SPECTROSCOPIC STUDIES AND KINETICS OF CYCLISATION OF N-CARBOBEZOXY-L--AMINO ACIDS WITH ETHANOIC-ANHYDRIDE.

BY

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ZARIA-NIGERIA.

MARCH, 1999.

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any form for a higher degree. All quotations are indicated and the sources of information are specifically acknowledged by means of references.

LADAN, Z

19:8:99

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CERTIFICATION

This thesis entitled, **"Spectroscopic Studies and Kinetics of Cyclisation of N - carbobenzoxy-1--amino acids with ethanoic anhydride"**, by Zakari Ladan meets the regulation governing the award of the Degree of Master of Science of Ahamdu Bello University, Zaria, Nigeria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is rightly and deservedly dedicated to my brothers (Amos, Billy) and Sisters (Ladi, Sarah).

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Finally, I wish to express my gratitude to the Management of NARICT for the grant provided for this programme.

ABSTRACT

The spectroscopic and kinetics of cyclisation of N-carbobenzoxy-L-a-amino acid derivatives to form their oxazol-5(4H)-ones were studied. The absorption maxima of the amino acid derivatives were found to be affected by the polarities of various solvents employed. Polar solvents shifted the $_{max}$ to longer wavelengths. The shifts have been attributed to either

n –> or transitions respectively.

The cyclisation rates of N-carbobenzoxy-L-a-amino acids studied were found to be influenced by the nature and size of the alkyl groups at the a-carbon as well as the substituent group at the nitrogen atom. The rate constants were affected by the size of the alkyl groups. The rate constants obtained from the linear plots of time versus logarithm of absorbances of each of the amino acids were used to correlate the linear free energy relationships using the Taft-equations. The steric and polar factor contributions were found to greatly influence the cyclisation rates.

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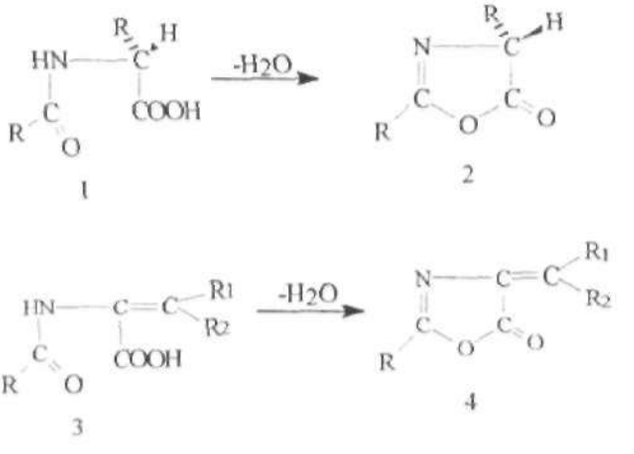
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CHAPTER ONE

1.0 INTRODUCTION

Oxazolones or azlactones may be considered as five membered heterocycles of α-acyl-amino acids (Roger *et al.*, 1946) which are conveniently classified into two groups, saturated and unsaturated (scheme I) based on their characteristic differences in properties.



1 2 3 4

Scheme I: R = CH_3O, C_6H_5O, $C_6H_5CH_2O$.

R^1 = R^2 = H, CH_3, CH_3CH_2, $(CH_3)_2CH$, $(CH_3)_2CHCH_2$, $C_6H_5CH_2$.

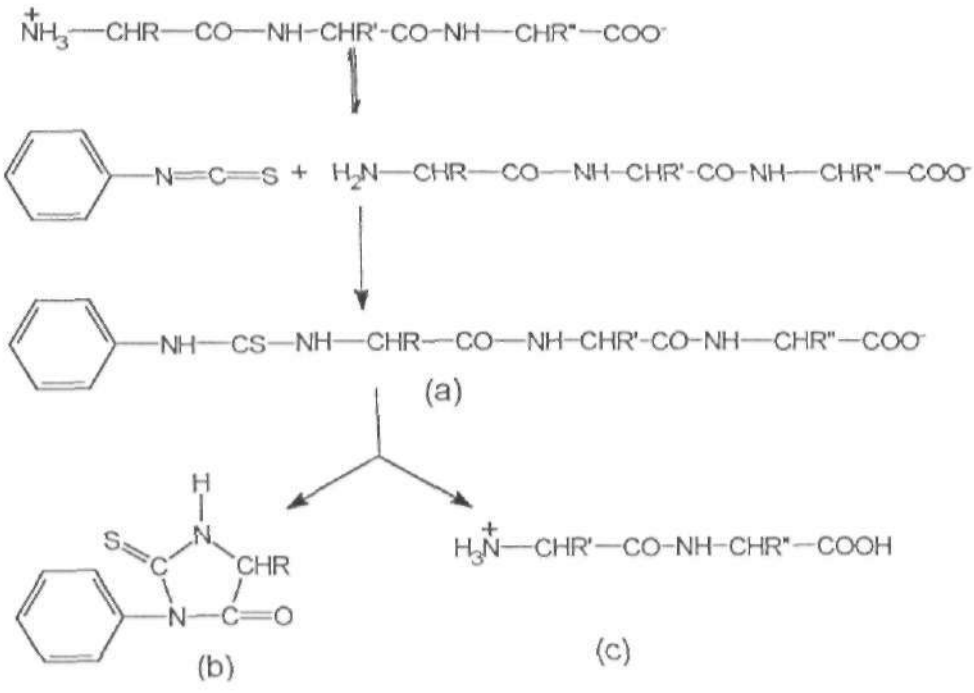
The first unsaturated oxazolone was prepared (Ploch, 1883) by the condensation of benzaldehyde with hippuric acid in the presence of ethanoic anhydride. However, the structure of the product was determined (Erlenmeyer, 1893) by extending the reaction to other alkanals (aromatic and

aliphatic). The structural determination led to the synthesis of α-keto and α-amino acids. The reaction of an alkanal with hippuric acid is usually referred to as the Erlenmeyer azlactone synthesis. Some workers (Mohr *et al.*, 1908 and 1910) prepared several compounds of saturated oxazolones by the action of ethanoic anhydride on α-acyl-amino acids. This reaction has been utilized in the synthesis of a variety of saturated oxazolones which have been used as intermediates in the synthesis of peptide (Bergmann and Grafe, 1930; Bergmann and Zervas, 1928).

1.1 THE EDMAN TYPE SEQUENCE ANALYSIS OF POLYPEPTIDES

The N-terminal residue of a polypeptide can be identified when a suitable substituent is attached on the α-amino group followed by the selective cleavage of the modified N-terminal residue. Repetition of such a method then provides a means for the stepwise degradation of a peptide and the progressive elucidation of its sequence. One of such procedures have been described in the literature (Thompson, 1960). However, the best known is due to Edman (1967) and involves the reaction between phenylisothiocyanate and the peptide at approximately pH 8. Reaction occurs with the unprotonated α-amino group and the solution is either buffered or kept at constant pH. The resultant N-phenylthiocarbamoyl derivative (a) is freed from

excess phenyl isothiocyanate by extraction with an organic solvent. Under suitable conditions of acid catalysis, it gives rise to the 3-phenyl-2-thiohydantion (b) corresponding to the N-terminal residue and the peptide (c) containing one residue less than the original.



Sequence analysis of polypeptides via N-thiobenzoyl derivatives (Hume, 1972) have been carried out. The work examined a variation of the Edman technique using a sequencing of peptides via their N-thiobenzoyl derivatives. The expected improvement on the Edman method in cleaving the N-terminal amino acids was realised in practice. Furthermore, a number of N-terminal analyses and the sequencing of a small peptide were performed which showed

good yields for the steps crucial to the determination of long sequences.

The length of any peptide sequence which can be determined by this technique depends upon the yield at each reaction step, this is illustrated by the following table below:

Yield of amino acid derivative after some steps of sequential degradation assuming certain yields at each step.

No. of Steps	Yield Per Step		
	99	95	90
10	90%	60%	35
50	60%	8%	0.5
100	36%	0.6%	0.003%
Amount of starting material σ	0.3μm	17μm	3500μm

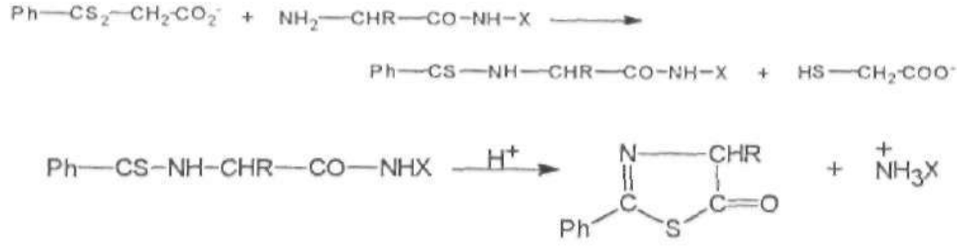
σ - for 100 degradations, if a minimum of 0.1μm of amino acid derivative is needed for identification.

Since very high yields are difficult to obtain in practice, it is often only possible to perform ten or

twenty steps using manual methods (Blomback *et al.*, 1966).

1.2 SEQUENCING VIA N-THIOACYL DERIVATIVES

This modification (Kenner and Khorana, 1952) of the Edman (1967) procedure was first reported by Barrett (1967).



The product is a 2-phenyl-(4H)-5-thiazolone, this may be identified directly or by thin layer chromatography after conversion to the thiobenzoyl amino acid anilide (TBA). Either way, the major difference is in the first step which is a thiobenzoylation involving two products, and a thiobenzoylating reagent which is water soluble.



The cleavage step is much more rapid than is the case with N-phenylthiocarbamoyl peptides where glycine as the N-

terminal residue on exposure to acid for three hours at 40°C (Tschenesche and Watchter, 1970) with thiobenzoyl glycyl peptides, takes place within twenty minutes at 25°C. This procedure works well for all amino acids including cystein, tryptophan, serine and threonine (Hume, 1972).

The results of this cleavage reaction supports the theoretically expected result that electron withdrawing substituents increase the ease of thiobenzoylation but decrease the ease of cleavage from the peptide. Conversely electron releasing substituents have the opposite effect. Such derivatives are useful in allowing milder cleavage condition.

1.3 AUTOMATION OF THE EDMAN METHOD OF SEQUENTIAL DEGRADATION

Edman and Begg (1967) described an automatic protein sequenator which performs only the coupling and cleavage steps collecting the 2-anilinothiazolones in a fraction collector for identification.

The basic principle of the instrument is that solutions of reactants and extracting solvents are spread in thin films inside a rotating cylindrical cup. Thus, the main processes of mixing, extraction, centrifugation and drying under vacuum are all carried out in the same reaction cup. The instrument is specifically suitable for long N-terminal

analyses of proteins. However, when used with shorter peptides, the loss of peptide on extraction becomes a problem. For this reason, complete degradation down to the C-terminal amino acid is rarely attempted. The overall yield per degradative cycle is 98% and the build up of products through very minor side reactions brings the sequenation to a halt.

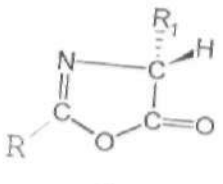
The starting material is dissolved in a volatile buffer solution [N,N,N',N'- tetrakis (2-hydroxypropyl) ethylene diammine (quadrolle)] in a cup and the normal reactants added in the correct order with extraction of excess reagents using various solvents. The spinning cup causes the extracting solvents to climb to the top of the cup where they are fed to the waste. Two dissolutions in hepta-fluorolatyric acid are used in order to overcome the problem of over-lapping derivatives. Less volatile buffers and reagents have to be used in order to reduce losses by evaporation. The spinning cup is covered by a bell jar and all the reactions are carried out in a nitrogen atmosphere. The protein often tends to form a glassy coating on the surface of the cup and desolution becomes difficult. The sequenation does not appear to be hindered in such cases as the protein continues to react even though it is in the solid phase.

A non-commercial instruments (Lynn and Bennett, 1972; Waterfield *et al.*, 1970) have been described which is based on the original design of Edman but incorporating some improvements in methods and materials. Edman reports the sequenation of 60 residues from the N-terminus of apomyoglobin D of the humpback whale, whilst others (Lynn, 1972; Niall and Edman, 1967) have found that only 25-30 steps are possible.

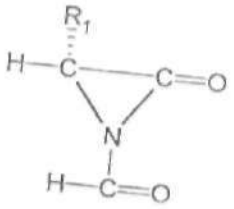
The Edman modified sequence analysis of polypeptides may also be used with N-carbobenzoxy - L α- amino acid derivatives just as N-thiobenzoyl-derivatives.

1.4 STRUCTURE OF OXAZOLONE

Several different structures have been suggested for the Oxazolones. Of these, only two (5 and 6) have received serious consideration.



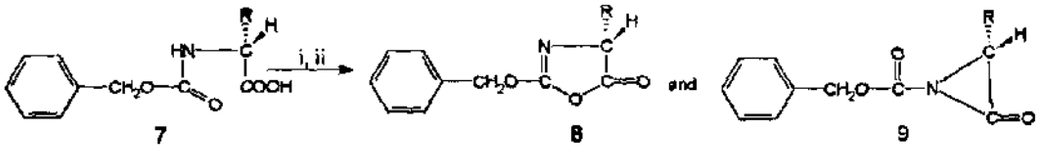
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6

The three-membered ring structure (6), called lactimide was initially proposed by Rebuffat, (1889) as the structure of Oxazolone and was accepted by Erlenmeyer, (1893). In 1900,

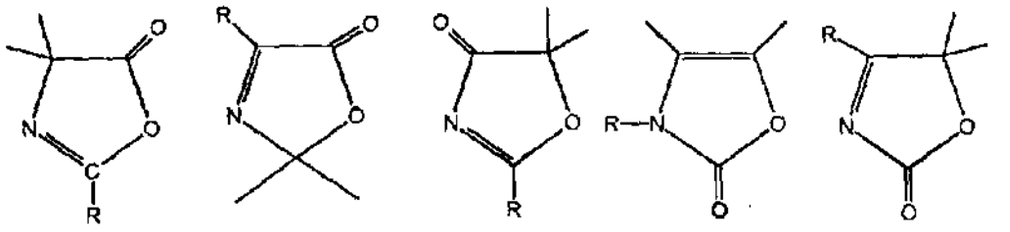
the three-membered ring structure was abandoned in favour of the five-membered ring (5). Although structure (5) has been accepted generally, the main argument in favour of (5) is from the observation that cyclodehydration of benzyloxy carbonyl-L-amino acid (7) does not give an alkoxy carbonyl aziridinone (9) as claimed by Miyoshi, (1970), scheme II. The product is in fact 2-benzyloxy-4-alkyloxazol-5(4H) one (8), (Jones and Witty, 1977).



Scheme II: Reagents: i, $COCl_2$, $SOCl_2$ or $POCl_3$ in Tetrahydrofuran at - 20°C.

ii, Et3N; R = alkyl groups.

There are five different structures that may be written for the Oxazolones based on the positions of the carbonyl (C=O) function and alkyl groups in the ring, namely:

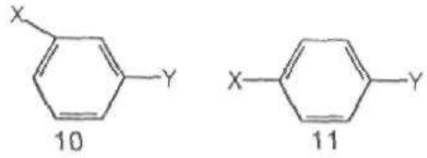


The 5(2H)-oxazolones are frequently referred to as pseudooxazolones, and there has been an increasing interest in the behaviour of these compounds (Filler and RaO, 1977). A number of studies of the 2(3H)-system previously in doubt, now seem firmly established. The 2 (5H)-oxazolones, however, remain unknown (Katritzky, 1965). The 5 (4H)-oxazolones are the familiar azlactones and it is with this type that most studies have been conducted.

