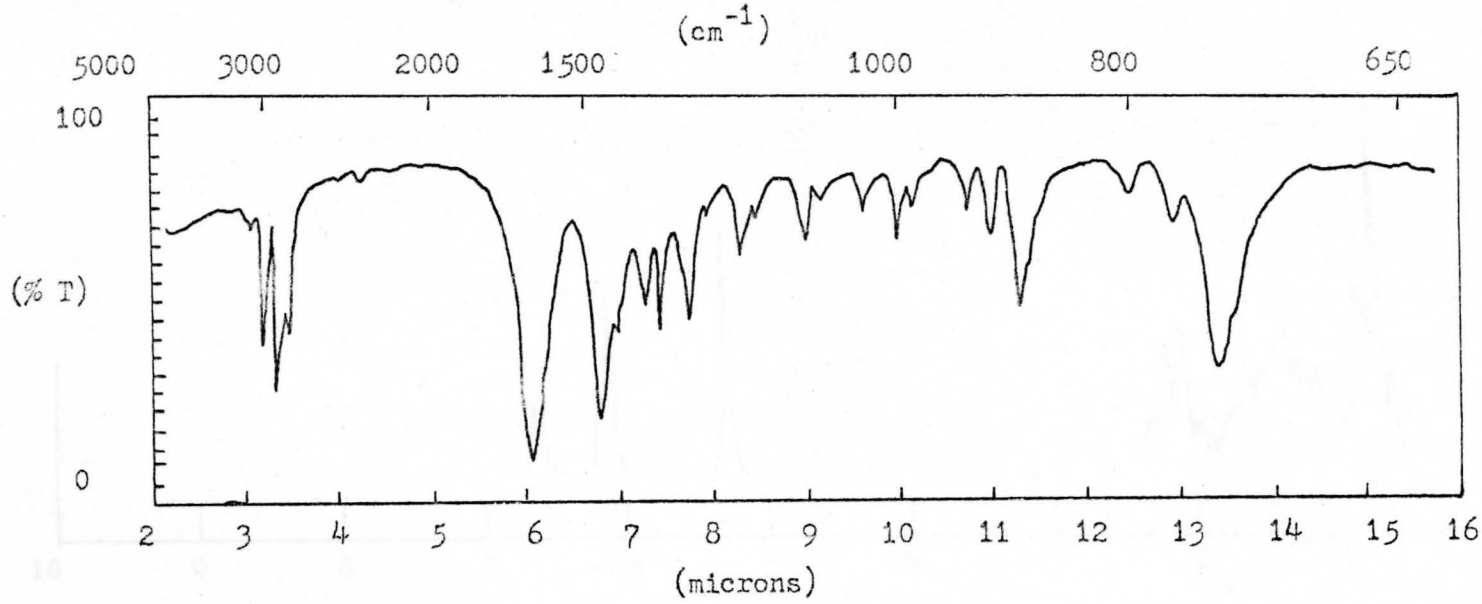


Figure 7. IR Spectrum of N,N'-Dipentanoyl-4-imidazoline.