1.5 LINEAR FREE-ENERGY RELATIONSHIP (LFER)

The determination of the electronic influence that substituents exert on the rate and course of a reaction has challenged chemists for years. One of the difficulties involved in determining electronic substituent effects is that if the substituent is located close to the reaction centre,polar as well as steric effect may affect the reaction so that electronic effects are masked. However, if the substituents are placed far away in order to avoid steric problem, the electronic effects will be severely attenuated.

The electronic influence of a substituent say X, can be assessed (Hammett, 1937) by studying reaction in a side chain at Y in benzene derivatives (10 and 11).



10 11

The substituent X is separated physically from the reaction site but its electron withdrawing influence is transmitted through the relatively polarisable π - electron system (March, 1992).

CHAPTER TWO

2.0 LITERATURE REVIEW

Most of the studies on the Chemistry of oxazolones up to 1954 stemmed from either interest in the role of oxazolones as precursors of α-amino acids and peptides or the monumental studies on penicillin (Clarke *et al*, 1949), which for a time, was thought to possess an oxazolone ring, rather than the correct β-lactam moiety. Since about 1955, a considerable number of new developments of wide synthetic, structural and mechanistic interest of oxazolones have been described. The significant features of these new advances are discussed.

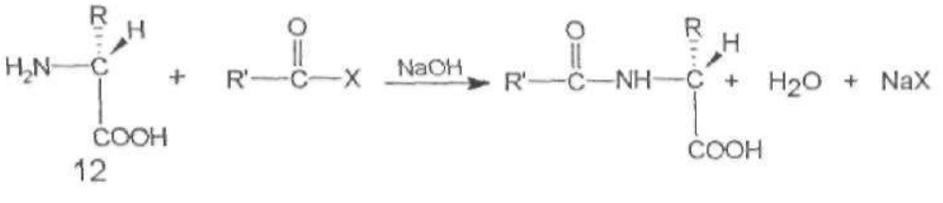
Oxazolones have been of interest mainly as intermediates in the synthesis of other compounds. Saturated oxazolones are obtained most often from the corresponding amino acids and are used in preparing derivatives of those amino acids. Unsaturated oxazolones, on the other hand, usually are prepared by condensing aldehyde with an acyl glycine and are used in the synthesis of the corresponding amino and keto acids. The synthesis of oxazolones can be achieved in two main stages, namely, acylation of α-amino acids to give the corresponding N-acyl-α-amino acids and the subsequent

cyclisation of the N-acyl-α-amino acids to their oxazolones.

2.1 ACYLATION OF α-AMINO ACIDS

Amino acids are bifunctional molecules carrying an amino group -NH2 which is alpha (α) to the carboxylic acid group (-COOH. The amino group may be protected using alkoxyhalides, acidhalides or carbobenzoxyhalides to form N-acyl - α-amino acids. The reaction is by the Schotten-Bauman type (Morrison and Boyd, 1987) in which the α-amino acid is suspended in an excess of 10% aqueous sodium hydroxide solution and a small excess of the acylating agent is added and the mixture vigorously shaken.

The reaction is outlined below:



12

R = alkyl, phenyl or benzyl

R' = alkoxy, benzoyl, benzyloxy

X = Cl, Br, I.

Acylation proceeds well under these conditions to produce the N-acyl α-amino acids. Yields in excess of 80% of theory are generally obtained.

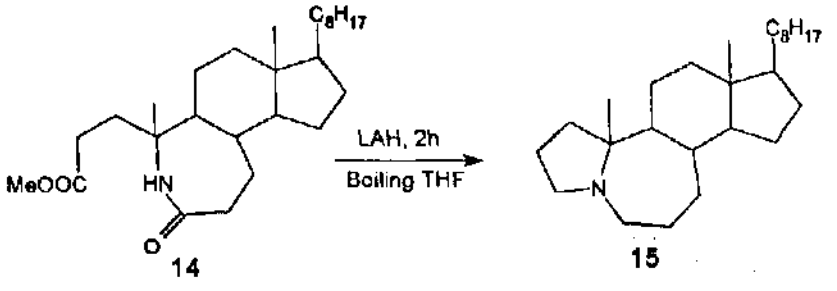
The extensive use of N-benzoyl - α - amino acids for characterisation purposes as well as starting materials in the synthesis of benzylated peptides employed in enzyme specificity studies may be attributed to the facility with which these compounds can be prepared, crystallized and purified (Goodman and Levine, 1964). The preparation of many N-acyl -α-amino acids via Schotten - Bauman type reaction have been achived by many workers (Carter and Stevens, 1940; Williams and Young, 1963; Carter *et al*, 1941).

2.2 GENERAL CYCLIZATION REACTIONS

There are various synthetic routes available for the cyclisation reactions of organic molecules to form products. Each method is based on factors such as good yield, relative ease of cyclisation and moderate reaction conditions. Some cyclisation reactions are outlined below:

2.2.1 REDUCTIVE N-CYCLISATION OF LACTAMOESTERS:

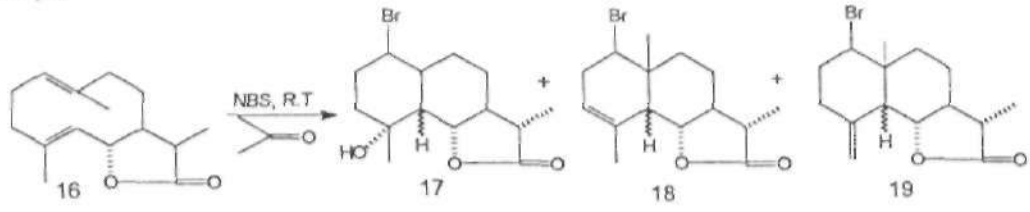
N-lactamoesters have been cyclised by lithium aluminium hydride reduction in boiling tetrahydrofuran (Rodewald and Morzycki, 1978) affording a single product, (15).



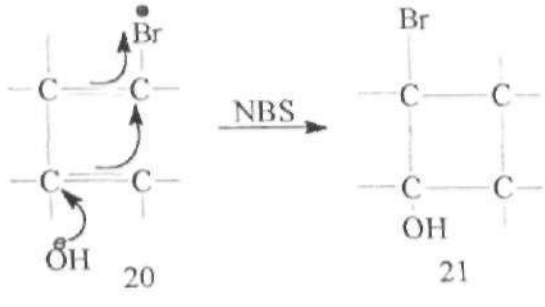
14 15

2.2.2 BROMINATIVE CYCLISATION OF DIHYDROCOSTUNOLIDE:

Treatment of dihydrocostunolide with NBS in aqueous acetone at room temperature furnished bromolactones (Tikan and Calvin, 1979).



It has been demonstrated that the course of carbocyclisation largely depends, among other factors, on the steric and conformational characteristics of the olefinic substrate which are controlled by the polarity of the solvent employed. If in a given substrate two olefinic systems are oriented in an overlapping manner, then the oxidative attack of NBS with concurrent cyclisation would yield the bromohydrin (scheme III).



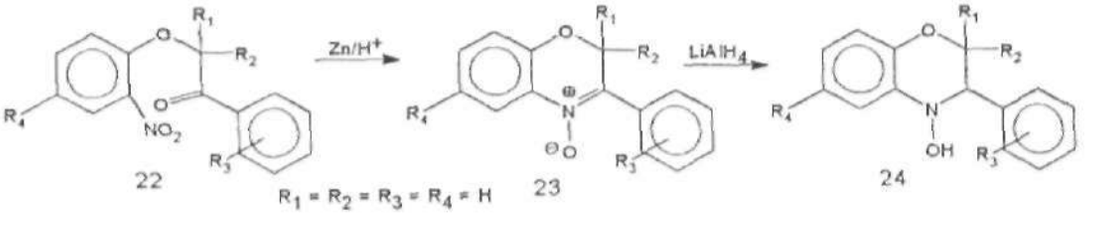
Scheme III:

The significant features of this reaction are formation

of a new C-C bond and the introduction of two functional groups in a stereospecific manner.

2.2.3. REDUCTIVE CYCLISATION OF *O*-NITROPHENOXYACETOPHONES:

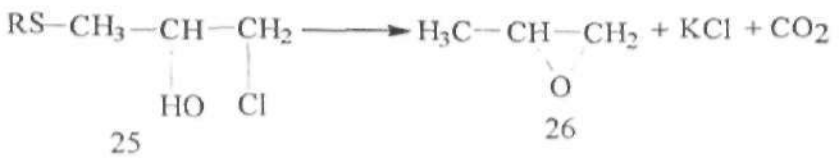
The reductive cyclisation of *O*-nitro-phenoxy acetophenones using ammonium chloride and zinc dust led to 3-phenyl-2H-1,4-benzoxazine-4-oxides with yields varying from 20 - 80%. The reaction follow a general pathway and intermediate mixtures of hydroxylamine and of another compound which proved to be a hydrated nitrone, were observed. These mixtures, when heated up to 50°C were completely transformed into compounds (24) characterised by ^{1}HNMR, elemental analysis and mass spectra (Paolo *et al*, 1979).



2.2.4. ASSYMETRIC CYCLISATION OF SOME CHLOROHYDRINS CATALYSED BY OPTICALLY ACTIVE COBALT (SALEN) TYPE COMPLEXES

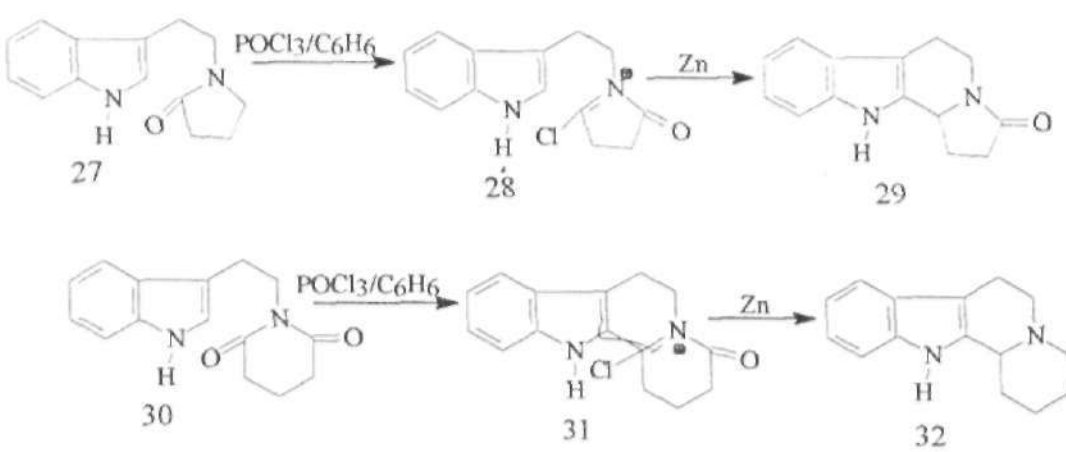
Optically active chloromethyloxirane can be obtained from 1,3-dichloro-2-propanol by a process of asymmetric synthesis. The highest enantiometric excess (e.e.) of

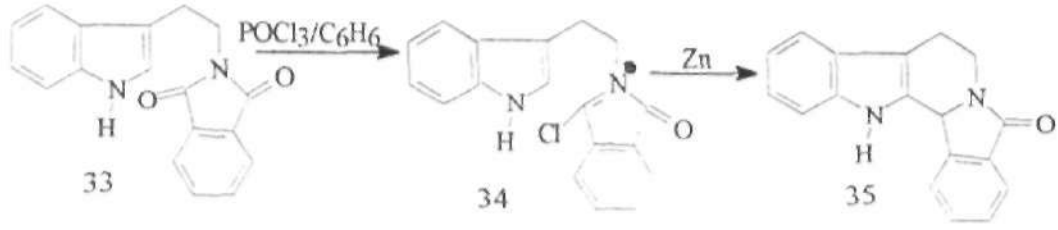
chloromethyloxirane that could be obtained is 67% using Co(II) (3,5 -Cl, Cl-sal.) (S-CH x DA) and K_2CO_3 as the catalyst and base respectively (Tsutomu *et al*, 1980).



2.2.5 REDUCTIVE CYCLISATION OF INDOLIC IMIDES – A NEW β-CARBOLINE SYNTHESIS

N-succinimidotryptamine (27) afforded (the corresponding complex (28) on refluxing with $POCl_3/C_6H_6$ for 1hr) β-carboline lactam (29) in 50% yields. N-glutarimido tryptamine (30), also afforded the corresponding cyclised lactam (32) in 60% yields. N-phthalimido tryptamine (33) on identical treatment with $POCl_3/C_6H_6$ followed by Zinc dust afforded the cyclised lactam (35) in 90% yields (Atta-Ur-Rahman and Waheed, 1977).

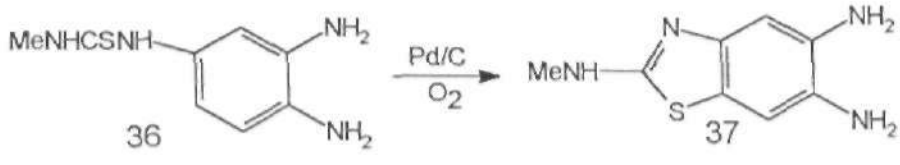




The reaction proceeds via uncyclised chloroimmonium intermediates 28, 31 and 34 rather than cyclised enamides. This is demonstrated by aqueous treatment of aliquots prior to Zinc dust treatment when the starting imides are recovered quantitatively and no cyclised enamides are detected.

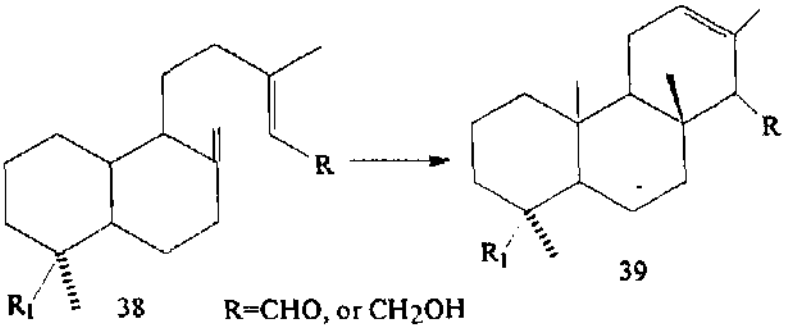
2.2.6. INTRAMOLECULAR OXIDATIVE CYCLISATION ON ORTHO-PHENYLENEDIAMINES:

A novel intramolecular oxidative cyclisation of 3,4-diaminophenylthiourea (36) to the 5,6-diaminobenzthiazole (37) is reported. This conversion can be brought about by Pd/C in the presence of atmospheric oxygen in acid solution (Rajappa and Sreenivasan, 1980).



2.2.7 ACID-CATALYSED CYCLISATION OF LABDANE SERIES II:

Influence of carbonyl group at position 15: Cyclisation in formic acid of 4β-carbomethoxy labda-8-(17) 13-diene leads to aldehyde of the pimaric and isopimaric series in contrast with known behaviour of the related methylester (dimethylagathate). Similar results have been found for the cyclisation of the corresponding methylketone (Fetizon and Ragoussis, 1978).



2.2.8. CYCLISATION OF N-ACYL-α-AMINO ACIDS TO FORM OXAZOLONES.

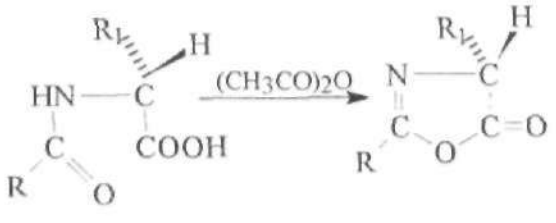
Several improved methods for the preparation of known oxazolones as well as some interesting new compounds of this type have been reported (Crawford and Little, 1959). Aromatic aldehydes react rapidly with hippuric acid in the presence of a sulphur trioxide-dimethylformamide complex (Baltazzi and Davis, 1962) to form oxazolones in 60-98% yield. By this

method, oxazolones containing free aromatic hydroxyl groups may be prepared from phenolic aldehydes, such as salicyaldehyde and vanillin. Previously, only the acetoxy compounds could be isolated, although, these may be deacetylated to the hydroxy analogs with cold concentrated sulphuric acid (Gupta, 1960).

While the classical Erlenmeyer synthesis of oxazolones (Condensation of aldehydes with hippuric or aceturic acid in the presence of ethanoic anhydride and sodium acetate) continues to be the most commonly used method of preparation, several recent variations deserve mention.

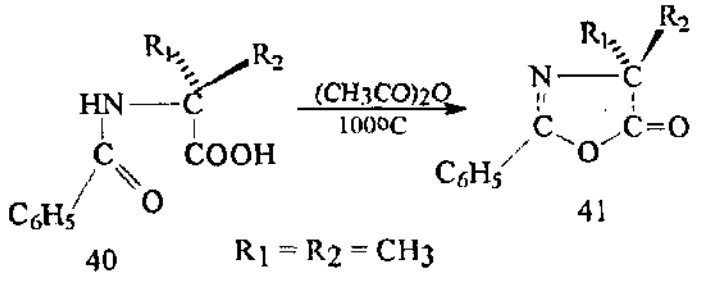
2.2.9 REACTION OF N-ACYL-α-AMINO ACID WITH ETHANOIC ANHYDRIDE

Reaction of N-acyl-α-amino acid with ethanoic anhydride, either alone or in the presence of ethanoic acid as solvent (Carter and Stevens, 1940).



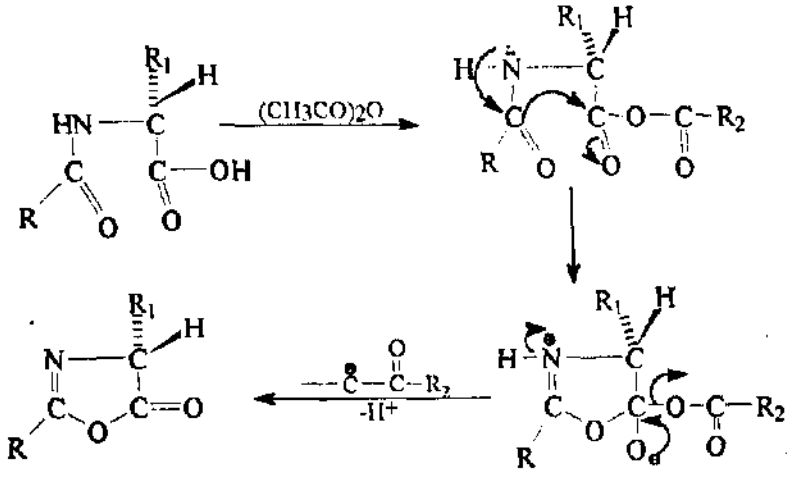
2.3.0 HEATING OF N-ACYL-α-AMINO ACIDS WITH ETHANOIC ANHYDRIDE

Many oxazolone derivatives have been prepared by heating N-acyl-α-amino acids in the presence of ethanoic anhydride to yield oxazolones (Mohr and Geis, 1908).



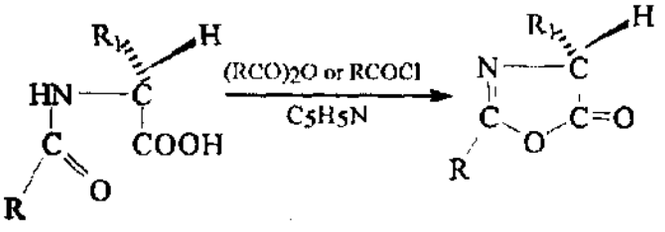
2.3.1 ELECTRONIC CHANGES INVOLVED IN THE FORMATION OF OXAZOLONES

The series of electronic changes involved in the formation of oxazolones have been formulated by Baker, Ollis, and Poole (Baker *et al*, 1950) using ethanoic - anhydride.



2.3.2 ACTION OF AN ACID ANHYDRIDE/ACID CHLORIDE ON N-ACYL-α-AMINO ACID

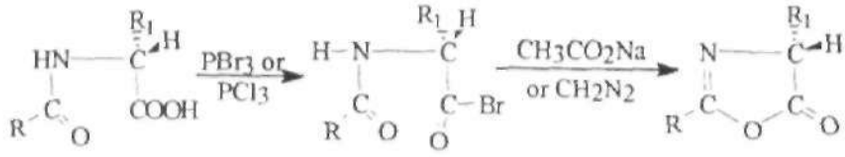
Action of an acid anhydride or acid chloride on N-acyl-α-amino acid in pyridine as the solvent (Carter *et al*, 1941).



N-benzoyl-L-α-alamine and N-benzoyl-L-α-phenyalanine have been cyclised by the use of ethanoic-anhydride in the presence of a tertiary anine or pyridine (Baltazzi, 1958; Carter *et al*, 1941).

2.3.3 REACTION OF N-ACYL-α-AMINO ACID WITH PHOSPHORUS TRIBROMIDE/PHOSPHORUS TRICHLORIDE

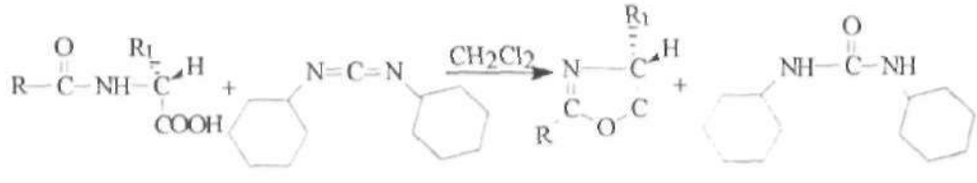
Reaction of an N-acyl-α-amino acid with phosphorous tribromide or phosphorus trichloride in the presence of diazomethane or sodium ethanoate in an organic solvent such as pyridine.



Cyclisation of N-benzoylglycine and N-benzoyl-L-α-phenylalanine to the corresponding 3-phenyloxazolin -5- one (R^1 = H, R = C_6H_5) and 2 - phenyl - 4 - benzyloxazo-5-one (R^1 = CH_2CH_5, R = C_6H_5) respectively have been reported (Karier and Widmer, 1925; Karier and Bussman, 1941).

2.3.4 REACTION OF AN N-ACYL-α-AMINO ACID WITH DICYCLOHEXYLCARBODIMIDE

Reaction of an N-acyl-α-amino acid with dicyclohexyl carbodiimide in an organic solvent such as dry dichloromethane or dry ethoxy-ethane (Goodman and McGahren, 1967).



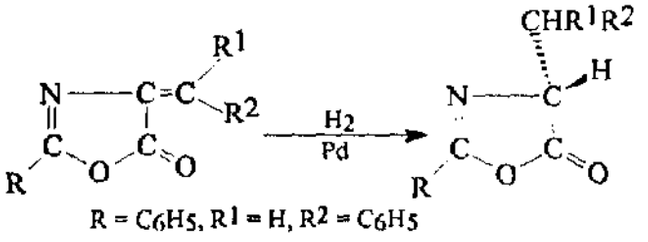
$R=C_6H_5$, $R^1=CH_3$

Goodman and McGahren in 1967, were able to obtain two peptide oxazol-5 (4H)-ones, 2-(1^1 benzyloxy carbonyl-amino - 1^1-methyl) ethyl-L-4-methyloxazol-5(4H)-one ($R^1=CH_3$) and 2-(1^1-benzyloxycarbonylamino-1^1-methyl)ethyl-L-4-benzyloxazol-5

(4H)-one ($R^1 = CH_2C_6H_5$) from the corresponding free N-acyl-α-amino acid using dicyclohexylcarbodiimide in dry ethoxyethane.

2.3.5. CATALYTIC REDUCTION OF UNSATURATED OXAZOLONES USING PALLADIUM AS A CATALYST

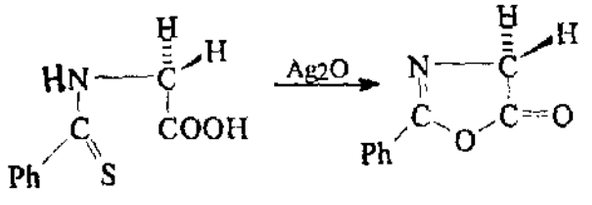
Catalytic reduction of the corresponding unsaturated oxazolones using palladium as a catalyst to afford saturated compounds.



Hydrogenation of 2-phenyl-4-benzylidene oxazol-5(4H)-one over Palladized charcoal to give the corresponding 2-phenyl-4-benzyloxazol-5(4H)-one has been reported (Cornforth, 1949).

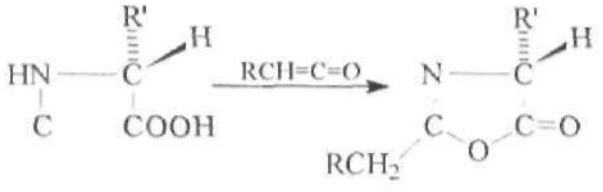
2.3.6 ACTION OF SILVER SALT OR OXIDE ON N-PHENYLTHIOACETYLOR N-THIOBENZOYL DERIVATIVES OF α-AMINO ACIDS

Action of silver salt or oxide on N-phenylthioacetyl or N-thiobenzoyl derivatives of α-amino acids, give oxazolone.



2.3.7 ACTION OF KETENE ON AN AQUEOUS SOLUTION OF α-AMINO ACIDS

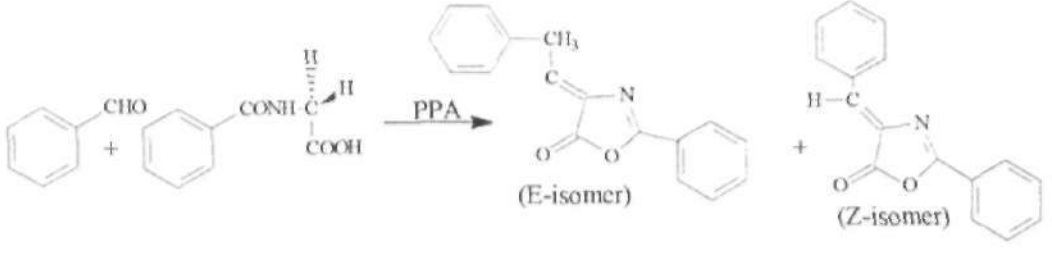
Action of ketene on an aqueous solution of an α-amino acids (Berger and Wolff, 1951).



N-acetyl-L-α-leucine (R = H, R^1 = CH_2CH (CH_3)) have been cyclised to the corresponding oxazol-5-(4H)-one by this method.

2.3.8 USE OF POLYPHOSPHORIC ACID:

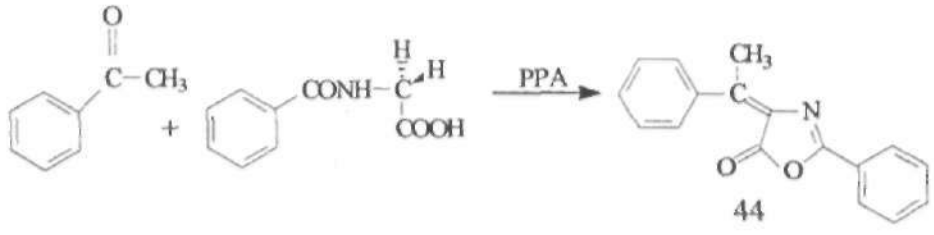
Rao (1975) has reported that aromatic aldehydes condense



with hippuric acid in polyphosphoric acid to give the generally inaccessible lower melting geometric isomers of 4-aryl-methylene-2-phenyl-2-oxazolin 5-ones.

The Erlenmeyer method and its minor variations, such as use of 4-chloromethylene or 4-ethoxy-methylene oxazolones,

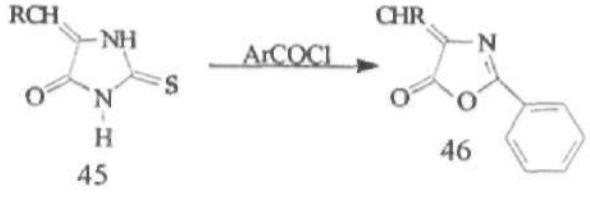
always afford the geometric isomers (Filler, 1965). A major advantage of this modification is that hydroxybenzylidene oxazolones can be prepared, and the acetoxy compound is obtained when ethanoic anhydride is used. Moreover, acetophenone and its ring substituted analogs react in the PPA system to give the hitherto unknown α-arythyllidene oxazolones (44).



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2.3.9 SYNTHESIS OF OXAZOLONE DERIVATIVES FROM OTHER HETEROCYCLES.

Heating 5-aryllidene-2-thiohydantoins (45) with a 2-4-fold excess of aroylchlorides in pyridine (Zubenko, 1968) gave 2-oxazolin-5-one derivatives.



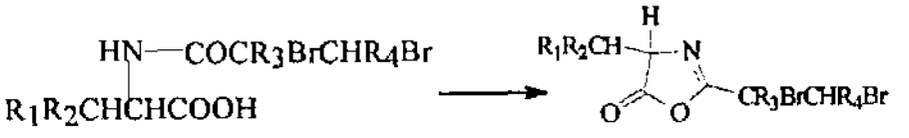
45 46

The advantage of this method is that it provides a convenient route to oxazolones bearing a substituted phenyl

group in the 2-position. Yields are high in the range of 79-93%.

2.4.0 APPLICATION OF BERGMANN'S REACTION TO THE SYNTHESIS OF OXAZOLONES.

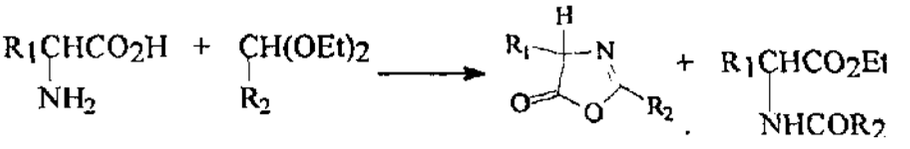
Bergmann's procedure has been utilised (Riodan and Sammer, 1971) for the synthesis of a series of oxazolones. Amino acids, such as phenyl alanine, tyrosine, valine, leucine and isoleucine were converted to N-(α-methylcinnamoyl) derivatives. Azlactonization followed by oxidation using pyridine hydrobromide perbromide gave the corresponding unsaturated oxazolone having the Z-configuration around the newly formed double bond in the 4-position.



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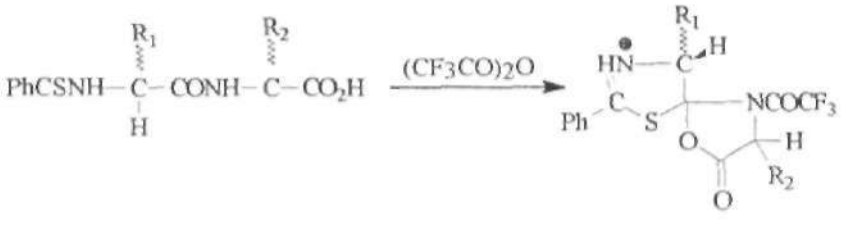
2.4.1. REACTIONS OF NEUTRAL AMINO ACIDS WITH ORTHOACETATE ESTERS

Neutral amino acids such as alanine, valine, isovaline and methionine react with orthoacetate esters in dimethyl acetamide to give mixtures of N-acetamido acid esters and saturated oxazolones (Ragozhin *et al*, 1971).



2.4.2. REACTIONS OF N-THIOBENZOYL-α-AMINO ACIDS WITH TRIFLUOROETHANOIC ANHYDRIDE

Reactions of N-thiobenzoyl -α-amino acids and related N-substituted thiobenzamides with trifluoroethanoic anhydride: Trifluoroethanoic anhydride has been used for the cyclisation of N-benzoyl-α-amino acid amides to 5-trifluoroacetamido-oxazolones (Barrett, 1978) and has been claimed to be effective for the cyclisation with concomitant yield.