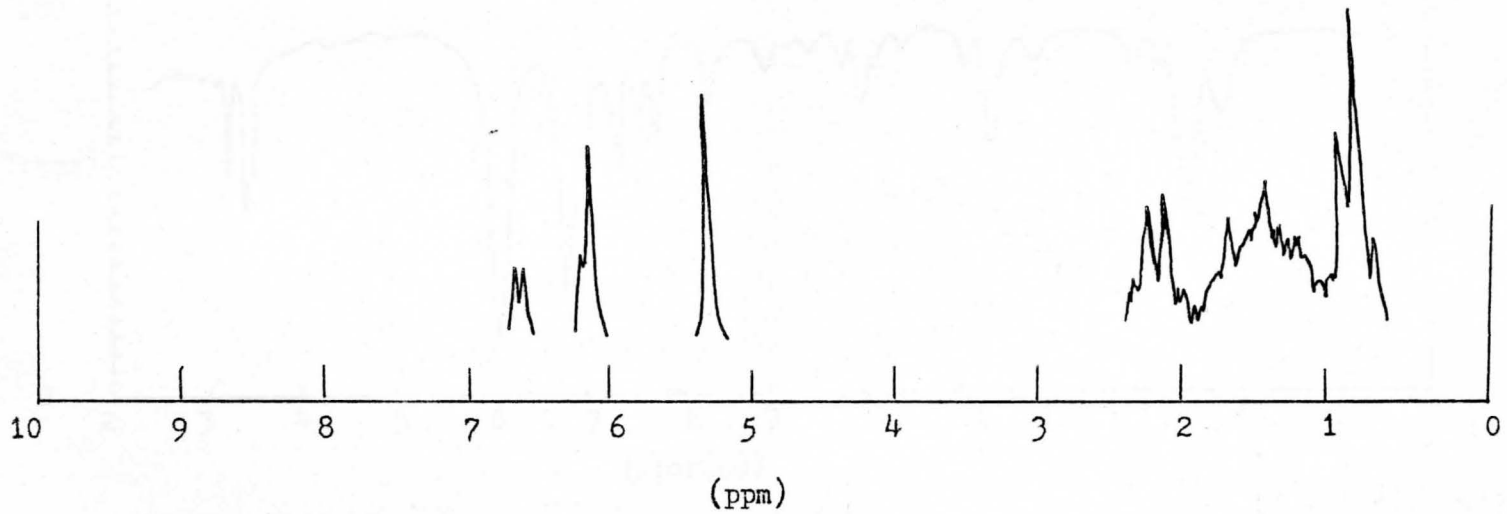


Figure 8. NMR Spectrum of N,N'-Dipentanoyl-4-imidazoline.