2.5 SPECTROSCOPIC METHODS

Spectroscopic methods provide a rapid and precise way of determining organic structural formulae and in contrast to the more classical chemical methods, only milligram quantities of compound are required as compared with other methods. Spectroscopic methods, such as nuclear magnetic resonance, infrared, ultraviolet and mass spectroscopy have become invaluable in a whole range of applications extending from compound identification or purity determination to such vastly more difficult problems as the structure elucidation of

complex natural products. (Scheinmann, 1970).

The spectroscopic methods employed in this study included ultraviolet, infrared and nuclear magnetic resonance spectroscopy.

2.5.1 ULTRAVIOLET SPECTROSCOPY

The absorption of ultraviolet radiation by an organic molecule leads to transitions among the electronic energy levels of the molecule. This transition leads to the promotion of electron in the bonding sigma (σ) orbitals, bonding Pi(π) orbitals and the non- bonding (n) electrons usually in the ground states to the anti-bonding sigma star (σ^*) and pi star (π^*) orbitals in the excited states. The non-bonding (n) electrons have no anti-bonding orbitals associated with them. Ultraviolet absorption promotes the electronic transitions $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$. The energy difference between the ground and excited states is given by DE. The DE values for transitions are in the order: $n \rightarrow \pi^* < \pi \rightarrow \pi^* < n \rightarrow \sigma^* << \sigma \rightarrow \sigma^*$.

The energy required for the $\sigma \rightarrow \sigma^*$ transition is very large, consequently, compound such as saturated hydrocarbons with no n electrons and only σ bonds do not absorb near-ultraviolet radiation (Pecksok *et al*, 1976).

Absorption of organic molecules are normally recorded in solution for quantitative studies. Beer's and Lamberts' laws are two early empirical laws which govern the absorption of light by molecules. Beer's law relates the absorption to the concentration of absorbing solute and Lambert's law relates the total absorption to the optical path length. They are most conveniently used as the combined Beer - Lambert law written as:

$$\text{Log } I_o/I = \varepsilon cl \text{ or } \varepsilon = A/cl$$

where I_o is the intensity of the incident light (or the light intensity passing through a reference cell).

I is the light transmitted through the sample solution.

Log I_o/I is the absorbance (A) of the solution, formerly called the optical density, O.D.

C is the concentration of solute (in $moldm^{-3}$)

l is the path length of the sample (in cm).

ε is the molar absorptivity (formally called the molecular extinction coefficient).

The molar absorptivity, ε is constant for a particular compound a given wavelength, and is most commonly expressed as ε_{max} (the molar absorptivity at an absorption band maxima). Since the values of ε_{max} can be very large, an alternative convention is to quote its logarithm (to the base 10), Log_{10} ε_{max}. which is defined as:

$$Log10\,\varepsilon = \frac{M \times A}{C \times l}$$

where A = absorbance, C = concentration (in g/litre), M = Molar Mass, and l = path length.

An absorption spectrum is obtained when $\log_{10}e$ is plotted against wavelength. In this case, lmax. (the maximum wavelength of absorption) can be measured.

2.5.2 SOLVENT EFFECT ON ULTRAVIOLET ABSORPTION SPECTRA

The role played by solvents in understanding organic reactions is very important. Solvent effect on the electronic absorption spectra of some organic compounds have been studied (Lyman *et al*, 1976; Martins and Ojokuku,1982). Solvents to be used are usually of highest purity grade and transparent over the desired range of wave lengths. The solvents used may be of low or high polarity. Non-polar solvents such as hexane gives fine structures while polar solvents gives abscured structures of the compound.

Frank-Condon principle can be used to explain solvent effects on $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. This principle states that during electronic transition, atoms do not move. Electrons, however, including those of the solvent molecules,

may reorganise. Most transitions result in an excited state and dipole-dipole interactions with solvent molecules which lower the energy of the excited state more than that of the ground state. Thus, it is usually observed that polar solution give longer wavelength maxima than do non-polar solutions. In other words, there is a small red-shift of the order of 10 to 20nm in going from non-polar solvent to more polar solvent. The $n \rightarrow \pi^*$ transitions show a solvent effect in the opposite direction (blue-shift) which could be due to the lesser extent to which solvents can form hydrogen bond with carbonyl groups in the excited state. The $n \rightarrow \pi^*$ absorption band is normally superimposed upon the end absorption of a much stronger band with its peak in the vacuum ultraviolet. This absorption band has been studied (Nagakura *et al*, 1964) and peaks found in the range 155 -165nm, with molar absorptivity from 2500 to 4200, in the spectra of the vapours of formic and acetic-acids and of ethyl acetate. Theoretical consideration led these authors to the conclusion that this band is of mixed character, involving intramolecular charge transfer from the singly bonded oxygen to the carbonyl group, combined with a smaller contribution from the transition of the carbonyl. When a long-wavelength band is moved to a shorter wavelength in changing the solvent from non-polar to a polar solvent a hypsochromic or blue-shift is said to have occurred. This shift has been

attributed to n → π* transition (McConnell, 1952). The n → π* transition (blue-shift) was first explained by McConnell on the basis of the general solvation hypothesis. The blue-shift band was attributed to hydrogen bond formed between the solute and the solvent molecules (Brealey and Kasha, 1952). The ultraviolet spectra of aliphatic carboxylic acids, ethyl esters and alkyl acetates have been determined for solution in hexane, acetonitrile and water (Lyman *et al*, 1976). They found the carbonyl n → π* transition for these compounds in the vicinity of 206nm under hydrogen bonding conditions, and around 212nm in the absence of hydrogen bonding. Also, electronic absorption spectra of N-alkoxythiocarbonyl-L-α-amino acids and a number of N-benzoyl-α-amino and amino acids have been studied (Martins and Yassin, 1979; Martins and Ojokuku, 1981) in various solvents. They found that each N-benzoyl-α-amino and imino acids displayed a single absorption peak with transitions observed between 195-250 nm (logε = 3.86-4.32). This was attributed to the π → π* transitions which is due to the conjugation of the carbonyl group with the benzene ring in the compounds. However, the low intensity bands in the region ≈ 280 nm (Logε = 2.15 - 2 - 2.36) was assigned n → π* transitions of the carbonyl functional group. The N-alkoxythiocarbonyl amino acids were interpreted on the

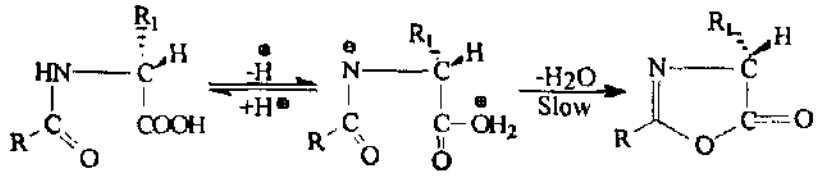
basis of solvational and conformational equilibria.

2.6 THIN LAYER CHROMATOGRAPHY

Chromatography includes simple separation techniques which is applied to processes involving distribution of a sample between a stationary phase and a mobile one. Purity is of paramount importance in identifying compounds through comparison of their properties with those of known substances. The experimental procedure involved in thin layer chromatography is described under the experimental section.

2.7 KINETICS OF CYCLISATION OF N-CARBOBENZOXY-L-α-AMINO ACIDS UNDER THE INFLUENCE OF ETHANOIC ANHYDRIDe

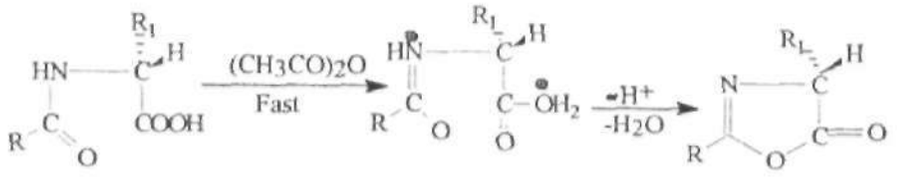
N-carbobenzoxy-L-α-amino acid derivatives can be cyclised to 2-benzyloxy-4-alkyloxazol-5(4H)-ones using ethanoic anhydride as cyclising agent. The mechanism of cyclisation of the amino acid to form oxazolones probably goes in the same manner as proposed by Chien and Kemp, (1967). They showed the existence of equilibrated amide anions as the reactive intermediates leading to the formation of oxazolone.



Scheme IV: R = $C_6H_5CH_2O$

R^1 = $PhCH_2$, CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$

It is also possible that the ethanoic anhydride may react as shown in the scheme below:



Scheme V:

In both cases, (Schemes IV & V) there is the removal of water molecule by the ethanoic anhydride. The two mechanisms shown therefore are possible for the cyclisation of N-carbobenzoxy-L-_-amino acids to form oxazolones. According to Chien and Kemp, the overall reaction is first order (Chien and Kemp, 1967).

Kinetic investigation involving N-alkoxythiocarbonyl-L-α-amino acids and N-acyl-α-amino acids in ethanoic anhydride as cyclising agent have earlier been reported by some workers (Martins and Yassin, 1979); (Martins and Ojokuku, 1981, 1982). They found that the rate of cyclisation largely depends among other factors, on the steric and the characteristic nature of the alkyl group at the 4-carbon position of the oxazolone. Ethanoic anhydride is a potent cyclising agent in

the preparation of oxazolones from α-acyl-amino acids (Roger *et al*,1946). Trifluoroethanoic anhydride has also been used as cyclising agent in the synthesis of 5-(2'-benzamido-acyloxy)thiazoles (Barrett, 1978) with N-thiobenzoyl-α-amino acids and related N-substituted thiobenzamides.

The present work constitutes an investigation on the kinetics of cyclisation of N-carbobenzoxy-L-α-amino acid derivatives to their corresponding oxazolones and the effect of alkyl groups on the cyclization rate.

2.7.1 DERIVATION OF FIRST - ORDER REACTION

The mathematical expression for first-order reaction can be obtained if a first order reaction is represented by:

$$A \rightarrow \text{Products.}$$

The rate of this reaction is directly proportional to the concentration of A. If C is the concentration of the reactant A at any time t, the rate of disappearance of A can be expressed mathematically as:

$$\frac{-dC}{dt} = k_1 C \qquad \ldots\ldots(i)$$

where k_1 is proportionality constant called the specific reaction rate or specific velocity constant for the first order rate equation. If C=1, k_1 becomes equal to the reaction

rate. The specific rate constant for a first order reaction is a constant characteristic of the reaction and varies with temperature, but is independent of concentration. Since *dc/dt* and C have the units of concentrations per time and concentration respectively, therefore, k_1 has the units of reciprocal time, (t^{-1}). If time is given in seconds or minutes, the units of k_1 are sec^{-1} or min^{-1} as the case may be. Equation (i) can also be written in another alternate and convenient form. Let a mol/litre be the initial concentration of A. After time t, let x mol per litre of A have decomposed into the products. Therefore, the remaining concentration of A at time t = (a - x) mol/litre.

Putting (a-x) for C into equation (i):

$$\frac{-d(a-x)}{dt} = k_1(a-x) \quad \text{.......} (ii)$$

Equation (ii) becomes:

$$\frac{dx}{dt} = k_1(a-x)$$

after differentiation.

$$\therefore \frac{dx}{dt} = k_1(a-x) \quad \text{.......} (iii)$$

This equation gives the rate of first order reaction in terms of initial concentration of the reactant and concentration of the reactant decomposed. Separating the variables and

integrating this equation between the limits x = 0 at t = 0 and x = x at time t; gives:

$$\ln \frac{a}{a-x} = k_1 t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (iv)$$

Equation (iv) is known as the kinetic equation for a reaction of the first order. This equation can also be written in the following alternate forms:

$$k_1 = \frac{2.303}{t} \log \frac{a}{a-x}$$

or

$$k_1 t = 2.303 \log \frac{a}{a-x} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (v)$$

This equation (v) can be verified by any of the following methods: Integration, Graphical and or fractional life methods. In each case, the value of k_1 can be determined.

2.7.2 APPLICATION OF THE RATE EQUATION TO CYCLISATION REACTION

The kinetics of cyclisation of N-carbobenzozy-L-α-amino acids can be followed by the ultraviolet method at a fixed wavelength which corresponds to the maximum absorbance value obtained during the cylisation reaction.

As the cyclisation reaction progressed, the position of maximum absorbance gradually increased or decreased as the case may be until a stationary value is obtained (Frost and Pearson, 1962). Let A_o and A_∞ be the absorbances at the start (t = 0) and when the reaction is complete respectively and At is the absorbance at any time t. Then $(A_o - A_\infty)$ is proportional to the initial amount of N-carbobenzoxy-L-α-amino acids and $(A_t - A_o)$ is proportional to the amount of N-carbobenzoxy-L-α-amino acids left at time t. Therefore, rate equation becomes:

$$k_1 = \frac{2.303}{t} \log A_o - A_\infty / A_t - A_\infty \quad \ldots\ldots(vi)$$

or

$$t = \frac{2.303}{k_1} \log A_\infty - A_o / A_\infty - A_o \quad \ldots\ldots(vii)$$

also,

$$k_1 t = 2.303 \log A_\infty - A_o / A_\infty - A_t$$

A plot of log $A_\infty - A_o/A_\infty - A_t$ versus time (t) is linear, the slope is equal to k/2.303, this enables the calculation of the rate constant for the forward reaction, (k_1 forward).

2.8 APPLICATION OF LINEAR FREE-ENERGY RELATIONSHIP (LFER)

It has been demonstrated (Hammett, 1940 and Taft, 1956) that the rates of reaction of individual members of a series

of related reagents with certain compounds are a function of polar and steric parameters of the reagents. The first attempt to give numerical values was that of Hammett. He set up the equation below:

$$\text{Log } k/k_o = \sigma\rho \quad \text{--------} \quad \text{(viii)}$$

where k_o is the rate constant or equilibrium constant, k is the constant for substituted compound, k_o is the constant for the unsubstituted compound. σ, is a constant characteristics of the substituent and ρ is a constant for a given reaction under a given set of conditions.

2.8.1 APPLICATION OF TAFT EQUATION TO PRESENT STUDY

Taft extended linear free-energy relationship to aliphatic series and found that the rate or equilibrium constants for a variety of reactions of aliphatic compounds conform to the equation:

$$\log k/k_o = \sigma^*\rho^* \quad \text{----------} \quad \text{(ix)}$$

or $$\log K/K_o = \sigma^*\rho^* \quad \text{---------} \quad \text{(x)}$$

Reaction series having essentially constant steric and resonance features would generally satisfy equation(ix and x). However, the condition of variable substituent effects (steric) in aliphatic reaction may lead to the failure of the equation. Rates for which polar effects of substituents are

quite negligible have been found to obey the expression below:

$$\log k/k_o = \delta Es \text{ --------- (xi)}$$

where k/k_o measures quantitatively, the total steric effect of a given substituent relative to the standard of reference, normally a methyl group. Es, is a steric substituent constant obtained (Taft, 1953) from rates of ester hydrolysis and esterification reactions. δ, is a reaction constant which depends only on the nature of the reaction series. Its value gives a measure of the relative susceptibility of the reaction series to steric effect of substitutents (Taft, 1953).

2.8.2 Objective of the Present Work

Yassin (1979) and Ojokuku (1981) reported the syntheses and Kinetic studies of the cyclisation of N-alkoxythiocarboyl-L-α-amino acids and N-benzoyl-α-amino acids respectively to their corresponding oxazol 5-4H)-one derivatives. They found that the rate of cyclisation largely depends among other factors, on the steric and the characteristic nature of the alkyl group at the α-carbon of the amino acids. Also, the measurement of the absorption spectra of these amino acid derivatives in different solvents (Yassin, 1979; Ojokuku, 1981) showed different absorption maxima on the solvents employed.

Based on these findings therefore, the present work is

aimed at confirming and extending these earlier studies of Yassin (1979), Ojokuku (1981) and Martins (1982) to investigate the effect of substitutents of the L-α-amino acid derivatives on the cyclisation rates of N-carbobenzoxy-L-α-amino acids. It is also aimed at studying the role of polar and steric factors (Linear free- energy relationships) on the formation of oxazol - 5 (4H)-one derivatives.

CHAPTER THREE

3.0. EXPERIMENTALS

3.1.0 MATERIALS AND METHODS

3.1.1 MATERIALS

3.1.2 REAGENTS AND CHEMICALS:

All Chemicals and reagents used were of analytical grades and were either obtained from Aldrich or BDH Limited. The N-carbobenzoxy-L-α-amino acids used were of highest purity grades (99%) commercially available from Sigma Chemical Company. The amino acid derivatives and solvents used include: N-carbobenzoxy-L-α-phenylalanine, N-Carbobenzoxy-L-α-leucine, N-carbobenzoxy-L-α-valine, N-carbobenzoxy-L-α-alanine, ethanoic anhydride, petroleum ether (40-60°C), methanol, chloroform, benzene, 1, 4-dioxan, water, tetrahydrofuran, dichloromethane and ethoxyethane.

3.1.3 PURIFICATION OF SOLVENTS

The following solvents were purified according to standard methods (Porrin *et al*, 1980).

Methanol	AnalaR grade
Ethanoic anhydride	BDH

Tetrahydrofuran	BDH
1,4-dioxan	"
Dichloromethane	Analar grade
Ethoxyethane	"
Benzene	"
Petroleum ether (40 - 60°C)	"
Distilled water	-

3.1.4 METHANOL

Freshly prepared calcium(II)oxide (10.6g, 0.19 mol) was placed in a 1-litre round bottomed-flask fitted with a double-surface reflux condenser. The methanol (400 cm^3) was added slowly through the condenser and the mixture refluxed for 1 hour. The product was distilled with the exclusion of moisture and the fractions that boiled between 62 - 63°C were collected and stored.

3.1.5 ETHANOIC ANHYDRIDE

Ethanoic anhydride (360 cm^3) was transferred to a 1-litre round bottomed-flask and distilled. The fractions that boiled at 139°C were collected and stored in a clean, dry conical flask.

3.1.6 TETRAHYDROFURAN

Tetrahydrofuran (THF), 500 cm^3) was transferred to a 2-

litre round-bottomed flask and (190g, 8.26mol) sodium metal added and the mixture refluxed for twenty hours. The tetrahydrofuran was distilled from sodium and the fractions that boiled at 65°C were collected and kept in a dark, clean dry bottle.

3.1.7 1,4-DIOXAN

Dioxan (250 cm^3) was transferred to a clean 500 cm^3 round bottomed flask (85 g, 3.70 mol) of sodium metal was added and the mixture refluxed for nine hours after which the sodium turned very bright. The dioxan was distilled from sodium and the fractions that boiled between 100 - 101°C were collected and kept in a clean dry bottle for use.

3.1.8 DICHLOROMETHANE

Dichloromethane (300 cm^3) was transferred into a 1-litre conical flask and anhydrous magnesium tetraoxosulphate (VI) (60 g, 0.5 mol) was added. The flask was closed tightly and allowed to stand for two days (48 hours) with occasional shaking. The mixture was filtered into a 1-litre round-bottomed flask and distilled. The fractions that boiled between 38 - 40°C were collected into a clean dry conical flask and stored.

3.1.9 ETHOXYETHANE

Ethoxyethane (360 cm^3) was transferred into a 1 - litre conical flask, and (90 g, 0.84mol) of anhydrous calcium chloride were added and the flask closed tightly and kept for five days during which it was shaken from time to time. The ether was then filtered into a clean, dry 1-litre conical flask. The flask was closed with a rubber stopper containing a drying tube of calcium chloride closed with glass wool plugs and left for 24hours. The drying tube was removed and the flask closed tightly.

3.2.0 BENZENE

Benzene (10 cm^3) was initially shaken with concentrated tetraoxosulphate (VI) acid (30 cm^3) to remove any sulphur compound that might be present. The mixture was then separated by the use of separating funnel and the benzene portion distilled. The fractions distilling between 80 - 81°C were collected and stored for use.

3.2.1 PETROLEUM ETHER

Petroleum ether (400 cm^3) each of 40 - 60°C and 60 - 80°C boiling range were transferred into a 1-litre round bottomed-flask and distilled. The fraction that boiled between 40 - 59°C and 61 - 79°C were collected and kept in two clean and dry conical flasks.

3.2.2 INSTRUMENTATION:

Melting points were determined on Gallenkamp electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded on an FT - AT1 Mattson Genesis Spectrophotometer as films or as Nujol mulls. ^{1}H NMR spectra were recorded on varian EM 360L (60 MHz) model, in carbon tetrachloride (tetramethyl silane as internal reference), unless otherwise reported. All chemical shifts were reported as ppm on the δ-scale. Kinetic studies were monitored on a PU8700 ultraviolet spectrophotometer.

Thin layer chromatography (tlc) was carried out on silica gel Grade 6. Solvent ratios were in volumes before mixing.

3.3 METHODS

3.3.1 KINETIC MEASUREMENTS:

The reactions were carried out under pseudo-first order conditions by keeping an excess of the amino acid over ethanoic anhydride. All reactions were carried out in $10cm^3$ volumetric flask at 25°C. Rate constants were computed from the linear plots of time against Log A_o-A_∞ /A_t-A_∞ or Log A_∞ - $A_o/A_\infty-A_t$ as the case may be. Duplicate kinetic runs showed the rate to be reproducible to within ± 4%.

3.3.2 REACTION OF N-CARBOBENZOXY-L-α-PHENYLALANINE WITH ETHANOIC ANHYDRIDE

N-carbobenzoxy-L-α-phenylalanine (0.02g, 0.000067 mole) was dissolved in 10cm^3 ethanoic anhydride in a 10cm^3 volumetric flask. 1cm ultraviolet silica cells were used to obtain the baseline of ethanoic anhydride. A Pu 8700 ultraviolet spectrophotometer was used to obtain the λ_{max}. of the N-carbobenzoxy-L-α-phenylalanine.

In a clean dry 10 cm^3 volumetric flask, (0.02g, 0.000067 mol) N-carbobenzoxy-L-α-phenylalanine was accurately weighed. Ethanoic anhydride was made up to the mark and the stopwatch started immediately. The solution was quickly transferred into a 1 cm-silica cell and the ultraviolet spectrum was run at intervals of five-minutes while the λ_{max} was fixed at 293 nm. The absorbance decreased with time until a stationary value was obtained.

The absorbance (A) at five-minutes intervals was calculated after subtracting the distances made by the baseline to get the correct absorbances of the N-carbobenzoxy-L-α-phenylalnine in ethanoic anhydride. A plot of the absorbance (A) against time (t) was made and extrapolated to t = 0 to give (A_o). The absorbance at infinity (A_∞) was obtained from the kinetic studies and the value (A_o-A_∞) was calculated. Various values of A_t - A_∞ were

calculated, where (A_t) is the absorbance at time (t). A plot of the time (t) against Log $A_o - A_\infty / A_t - A_\infty$ was made. A straight-line graph whose slope equals 2.303/k afforded the calculation of rate constant (k) for the cyclisation of N-carbobenzoxy-L-α-phenylalanine.

Table 1: KINETIC DATA OF N-CARBOBENZOXY-L-α-PHENYLALANINE WITH ETHANOIC ANHYDRIDE AT FIXED λ_{max}. (293 nm)

Time(min.)	Absorbance
0	-
5	0.023
10	0.021
15	0.019
20	0.018
25	0.017
30	0.016
35	0.015
40	0.014
45	0.014
50	0.014
55	0.014
60	0.014

A_∞ = 0.012, Fig. 1

3.3.3 N-CARBOBENZOXY-L-α-ALANINE

The above procedure was repeated for the cyclisation of N-carbobenzoxy-L-α- alanine using ethanoic anhydride thus: N-carbobenzoxy-L-α-alanine (0.03g, 0.00013mol) was weighed and dissolved in a 10 cm^3 volumetric flask at 25°C. The fixed λ_{max} was 261 nm.

The rate constant (k) for cyclisation of N-carbobenzoxy-L-α-alanine under the influence of ethanoic anhydride was calculated.

3.3.4 N-CARBOBENZOXY-L-α-LEUCINE:

N-carbobenzoxy-L-α-leucine (0.08g, 0.0003mol) was weighed and dissolved in a 10 ml volumetric flask. The fixed λ_{max}. was 294 nm.

Table 2: KINETIC DATA N-CARBOBENZOXY-L-α-ALANINE WITH ETHANOIC ANHYDRIDE AT FIXED λ_{max}. (261 nm)

Time (min.)	Absorbance
0	-
5	0.0150
10	0.0185
15	0.0230
20	0.0250
25	0.0275
30	0.0290
35	0.0300
40	0.0310
45	0.0310
50	0.0310
55	0.0310
60	0.0310
65	0.0310

A_∞ = 0.0330, Fig. 2

Table 3: KINETIC DATA OF N-CARBOBENZOXY-L-α-LEUCINE WITH ETHANOIC ANHYDRIDE AT FIXED $\lambda_{max.}$ (294 nm)

Time (min.)	Absorbance
0	-
5	0.605
10	0.743
15	0.891
20	1.086
25	1.210
30	1.254
35	1.255
40	1.255
45	1.256
50	1.256
55	1.256
60	1.256
65	1.256

A_{∞} = 1.316, Fig. 3

The rate constant (k) for cyclisation of N-carbobenzoxy-L-α-leucine under the influence of ethanoic anhydride was calculated.

3.3.5 N-CARBOBENZOXY-L-α-VALINE:

N-carbobenzoxy-L-α-valine (0.05g, 0.00019 mol) was weighed and dissolved in a $10cm^3$ volumetric flask. The fixed λ_{max} was 217nm.

Table 4: KINETIC DATA OF N-CARBOBENZOXY-L-α-VALINE WITH ETHANOIC ANHYDRIDE AT FIXED λmax. (217nm)

Time (min.)	Absorbance
0	-
5	0.018
10	0.029
15	0.041
20	0.052
25	0.058
30	0.063
35	0.064
40	0.065
45	0.066
50	0.066
55	0.066
60	0.066
65	0.066
70	0.066

A_∞ = 0.068, Fig. 4

The rate constant (k) for the cyclisation of N-Carbobenzoxy-L-α-valine under the influence of ethanoic anhydride was calculated.

3.4.0 PREPARATION OF 2-BENZYLOXY-4-ALKYLOXAZOL-5 (4H)-ONE DERIVATIVES:

3.4.1 N-CARBOBENZOXY-L-α-LEUCINE:

N-carbobenzoxy-L-α-leucine (0.52g, 0.00196mol) in 30 cm^3 anhydrous ethanoic anhydride was evaporated *in vacuo* after 2-3 hours at 25°C. The residue was triturated in excess aqueous sodium bicarbonate and the resulting suspension extracted with anhydrous ether. The ethereal solution was dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to afford (0.34g, 0.00137mol) a white solid (melting point = 152° - 155°C) which was recrystallized from petroleum ether (melting point = 148 - 149°C) and characterised as the N-benzyloxyoxazolone on the basis of IR and ^1H NMR spectra (2-benzyloxy-4-isobutyloxazol-5 (4H)-one), IR: 3010cm^{-1} (υC-H, aromatic, sharp), 2900cm^{-1} (υC-H, alkane, sharp), 1750cm^{-1} (υC=O, carbonyl, sharp) 1652cm^{-1} (υC=N, sharp), 1132cm^{-1} (υC-O, ether, sharp). ^1H NMR (CCl_4): δ = 7.3 (s = 5H), 4.3. (s,2H), 1.5 (t, 1H), 1.0(s).

3.4.2 N-CARBOBENZOXY-L-α-ALANINE:

N-carbobenzoxy-L-α-alanine (0.30g, 00134mol) was treated in the same manner as above to afford (0.17g, 0.00083mol). The product was recrystallized from ethanol to afford white crystals, melting point = 96 - 98°C. IR: 3020cm^{-1} (υc-H, aromatic, sharp), 1749cm^{-1} (υC=0, carbonyl, sharp), 1650cm^{-1} (υC=N, sharp), 1100cm^{-1}) (υC-N). ^1H NMR (CCl_4): δ = 7.7(S, 5H), 4.1 (5,2H), 1.4 (9,1H), 0.9 (d, 3H).

3.4.3 N-CARBOBENZOXY-L-α-PHENYLALANINE:

N-carbobenzoxy-L-α-phenylalanine (0.24g, 0.0008mol) was treated as above to afford (0.14g, 0.00050mol) a syrupy residue which was recrystallized from petroleum ether (60-80°C) to afford white long needles, melting point = 75 - 76°C. IR: 3030cm^{-1} (υC-H, aromatic sharp), 2890cm^{-1} (υC-H, alkane, sharp), 1736cm^{-1} (υC=0, carbonyl, medium), 1129cm^{-1} (υC-0, ether, sharp). ^1H NMR (CCl_4): δ = 7.5 (S, 5H), 4.5 (s, 2H), 3.6 (t, 1H).

3.4.4 N-CARBOBENZOXY-L-α-VALINE:

N-carbobenzoxy-L-α-valine (0.46g, 0.0018 mol) was treated as above to afford (0.24g, 0.001 mol) a solid,

melting point = 102 - 103°C.

IR: 3000cm^{-1} (υC-H aromatic, sharp), 1748cm^{-1} (υC=O, carbonyl, sharp), 2898cm^{-1} (υc-H, alkane, sharp), 1648cm^{-1} (υC=N, sharp), 1055cm^{-1} (υC-N), 1130cm^{-1} (υC-O, ether, sharp). ^{1}HNMR (CCl_4): δ = 7.4(5.5H) 5.1 (5, 1H), 2.0 (t, 1H), 1.7(9,1H), 0.9(d, 3H).

Thin layer chromatography was employed to examine the purity of the starting material and products formed. Solvents used were chloroform and benzene in a ratio of 3:2, and chloroform-ether in the ratio of 4:1.

3.5.0 SPECTROSCOPIC ANALYSIS

The absorption spectra of N-carbobenzoxy-L-α-amino acids were determined on a PU 8700 Ultraviolet spectrophotometer using 1cm silica cells. Absorption spectra were measured in solutions of concentration 2.41-17.6g/L. Prior to each measurement, a baseline was established by a blank scan through the spectral region of interest using solvent in both cells each time and corrections were made on the actual results obtained. All the measurements were carried out at 25°C. The spectrophotometer was calibrated using standard dichromate solution and the appropriate correction made.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1.1 Properties of N-carbobenzoxy-L-α-amino acids:

N-carbobenzoxy-L-α-amino acids namely: N-carbobenzoxy-L-α-phenylalanine, alanine and valine used as starting materials are white crystalline solids, where as, N-carbobenzoxy-L-α-leucine is a colourless viscous liquid. The respective melting points of the solid compounds are recorded in Table 5.

The infrared and nuclear magnetic resonance spectra of the compounds are shown in Figs. 14 - 21 and Figs 22 - 29 respectively.

4.1.2 THIN LAYER CHROMATOGRAPHY:

Thin layer chromatography (tlc) was employed to ascertain the purity of the starting materials. Mixture of solvents of different polarities were used since they gave better separation than chemically homogenous solvents (Ehrhardt, (1962). The solvents used were chloroform and benzene, chloroform and ether and were mixed in the ratios of 3:2, 4:1. The experimental details have been described in Chapter two.