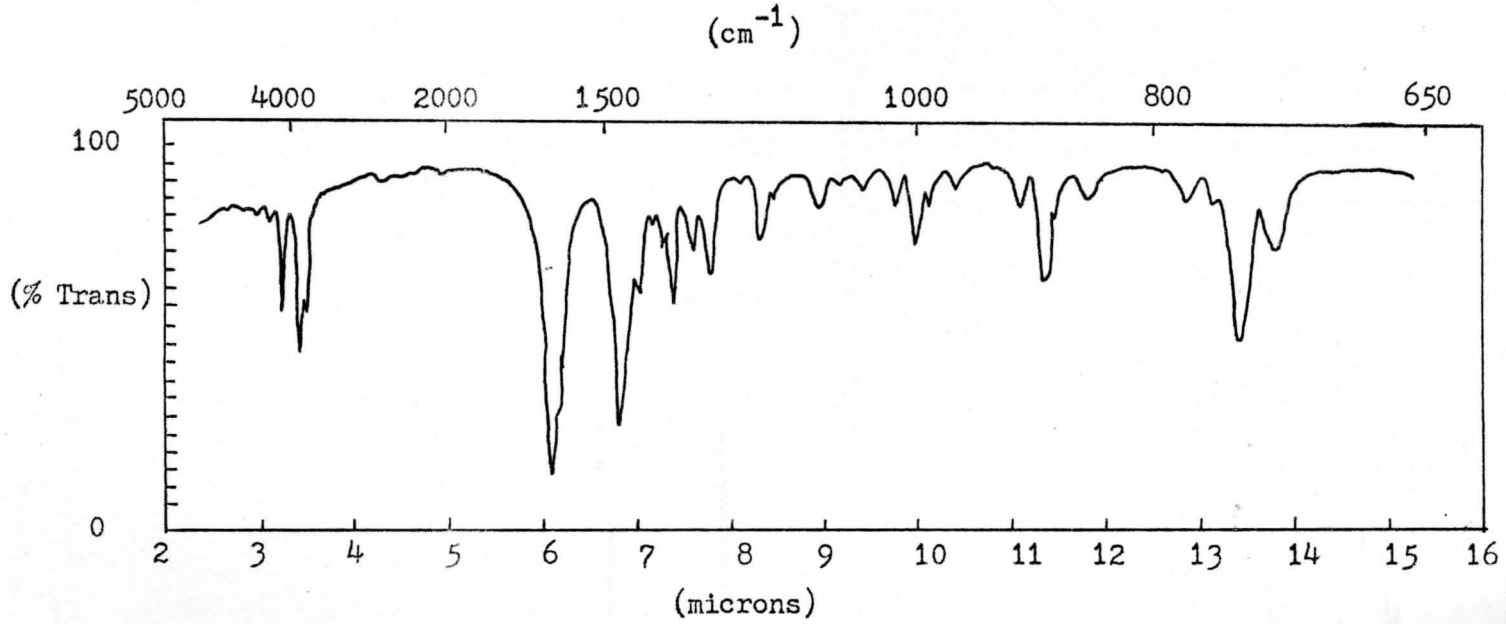


Figure 9. IR Spectrum of N,N'-Dihexanoyl-4-imidazoline