The R_f values for each N-carbobenzoxy-L-α-amino acid

derivatives was determined under the same condition (25°C) and all the compounds examined gave one spot each. The R_f-values for N-carbobenzoxy-L-α- alanine is 0.62, while N-carbobenzoxy-L-α-phenylalanine has a value of 0.53. N-carbobenzoxy-L-α-valine and L-α-leucine gave R_f-values of 0.69 and 0.75 respectively. These results suggest that the R_f-values depend on the nature of the alkyl substituents (R) at the 4-carbon position or the α-carbon. Also, it shows that as the size of the alkyl group increases, the R_f-values also increases. However, there is a reverse trend with alkyl groups, such as, benzyl ($PhCH_2$). This gave the lowest R_f-value indicating that the N-carbobenzoxyl-L-α-phenyl alanine may be the most polar of the N-carbobenzoxy-L-α-amino acid derivatives studied. The R_f-values of the product [2-benzyloxy-4-alkyloxazol-5(4H)-one] (Table 10) showed that the R_f-values are higher than the individual amino acid derivatives. This therefore implies that the products formed are less polar than the starting materials. One of the products [2-benzyloxy-4-isobutyl oxazol-5(4H)-one] gave the highest R_f-value of 0.92 indicating that it is the least polar compound.

4.1.3 SOLVENT EFFECTS ON THE ULTRAVIOLET ABSORPTION SPECTRA OF N-CARBOBENZOXY-L-α-AMINO ACIDS:

The ultraviolet absorption spectra of N-carbobenzoxy-L-α-amino acids were investigated in various solvents. Solvents used included: distilled water, methanol, ethanoic anhydride, dichloromethane, tetrahydrofuran, ethoxyethane and 1,4-dioxan. The N-carbobenzoxy-L-α-amino acid derivatives were found to exhibit different absorption maxima in the solvents employed (Table 8) which is similar to the results of other investigators (Martins and Yassin, 1979; Martins and Ojokuku, 1981) Using N-alkoxythiocarbonyl and N-benzoyl-L-α-amino acids respectively.

The absorption spectra of N-carbobenzoxy-L-α-phenylalanine in methanol, distilled water and ethanoic-anhydride is red-shifted. This may be due to $\pi \rightarrow \pi^*$ transitions. Solvation by a polar solvent (distilled water of methanol) stabilises π, π^* and n orbitals. However, the stabilisation of non-bonding orbitals is affected with hydrogen bonding solvents such as water or methanol. Therefore, π^* orbitals are more stabilised by solvation than π orbitals probably because, the π^* orbitals are more polar. This results in the lowering of the energy of $\pi \rightarrow \pi^*$ transitions with solvation, the transition therefore, is red-shifted while the energy of the spectra may be attributed to the dispersion

effects of the solvents. These effects for non-polar solvents with low dielectric constants are generally sufficiently small in comparison with dipole-dipole and hydrogen bonding effects. Hydrogen bonding effects is pronounced when water is used as solvent with the amino acids since non-bonding pair of electrons of the carbonyl is the acceptor site for the hydrogen bond. It has been shown (Brealey and Kasha, 1955) that the excitation energy of an $n \rightarrow \pi^*$ transition in a hydrogen bonding solvent includes the energy which must be added to break the hydrogen bond. In water, this hydrogen bond energy constitutes most of the energy difference of the blue-shift from a non-polar solvent.

From the absorption spectra obtained (Figs 9 - 12), there is shift of wavelength (absorption maxima) from longer to shorter regions as the polarity of the solvent increases. The absorption maxima of non-polar solvents, for example, tetrahydrofuran, diethylether, dichloromethane showed red-shift compared to the absorption maxima in polar solvents (methanol or distilled water.) The blue-shift in the $n \rightarrow \pi^*$ absorption bands can be attributed to the effects of both electrostatic and hydrogen bonding interaction on the medium used in the dissolution of the N-carbobenzoxy-L-α-amino acids. Blue-shift phenomena in $n \rightarrow \pi^*$ transitions have been explained (McConnell, 1952; Mitossun *et al*, 1960) on the basis of solvation hypothesis and hydrogen bonding.

They explained that blue-shifts originated from solvent molecules orientating themselves around the solute molecules in order to fit with the ground state charge distribution of the solute molecules. On excitation, if the charge distribution of the solute changed markedly as in the case of $n \rightarrow \pi^*$ transition, the solvent molecules would have the position and orientation to bind most strongly with the excited state distribution. This would give rise to a blue-shift phenomena, since a more polar solvent relative to a non-polar one would give a greater solvation energy for the ground state of the solute than the excited state.

A $\pi \rightarrow \pi^*$ transitions has absorption occurring in the far ultraviolet region. Conjugation of double bonds, however, lowers the energy required for the transition, and the absorption moves to longer wavelengths. The medium intensity short wavelength bands in the region 195 - 250nm (Martins and Ojokuku, 1982) has been attributed to the $\pi \rightarrow \pi^*$ transitions which is due to the conjugation of the carbonyl group with the benzene ring in the N-benzoyl-L-α- amino acids. Such conjugation does not exist in carbobenzoyl-L-α amino acids, since the methylene group is bridged between the benzene and the carboxyl functional group. The absorption due to the transition of the amino acids studied therefore, can be assigned $n \rightarrow \pi^*$ transition of a weak carbonyl while the $\pi \rightarrow \pi^*$ transition band is characterised by a large

blue - shift on changing the solvent from non-polar to polar solvent.

The band intensities were found to vary in the solvents employed. Distilled water, a polar solvent, gave the least intense peak (Fig. 9 - 12) compared to other solvents. This is attributable to the partial solubility of the amino acid in distilled water.

4.1.4 THE KINETICS OF CYCLISATION OF N-CARBOBENZOXY-L-α-AMINO ACIDS:

The rate of the cyclisation of N-carbobenzoxy-L-α-amino acids were followed by ultraviolet spectroscopic method at fixed wavelengths ($\lambda_{max.}$) using purified ethanoic anhydride as the cyclising agent. The position of the maximum absorbance (λ_{max}) exhibited by each of the compounds gradually increased or decreased as cyclisation progressed until a stationary value of absorbance was obtained (Frost and Pearson, 1962) indicating the completion of the reaction.

Using the relationship,

$$\frac{2.303}{k}\log(A_\infty - A_o)/(A_\infty - A_t) \quad or \quad t = \frac{2.303}{k}\log(A_o - A_\infty)/(A_o - A_t)$$

where t is the time in minute,. k is the rate constant, A_o, A_t and A_∞ are the absorbances at the beginning, t = 0, at a particular time t, and at the end of the reaction, t = respectively (Equation vii), a plot of t versus log $(A_\infty - A_o)/(A_\infty - A_t)$ gave a straight line obtained by the least square analysis method. The rate constant k for the reaction was then calculated from the slope of the straight line graph for each N-carbobenzoxy-L-α amino derivatives (Figs 5 - 8). The rate constants, k, for the formation of 2-benzyloxy-4-alkyloxazol-5(4H)-one are recorded in Table 9.

4.2 DISCUSSION OF THE KINETICS DATA.

The rate of formation of oxazol-5(4H)-one depends on the nature of the substituents on the 2- and 4- positions of the oxazolone. In the present work, the substituent at the 2-position is the same (benzyloxy group) in all the amino acids studied. Therefore, the difference in the rates of cyclisation of the amino acids to their respective oxazol-5-(4H)-ones may be attributed to the nature and influence of the substituents (alkyl groups) at the 4-position.

The following trends were observed in the results obtained:

i. N-carbobenzoxy-L-α-phenylalanine (R^1=$PhCH_2$) gave the lowest rate constant for its cyclisation to oxazolone, Table 9.

ii. When R^1 = CH_3, $CH(CH_3)_2$ and $CH_2CH(CH_3)_2$ corresponding to N-carbobenzoxy-L-α-alanine, valine and leucine, there is a regular increase in the rate constants (Table 9) in the order $CH_2CH(CH_3)_2$ > $CH(CH_3)_2$ > CH_3 > $CH_2C_6H_5$

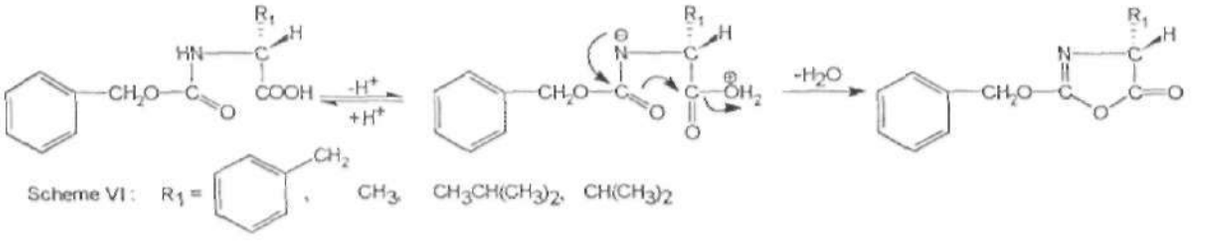
The lowest rate constant of the amino acid containing R_1=$PhCH_2$, may be attributed to the strong electron withdrawing tendency of the benzyl group, which reduces the electron density on the carbon at the 4-position. The demand of electrons at the 4-carbon centre becomes more and therefore, may be responsible for the fast cyclisation to form the oxazol-5- (4H)-ones. Also, alkyl groups that exhibit +I effect (electron donors) may be responsible for the high rate constants indicating less demand of electrons at the 4-position.

The rate of cyclisation is influenced by the nature and size of alkyl groups in the N-carbobenzoxy-L-α-amino acids. Such alkly groups which may be electron withdrawing (-I effect) or electron donating (+I effect) either retard or enhance the cyclisation rates of the amino acid derivatives under the influence of ethanoic anhydride. Differences in the +I effect between methyl and ethyl groups is insignificant. However, benzyl

groups are found to exhibit strong -I effect (Jackson, 1972), this could be attributed to the unsaturated carbon atom of the benzene ring which is a better electron withdrawing group than the corresponding saturated carbon atom of the alkane. It is therefore, not surprising that the rate constant of cyclisation of N-carbobenzoxy-L-αamino acid derivative containing $R^1 = CH_2Ph$ under the influence of ethanoic anhydride has the lowest rate constant than the correspounding alkyl groups with +I effect.

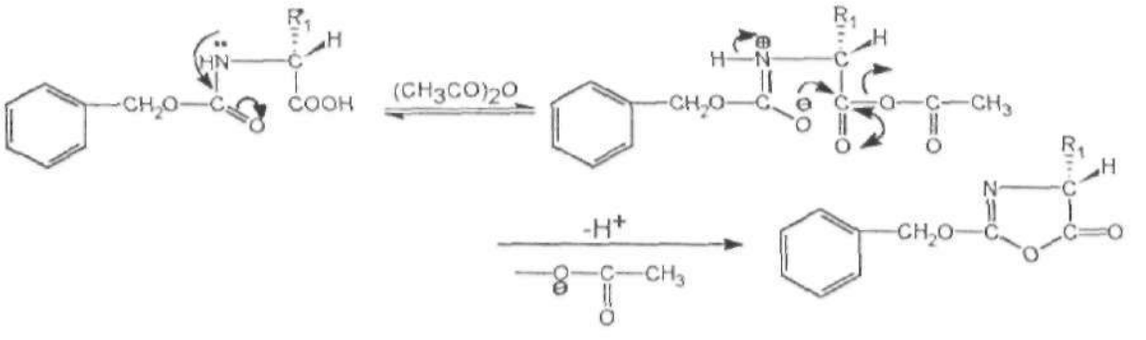
The linear correlation (Figs. 5-8) may imply that all the N-Carbobenzoxy-L-α- amino acids are cyclised by same mechanism and the changes in the rates are governed by the nature and size of the R-group. Some workers (Martins and Yassin, 1979; Martins and Ojokuku 1981) have observed an increase in the rate of cyclisation of amino acid derivatives of N-alkoxythiocarbonyl and N-benzoyl-under the influence of ethanoic anhydride as the size of the alkyl group increases. They attributed such increase in the rate constants to the +I effect of the alkyl groups. The kinetics of the cyclisation of N-carbobenzoxy-L-α-amino acid derivatives under the influence of ethanoic anhydride (Figs 1 - 4) show a linear relationship of absorbance with time until a constant absorbance was obtained indicating the completion of the reaction. However, Fig. 1 showed a decrease of absorbance as time increases. The following mechanisms are proposed for the

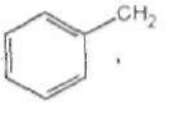
cyclisation of N-carbobenzoxy-L-α-amino acids (schemes VI and VII) under the influence of ethanoic anhydride.



Scheme VI: R_1 = , CH_3, $CH_3CH(CH_3)_2$, $CH(CH_3)_2$

The mechanism of the cyclisation proceeds in a manner similar to oxazolone analogues (Kemp and Chien, 1967). However, the mechanism for the ethanoic anhydride cyclisation of N-carbobenzoxy-L-α-amino acids probably proceeds by a concerted mechanism (Scheme VII).



Scheme VII: R_1 =  , CH_3, $CH_3CH(CH_3)_2$, $CH(CH_3)_2$

The intermediate moiety cyclises with the abstraction of a proton on the nitrogen and the subsequent elimination of an

acetyl molecule to afford the oxazol-5(4H)-one (Scheme VII). The two mechanisms (Schemes VI and VII) are possible for the cyclisation of N-carbobenzoxy-L-α-amino acids using ethanoic anhydride as cyclising agent. However, Scheme VII, is probably a better mechanistic route for the formation of oxazolones since the cyclisation involves an attack of the lone pairs of electrons of nitrogen on the carbonyl functional group with simultaneous acetylation of the hydroxyl group by the ethanoic anhydride to form the intermediate moiety which cyclises via a concerted mechanism to afford the oxazolones.

4.3 PREPAPRATION AND SPECTROSCOPIC PROPERTIES OF 2-BENZYLOXY-4-ALKYLOXAZOL-5(4H)-ONE

A number of 2-benzyloxy-4-alkyloxazol-5(4H)-one and its derivatives were synthesised from the corresponding N-carbobenzoxy-L-α-amino acids using ethanoic anhydride (Carter and Risser, 1941) at room temperature (25°C). All the experimental procedures are described in the experimental section.

The cyclodehydration of N-carbobenzoxy-L-α-amino acids under the influence of ethanoic anhydride afforded 2-benzyloxy-4-alkyloxazol-5(4H)-ones in good yields (Table 9). The 2-benzyloxy-4-alkyloxazol-5(4H)-ones are white crystalline solids with low

melting points Table 10. Their R_f-values are higher than the starting materials indicating that they are less polar compounds compared to the starting materials.

The infrared and proton nuclear magnetic resonance spectra of N-benzyloxyoxazol-5(4H)-one and N-carbobenzoxy-L-α-amino acids in liquid state and in solution (Tables 6 and 7) are displayed. The alkyl groups on the oxazolone ring show different bands depending on their nature in the ring. The carbonyl stretching vibration (1749-1750cm^{-1}) is strong and sharp when the R group corresponds to CH_3, $CH_2CH(CH_3)_2$ or $CH(CH_3)_2$. However, when R=$PhCH_2$, a medium carbonyl stretching vibration (1736cm^{-1}) is observed. This shows that the different substituents, (alkyl groups) affect the band intensities of other functional groups this may depend on whether such substituents are electron withdrawing donating groups. Benzyl group (R=$PhCH_2$) exhibit strong -I effect and therefore may be responsible for the medium intensity band of the carbonyl stretching vibration at 1736 cm^{-1}.

The higher frequency bands at about 1380 - 1390 cm^{-1} is attributed to methyl symmetrical bending modes, and that at about 940 cm^{-1} to the υC-C vibrations. Other absorption at 1200-1100cm^{-1} and 1030 cm^{-1} are probably due to methyl rocking modes. The most characteristic bands related to the various substitution pattern of the oxazolones is the υC-H vibration (2910 cm^{-1}, 2890 cm^{-1},

2870 cm^{-1} and 2900 cm^{-1}) due to alkyl groups at the carbon 4-position. The ring stretching frequencies differ as the substituents on the ring are varied. In-plane ring deformation bands were found in the region 1050 - 500 cm^{-1} and the two out-of-plane deformation in the region 600-400 cm^{-1}. The absorption bands in the range 1580 - 1675 cm^{-1} is attributed to the C=N bond. The absence of N-H stretching vibration in 2-benzyloxy oxazol-5(4H)-one and the signal of C=N at 1648 - 1652 cm^{-1}, Table 11, indicates that N-carbobenzoxy-L-α-amino acids were cyclised by the ethanoic anhydride. The proton nuclear magnetic resonance spectra of the 2-benzyloxy oxazol-5 (4H)-ones confirmed the absence of the nitrogen proton which further agrees that a proton abstraction took place to form the product.

Due to the presence of two different heteroatoms (N and O) in the oxazolone ring, the disymmetry of the oxazolone nucleus affects the values of the chemical shifts of the protons of the carbon atoms (Pouchan *et al*, 1976) in the ring. the signals of the proton at carbon 4-position (methine protons) absorb at a lower field (δ = 1.23 - 1.81) than do others. The presence of an electron donating substituent, such as a methyl group, causes an up field shift of the protons in the oxazolone ring. This effect is stronger when R group is larger or bulkier indicating that the substituent has the greatest influence (Katritzky, 1963). The shift due to the substituents, can be evaluated relative to the

chemical shift of the parent alkyl. Strong deshielding shifts due to the negative I effects was observed when R=$PhCH_2$.

4.4 TAFT POLAR-FREE ENERGY RELATIONSHIPS

4.4.1 Effect of Polar and steric factors on rates:

Treatment of the kinetic data obtained for the cyclisation reaction of N-carbobenzoxy-L-α-amino acid derivatives under the influence of ethanoic anhydride, in a manner similar to that described by Taft for other reaction, makes it possible to separate the polar and steric factors which influence the rates. The Linear relationship between log k/k_o and δ^* values (Fig. 13) may be described by equation (xiv).

It has been found from the study of other reactions (Friedman and Sigel, 1966; Friedman and Wall, 1964 and Ojokuku *et. al.*, 1993) involving amino acids of varying structures, that the linear plots of sterically similar series are parallel, that is, the slopes of the lines are nearly identical.

The polar reaction parameter ε^* gives a measure of sensitivities of rates to substituents on the L-α-amino acids and the polar substituent parameter δ^*, defined as the ratio in rate constants of substituted L-α-amino acid and alanine gives a quantitative measurement of the change in substituent of the

amino acid as they are varied at the α-carbon of alanine. A very good linear correlation (Fig 13), with a negative slope (-0.436) obtained from least squares methods ($r = 0.97$, $n = 4$) reveal that the cyclisation reaction of N-carbobenzoxy-L-αamino acid derivatives probably involves a cyclic transition state (Chien and Kemp, 1967) which has a greater demand for electrons than the reactants. Also, the magnitude and sign of ρ^* may not only depend on the nature and size of the alkyl substituent (R) at the α-carbon, but probably by steric factors as well.

There was a correlation between steric substituent constants, Es and the log k/k_o values by least squares analysis ($r = 0.049$, $n = 4$). However, the steric substituent Es, depends on the size and steric requirements of the substituent in the reaction. Increase in the size of the alkyl substituent group causes an increase in the rate constants of the cyclisation of N-carbobenzoxy-L-α-amino acid (Polar factor) as well as a change in the steric environment (Steric factor). The effect of polar factor resulting from the change in the rate constants is quantitatively given by $e^*\delta^*$ (Zakhary and Iskander, 1978) and that of steric factor by Es.

4.5 CONCLUSION/SUGGESTION

The kinetics of cyclisation of N-carbpbenzoxy-L-α-amino acids under the influence of ethanoic anhydride to form their oxazolones was carried out at room temperature (25°C). The oxazolone structures assigned were supported by infrared and proton nuclear magnetic resonance spectroscopy. Their R_f-values from thin layer chromatography showed the oxazolones to have higher R_f-values than the corresponding amino acid derivatives with single spots of each compound.

Rate constants for the cyclisation reaction were found to increase with increase in the size of the alkyl group, that is, alkyl groups with +I effect. However, R group with -I effect gave low rate constant values.

On the basis of results obtained using linear free energy relationships (Taft-polar free energy relationship) both polar and steric factors influence rates in the cyclisation reaction to form the oxazol-5 (4H)-ones. Two important conclusions may therefore be drawn from the fact that good correlations are obtained:

(i) that the mechanism of the cyclisation of N-carbobenzoxy-L-α-amino acids investigated follow the same mechanism.

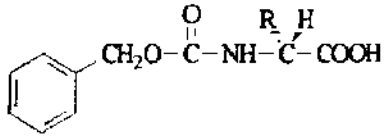
(ii) that there is no large differences of rate constants as steric hindrance becomes more on the α-carbon of the series of

L-α-amino acids studied.

The absorption maxima of N-carbobenzoxy-L-α-amino acids were affected by various solvents used resulting to either $\pi \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transitions as the case may be.

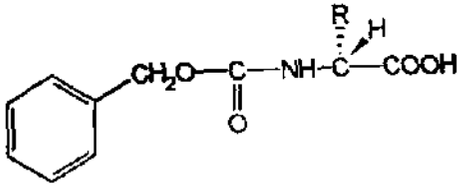
It is hereby suggested that there is the need to study the activation energy of the cyclisation reaction using the Arrhenius equation. Furthermore, there is the need to study the kinetics of racemisation and hydrolysis of oxazol-5(4H)-ones with different amino acid derivatives.

Table 5: **PHYSICAL PROPERTIES OF N-CARBOBENZOXY-L-α-AMINO ACID**



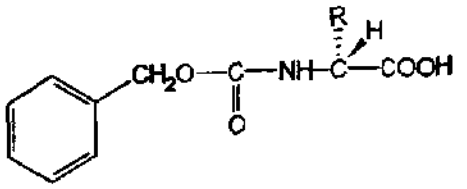
R	Physical state	Melting point (°C)	R_f-Value
CH3	Solid (crystals)	77 -78	0.62
CH(CH3)2	Solid (crystals)	80 -81	0.69
CH2CH(CH3)2	Liquid (syrupy)	-	0.75
CH2C6H5	Solid (crystals)	79 -81	0.53

TABLE 6: PROTON NUCLEAR MAGNETIC SPECTRA OF N-CARBOBENZOXY-L-α-AMINO ACIDS



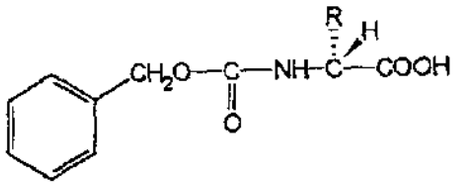
PROTON TYPE	δ-VALUE (PPM)				REMARKS			
	R=$CH_2CH(CH_3)_2$	R=CH_3	R=$CH_2C_6H_5$	R=$CH(CH_3)_2$	R=$CH_2CH(CH_3)_2$	R=CH_3	R=$CH_2C_6H_5$	R=$CH(CH_3)_2$
COOH (carboxylic acid).	10.31	10.33	10.28	10.32	Singlet	Singlet	Singlet	Singlet
C_6H_5 (aromatic)	7.30	7.60	7.25	7.50	Singlet	Singlet	Singlet	Singlet
NH (imino)	5.10	5.40	5.00	5.30	Singlet	Singlet	Singlet	Singlet
CH (methine)	3.30	3.00	3.90	3.10	Broad	Singlet	Singlet	Singlet
CH_2 (methine)	1.50	-	1.40	1.20	--	--	Singlet	doublet
CH_3 (methyl)	1.50	1.70	-	1.90	--	doublet	Singlet	singlet
$CH_2CH(CH_3)_2$ (isobutyl)	0.90	-	-	-	Doublet	doublet	Singlet	-
$CH(CH_3)_2$ (isopropyl)	-	-	-	0.80	Singlet	singlet	Singlet	doublet

Table 7: **INFRARED SPECTRA OF N-CARBOBENZOXY-L-α-AMINO ACIDS.**



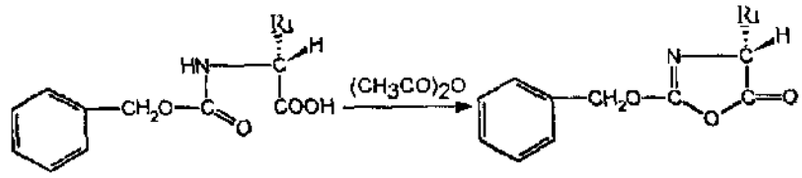
FUNCTIONAL GROUP	CLASS	REMARKS		R=$C_6H_5CH_2$	R=$CH_2CH(CH_3)_2$	R=CH_3	R=$CH(CH_3)_2$
C=O	Carbonyl	Sharp, stretching vibration	wave numbers (cm^{-1})	1795	1724	1745	1730
N-H	anines	Sharp stretching vibration	"	3333	3322	3334	3330
C-H	aromatic			3030	3034	3051	3044
-C=C-	aromatic	weak, stretching vibration	"	1536	1531	1535	1534
O-H	carboxylic	medium vibration	"	1462	1454	1463	1452
C-H	acids	weak vibration	"	2958	2888	2893	2898
C-N	alkane	sharp vibration	"	1375	1345	1374	1340
C-O	-	weak vibration	"	1252	1265	1291	1272
	ester	weak vibration	"				
			"				

Table 8: **ULTRAVIOLET SPECTRA OF N-CARBOBENZOXY-L-α-AMINO ACIDS**



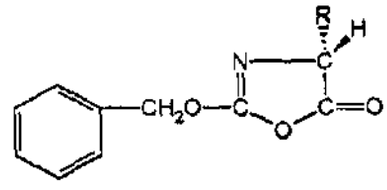
SOLVENT	$R=CH_3$		$R=CH_2CH(CH_3)_2$		$R=CH_2C_6H_5$		$R=CH(CH_3)_2$	
	λ_{max}	Logε	λ_{max} (nm)	Logε	λ_{max}	Logε	λ_{max} (nm)	Logε
Distilled Water	269	4.06	292	4.73	258	1.03	205	12.3
methanol	263	5.06	298	26.57	255	1.26	213	18.88
1,4-Dioxan	262	5.56	295	22.91	-	-	260	22.66
Tetrahydrofuran	296	6.00	295	16.60	-	-	248	20.56
Dichloromethane	293	10.14	302	26.98	-	-	259	14.79
Ethoxyethane	281	4.20	295	27.77	-	-	220	11.04
Ethanoic anhydride	261	2.04	294	17.75	293	1.05	217	1.89

Table 9: RATE CONSTANTS OF CYCLISATION OF N-CARBOBENZOXY-L-α-AMINO ACIDS UNDER THE INFLUENCE OF ETHANOIC ANHYDRIDE AT 25°C.



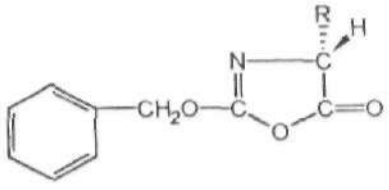
N-carbobenzoxy-L-α-amino acids	R^1	Yield (%)	$\lambda_{max.}$ (nm) Fixed)	Rate Constant (min^{-1})x10^{-2}
L-α-alanine	CH_3	56.60	261	5.40
L-α-Valine	$CH(CH_3)_2$	52.00	217	6.91
L-α-leucine	$CH_2CH(CH_3)_2$	65.40	294	7.60
L-α-phenylalanine	$CH_2C_6H_5$	58.00	293	4.61

Table 10: PHYSICAL PROPERTIES OF 2-BENZYLOXY-4-ALKYLOXAZOL-5(4H)-ONE.



R	Physical state	Melting Point (°C)	R_f-value
CH_3	Solid	65	0.81
$CH(CH_3)_2$	Solid	60	0.78
$CH_2CH(CH_3)_2$	Solid	52	0.92
$CH_2C_6H_5$	Solid	72	0.76

Table 11: IR AND H^1NMR OF 2-BENZYLOXY-4-ALKYLOXAZOL-5(4H)-ONE



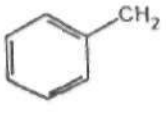
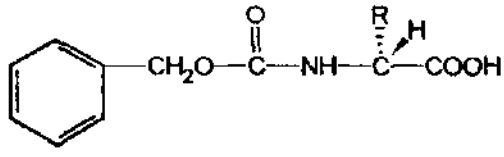
R	IR, V(cm^{-1})	^{1}HNMR δ-(ppm)
CH_3	C=O , 1749 C-O , 1154 C=N , 1650 C-H , 2910 C-H , 1100 C-H , 3020	0.9 (d,3H) 1.4 (9,1H) 7.7 (s,5H) 4.1 (s,2H)
	C=O , 1736 C-H , 2890 C=N , 1648 C-O , 1129 C-H 3030 C-N 1109	3.6 (t, 1H) 4.5 (s, 2H) 7.5 (s, 5H)
$CH_2CH(CH_3)_2$	C=O , 1750 C=N , 1652 C-O , 1132 C-H , 3010 C-N , 1058 C-H , 2900	1.0 (d, 3H) 1.5 (9, 1H) 2.3 (t, 1H) 4.4 (s, 2H) 7.3 (s, 5H)
$CH(CH_3)_2$	C=O , 1748 C-O , 1130 C-H , 3000 C=N , 1648 C-N , 1055 C-H , 2898	0.9 (d, 3H) 1.7 (9, 1H) 2.0 (t, 1H) 5.1 (s, 1H) 7.4 (s, 5H)

Table 12: PARAMETERS USED TO CORRELATE KINETIC DATA OF N-CARBOBENZOXY-L-α-AMINO ACID DERIVATIVES AT 298K.



R	Rate Constant (min^{-1})	Log k/k_o	δ^*	E_s
CH_3	5.40	0.00	0.00	0.00
$CH(CH_3)_2$	6.91	0.11	-0.19	-0.47
$CH_2CH(CH_3)_3$	7.60	0.15	-0.31	-0.35
$CH_2C_6H_5$	4.61	-0.07	0.22	-0.38

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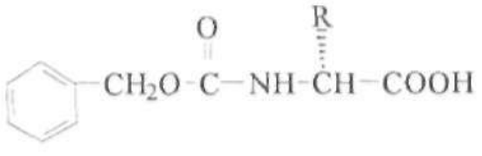
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APPENDIX

LINEAR REGRESSION (LEAST SQUARES) ANALYSIS OF DATA

Regression gives the best fitting relation between two quantities, one of which is liable to chance error. In this work, the values of the rate constant (log k/k_o) measured and for given values of σ^* or Es are computed.

Parameters used to correlate kinetic data of N-carbobenzoxy-L-α-amino acid derivatives at 298K.



R	X(σ^*)	X^2(σ^*)	Y(logk/k_o)	Y^2 (logk/k_o)^2	XY
CH_3	0.0000	-	0.0000	-	-
$CH(CH_3)_2$	-0.1900	0.0361	0.1100	0.0121	-0.0209
$CH_2CH(CH_3)_3$	-0.3100	0.0961	0.1500	0.0225	-0.0465
$CH_2C_6H_5$	0.2150	0.0462	-0.0700	0.0049	-0.0151
	Σx=0.2850	Σx^2=0.1784	Σx=0.1900	Σx^2=0.0395	Σxy=0.0825

There are four pairs of values of X and Y, therefore n = 4

$$\overline{X} = \frac{\Sigma x}{n} = \frac{-0.2850}{4}$$

$= -0.0713$

$$\overline{Y} = \frac{\Sigma x}{n} = \frac{0.1900}{4}$$
$$= 0.0475$$

Regression line equation is given as:

$$y - \overline{Y} = b(x - \overline{X})$$

Where b = regression coefficient (Y/X).