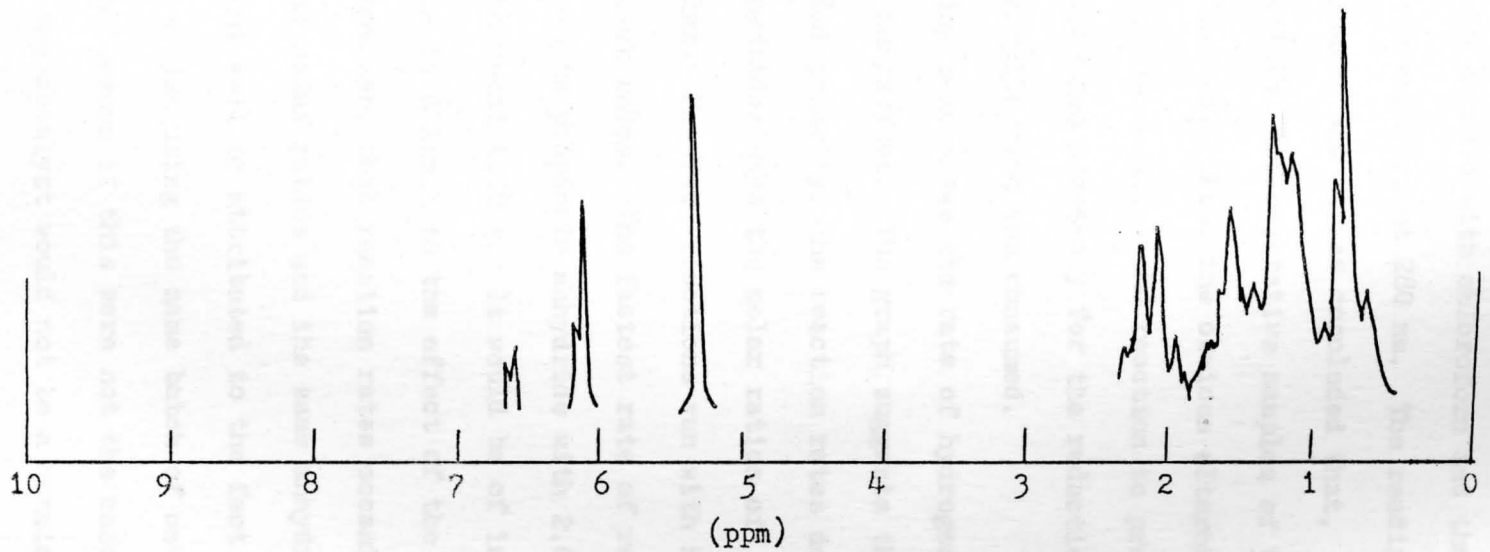
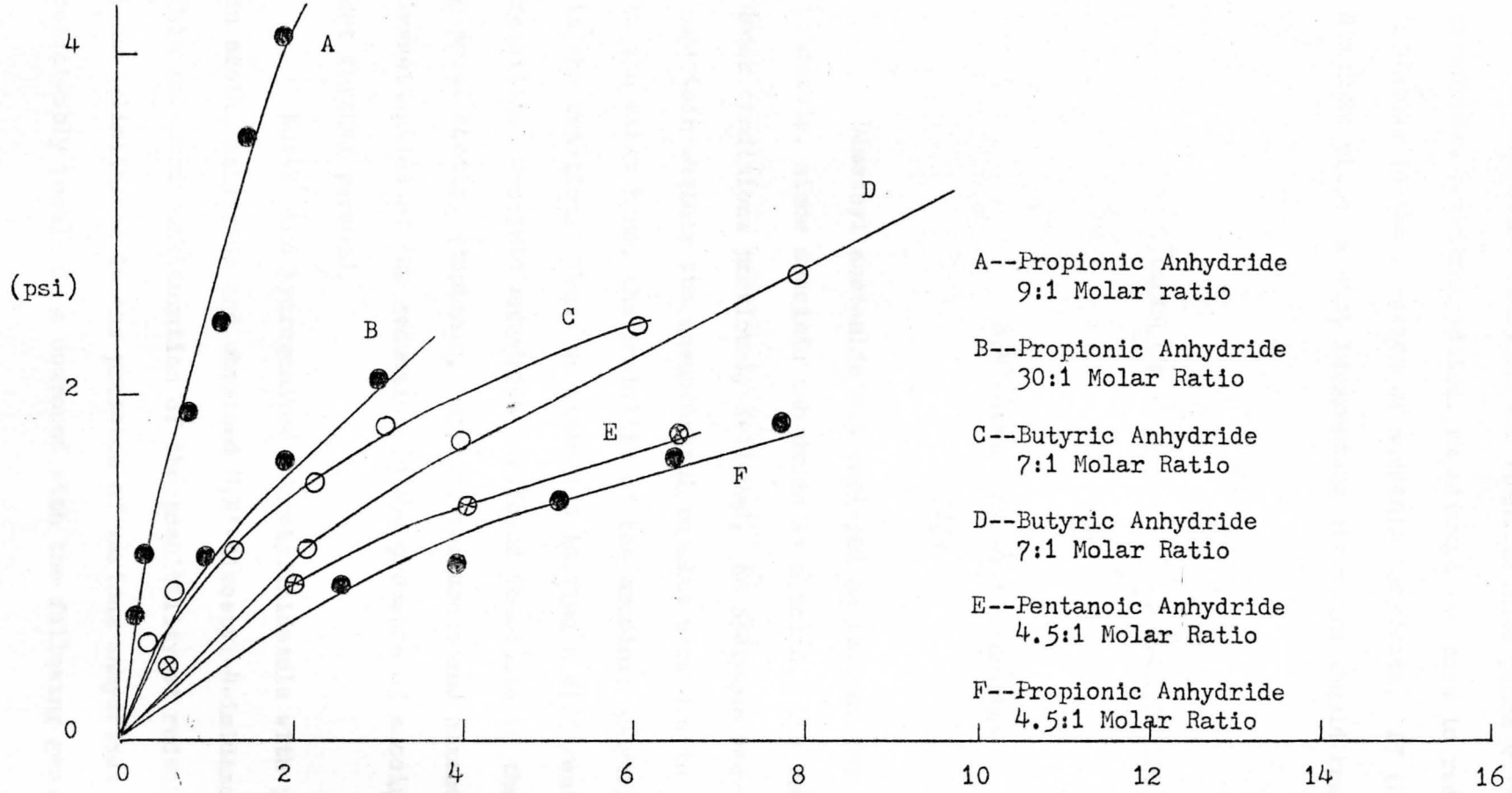


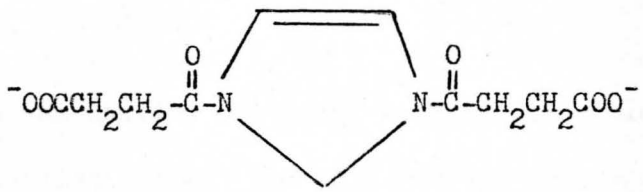
Figure 10. NMR Spectrum of N,N'-Dihexanoyl-4-imidazoline

material began to decrease. That should have been the point where the maximum amount of imidazoline was present. Small aliquots of the reaction mixture were removed from the reaction flask, at intervals, while stirring. The aliquots were diluted with chloroform and the Beckman-25 used for the absorbance readings at 280 nm. The readings did not change in a consistent manner and it was concluded that, because of the product's insolubility, representative samples of the reaction mixture could not be obtained. Thus, the obvious alternative, and the one chosen for this work, was to allow the reaction to proceed until the calculated amount of hydrogen necessary for the reduction of one double bond of the heterocyclic ring was consumed.

The graph on the following page shows the rate of hydrogenation of imidazole in three different anhydrides. The graph suggests that, despite the differences in solvent polarity, the reaction rates do not differ significantly between anhydrides when the molar ratios of anhydride to imidazole are similar. However, reactions run with higher molar ratios appear to have faster rates. The fastest rate of reaction was the hydrogenation of imidazole in propionic anhydride with 2.00 g of platinum oxide in place of the usual 1.00 g. It would be of interest to determine whether or not there is a limit to the effect of the catalyst on the rate. It was observed, however, that reaction rates occasionally varied markedly even when similar molar ratios and the same anhydrides were used. This observation might well be attributed to the fact that the reactions were not all carried out using the same batch of catalyst. It would have been far more advantageous if this were not the case, so that the variable activities of the catalyst would not be a consideration. However, preparation of larger batches of catalyst with the equipment available was inconvenient.


4
2
0
(psi)
A
B
C
D
E
F
0
2
4
6
8
10
12
14
16
A--Propionic Anhydride
9:1 Molar ratio
B--Propionic Anhydride
30:1 Molar Ratio
C--Butyric Anhydride
7:1 Molar Ratio
D--Butyric Anhydride
7:1 Molar Ratio
E--Pentanoic Anhydride
4.5:1 Molar Ratio
F--Propionic Anhydride
4.5:1 Molar Ratio

Since the reaction was found to take place with the four anhydrides previously cited, an attempt was made to reduce imidazole in the presence of succinic anhydride. If the reaction did take place, a most interesting structure should result:

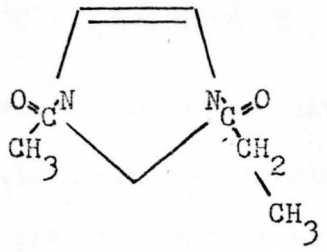


N,N'-Disuccinyl-4-imidazoline

XIV

Dimethyl acetamide was employed as solvent for the anhydride and imidazole, since succinic anhydride is a solid. The reaction was attempted under conditions previously followed. No hydrogen was consumed. It is uncertain whether the unsuccessful results were due to the solvent or, on the other hand, the inability of the succinic anhydride to take part in the reaction. Thus, we attempted to find a different solvent for the reaction. Succinic anhydride was found insoluble in the following acids: glacial acetic, propionic, butyric, pentanoic and hexanoic. The investigation of the reduction in the presence of succinic anhydride was not further pursued.

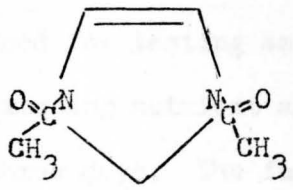
Kurek[6] had hydrogenated 1-acetylimidazole with platinum oxide in acetic anhydride and obtained N,N'-diacetyl-4-imidazoline in 25% yield. This prompted consideration of the possibility of reducing 1-acetylimidazole in the presence of various anhydrides which could conceivably result in a compound with the following general structure:



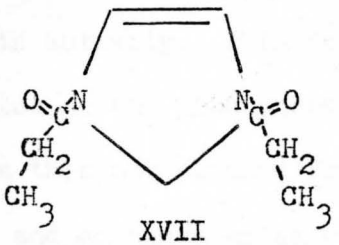
N-acetyl,N'-propionyl-4-imidazoline

XV

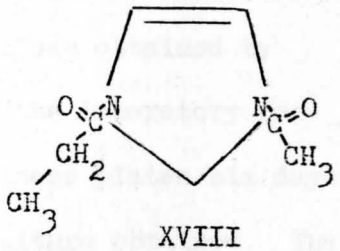
Thus, a reaction was carried out using 1-acetylimidazole, propionic anhydride and platinum oxide. The sole product recovered was N,N'-dipropionyl-4-imidazoline, identified by infra-red spectrum and melting point. From these observations, it appears that the acetyl group is removed during the reaction. Further work on this synthesis might involve carrying the reaction out in the presence of two different anhydrides. In this case, three products would be expected.



XVI



XVII



XVIII

Finally, the reduction of imidazole in heptafluorobutyric anhydride should be repeated. The results obtained in this investigation are questionable, since the reaction mixture stood for several days while the hydrogenator was repaired.

Antifungal Studies

Mod, Magne and Sumrell[10] studied the antimycotic activity of several N,N'-diacylimidazolidines, some of which correspond to the N,N'-diacyl-4-imidazolines prepared in this work. The imidazolidines prepared were tested on the organisms T. rubrum, M. gypseum, A. flavus, and T. violaceum. The results showed N,N'-dihexanoylimidazolidine to be the most effective inhibitor against all four organisms, with zones of inhibition at least 0.5 cm beyond the compounds periphery on the culture plate.

Since T. rubrum, M. gypseum and T. violaceum are all known pathogens, the more benign A. flavus was chosen for the investigation of the antimycotic activity of the new imidazolines. It was obtained from a commercial culture and subcultures of the fungus were made using nutrient agar plates. Another fungus of the Aspergillus genus was also used for testing antimycotic activity. This fungus was obtained by exposing nutrient agar plates to the atmosphere in the laboratory for three days. The fungus was then subcultured from these plates six days after the initial exposure and an uncontaminated culture obtained. The identification of the genus was made by Dr. John Yemma,[22] but the species was not determined.

A sterilized metal loop was used to transfer the organisms to the test plates of nutrient agar. Immediately after innoculation, the compounds that were to be tested were introduced to the innoculated plates.

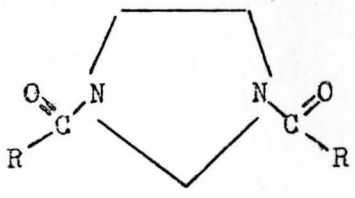
The initial testing was performed using discs which had been soaked in chloroform solutions of the compounds and then dried.

Two to six discs were applied to each plate. The molar concentrations of the chloroform solutions of the 4-imidazolines were between 5.5×10^{-3} M and 5.5×10^{-1} M. A sample of N,N'-dipentanoyl-4-imidazoline was not available for the initial test. Also, A. flavus, which was purchased, had not yet arrived and was studied later but not in the initial experiment.

The plates were checked 48 and 72 hours after innoculation. No signs of inhibition were present on any of the cultures. Fungus appeared to have grown over the paper discs. It was concluded that higher concentrations of the compounds should be used.

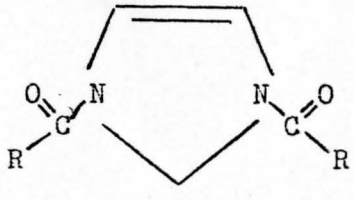
In the second and final tests both fungus organisms were used. N,N'-dipentanoyl-4-imidazoline was also available for the test. The compounds were applied to the freshly innoculated nutrient agar plates in samples of approximately 5 mg. Again, the cultures were allowed to grow for 48 hours. At that time they were checked for inhibition. None of the compounds appeared to inhibit the growth of the fungus. The cultures were checked again 24 hours later with similar results.

The results of these two investigations indicate that the 4-imidazolines tested do not possess the antimycotic activity of the imidazolidines. In that light, it is interesting to recall the subtle difference between the two structures.



N,N'-diacylimidazolidine

XIX



N,N'-diacyl-4-imidazoline

XX

Of course, it should be emphasized that only one of the fungi tested with the imidazolidines was used in this investigation. Also, some question might arise from the fact that Mod, Magne and Sumrell[10] employed a somewhat different method for the application of the compounds to the cultures. Although probably unlikely, it is conceivable that such a difference in method could offer argument against the comparison of results with the two series of compounds, imidazolines and imidazolidines, discussed above.

The fact that the N,N'-diacyl-4-imidazolines are less soluble in the agar medium than the N,N'-diacylimidazolidines might also account for the difference in activities. The inability to diffuse through the nutrient agar would greatly reduce the ability of the compounds to affect the fungus.

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