Regression coefficient,

$$b = \frac{\frac{\Sigma XY - \Sigma X \times \Sigma Y}{N}}{\frac{\Sigma X^2 - \Sigma^2(X)}{N}}$$

Correlation coefficient,

$$r = \frac{\frac{\Sigma XY - \Sigma X . \Sigma Y}{N}}{\sqrt{\left((\Sigma X^2 - \frac{\Sigma X^2}{N})(\Sigma X^2 - \frac{\Sigma Y^2}{N})\right)}}$$

$$\therefore \quad r = \frac{\frac{-0.0825 - (0.2850 \times 0.1900)}{4}}{\sqrt{\left(0.1784 - \frac{0.1784}{4}\right)\left(0.1784 - \frac{0.0395}{4}\right)}}$$

$$= \frac{0.0690}{0.0711} = 0.97$$

Degrees of freedom, N-2, since N=4

$\therefore$ 4-2 = 2

Theoretical value for degrees of freedom = 0.97

Value obtained from calculation = 0.97

This value (0.97) is greater than the theoretical value of r for P^1 = 0.05 and (degree of freedom) = 2. There is therefore, a significant correlation between X and Y or σ^* and log k/k_s, therefore, it is worth proceeding with the regression analysis.

To find b:

$$b = \frac{\Sigma XY - \frac{\Sigma X . \Sigma Y}{N}}{\Sigma X^2 - \frac{\Sigma^2 (X)}{N}}$$

$$b = -0.08245 - \frac{\frac{(-0.2850 \times 0.1900)}{4}}{\frac{0.1784 - (-0.2850)^2}{4}}$$

$$= \frac{-0.08245 + 0.0135}{0.1784 - 0.0203}$$

$$= \frac{-0.0689}{0.1581} \qquad \therefore \quad b = -0.436$$

The regression line equation is y-0.0475 = -0.436[x-(-0.0713)] becomes : y = 0.436x + 0.0164

R	X(σ*)	Y(logk/k_o)
CH_3	0	0.0164
$CH(CH_3)_2$	-0.19	0.0992
$CH_2CH(CH_3)_3$	-0.31	0.1516
$CH_2C_6H_5$	+0.215	-0.0773

See figure 13

(r = 0.97, n = 4)

Parameters used to correlate kinetic data of N-carbobenzoxy-L-α-amino acid derivatives at 298K.

R	Y(logk/k_o)	Y^2X	X(Es)	X^2	XY
CH_3	0.0000	0.0000	0.0000	-	-
$CH(CH_3)_2$	0.1100	0.0121	-0.4700	0.2209	-0.0517
$CH_2CH(CH_3)_2$	0.1500	0.0225	-0.3500	0.1225	-0.0525
$CH_2C_6H_5$	-0.0700	0.0049	-0.3800	0.1444	+0.0266
	ΣY=0.1900	ΣY^2=0.0395	ΣX=-1.200	ΣX^2=0.4878	ΣX=-0.0776

$$Y = \frac{\Sigma Y}{n} = \frac{\Sigma X}{n}$$

$$= \frac{0.1900}{4} = \frac{-1.200}{4}$$

$$\therefore \quad Y = 0.0475 = -0.3000$$

Correlation coefficient,

$$r = \Big/ (\Sigma x^2 - \frac{\Sigma x^2}{N})(\Sigma x^2 - \frac{\Sigma y^2}{N})$$

$$= \Big/ (0.4878 - \frac{0.4878}{4})(0.4878 - \frac{0.0395}{4})$$

$$= \frac{-0.0206}{0.4182}$$

$$\therefore \quad r = -0.049$$

FIG.1: CYCLISATION OF N-CARBOBENZOXY-L-α-PHENYL ALANINE UNDER THE INFLUENCE OF ETHANOIC-ANHYDRIDE

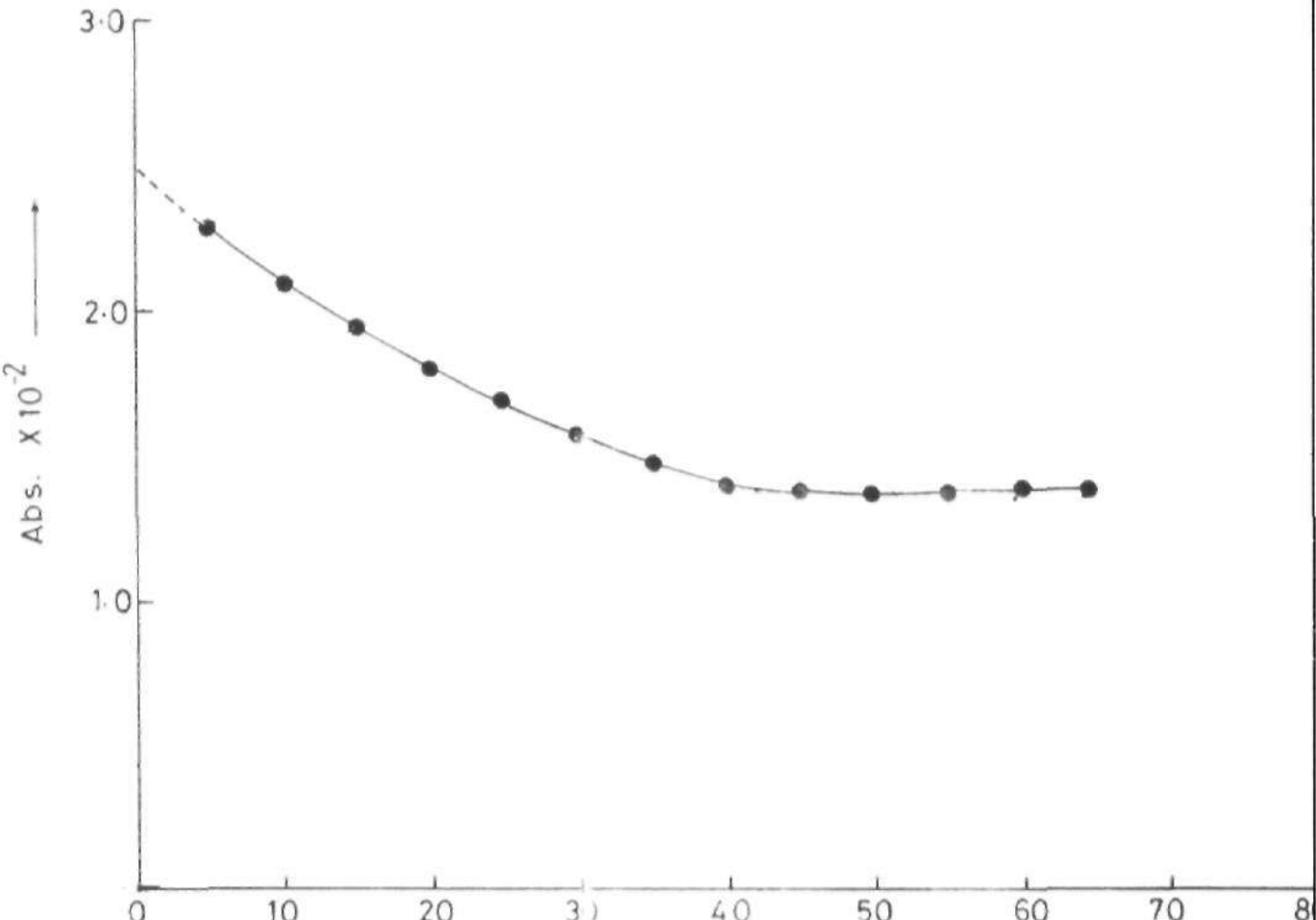


FIG. 2: CYCLISATION OF N-CARBOBENZOXY-L-α-ALANINE UNDER THE INFLUENCE OF ETHANOIC-ANHYDRIDE

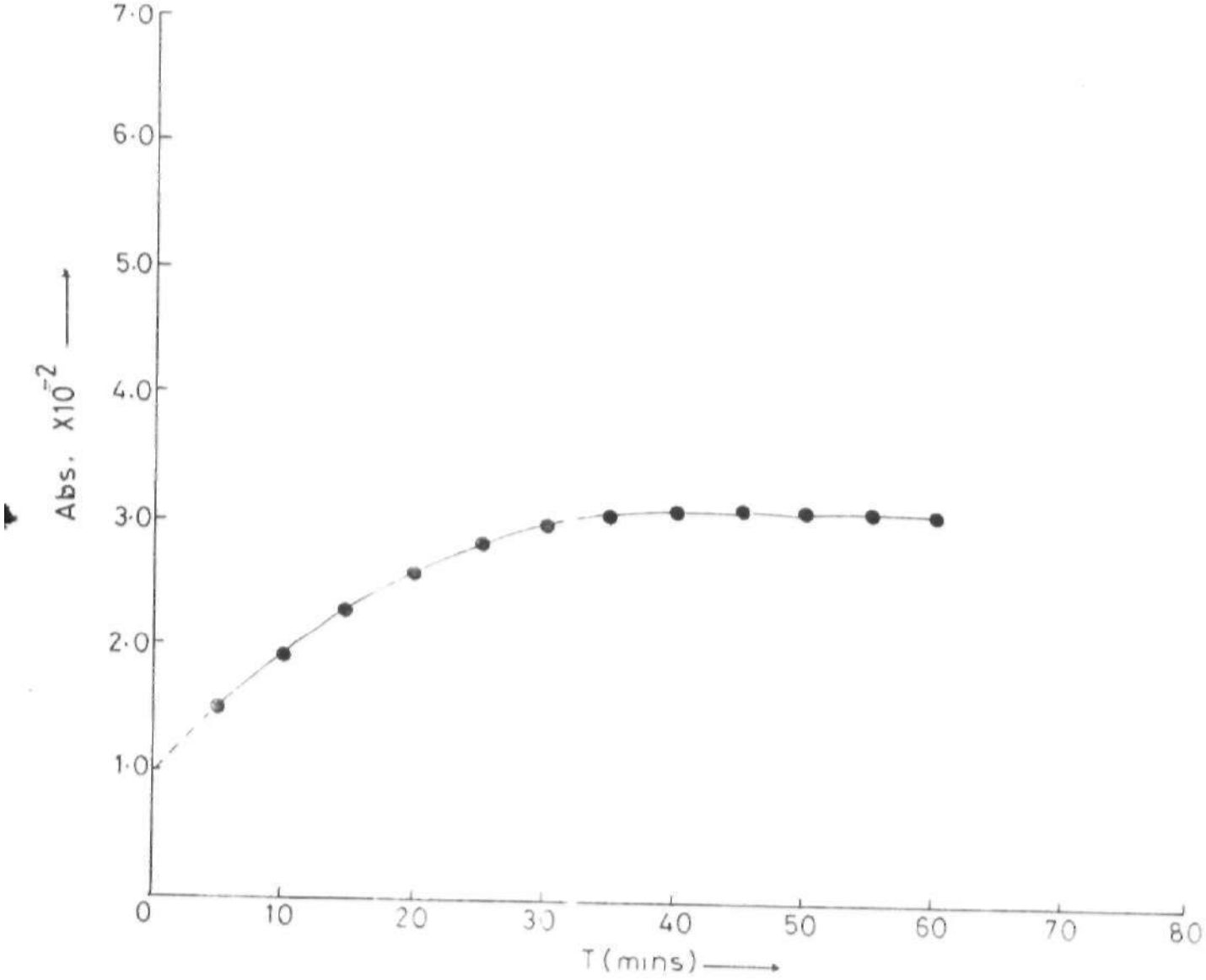


FIG.3: CYCLISATION OF N-CARBOBENZOXY-L-α-LEUCINE UNDER THE INFLUENCE OF ETHANOIC-ANHYDRIDE

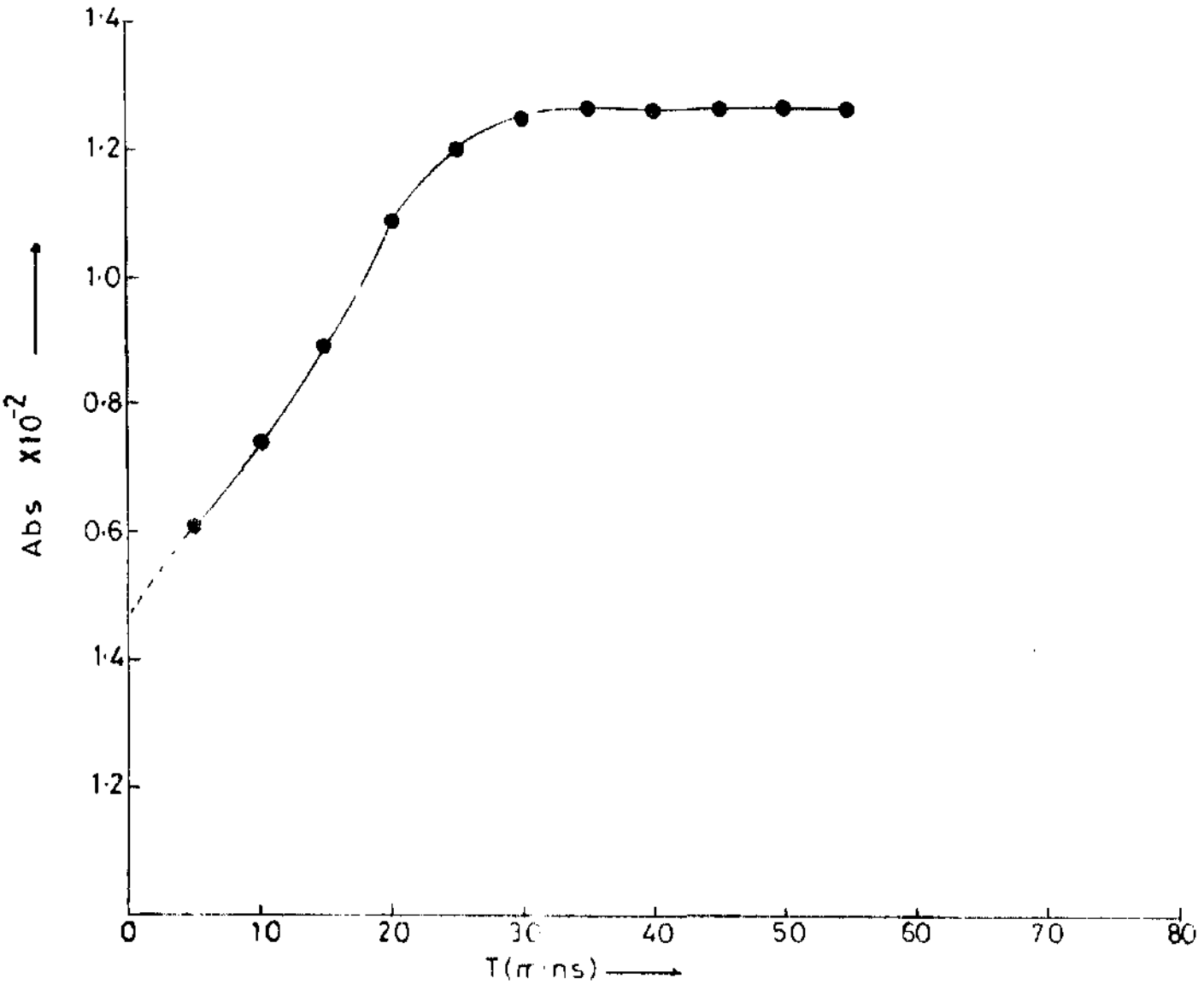


FIG 4: CYCLISATION OF N-CARBOBENZOXY-L-α-VALINE UNDER THE INFLUENCE OF ETHANOIC-ANHYDRIDE

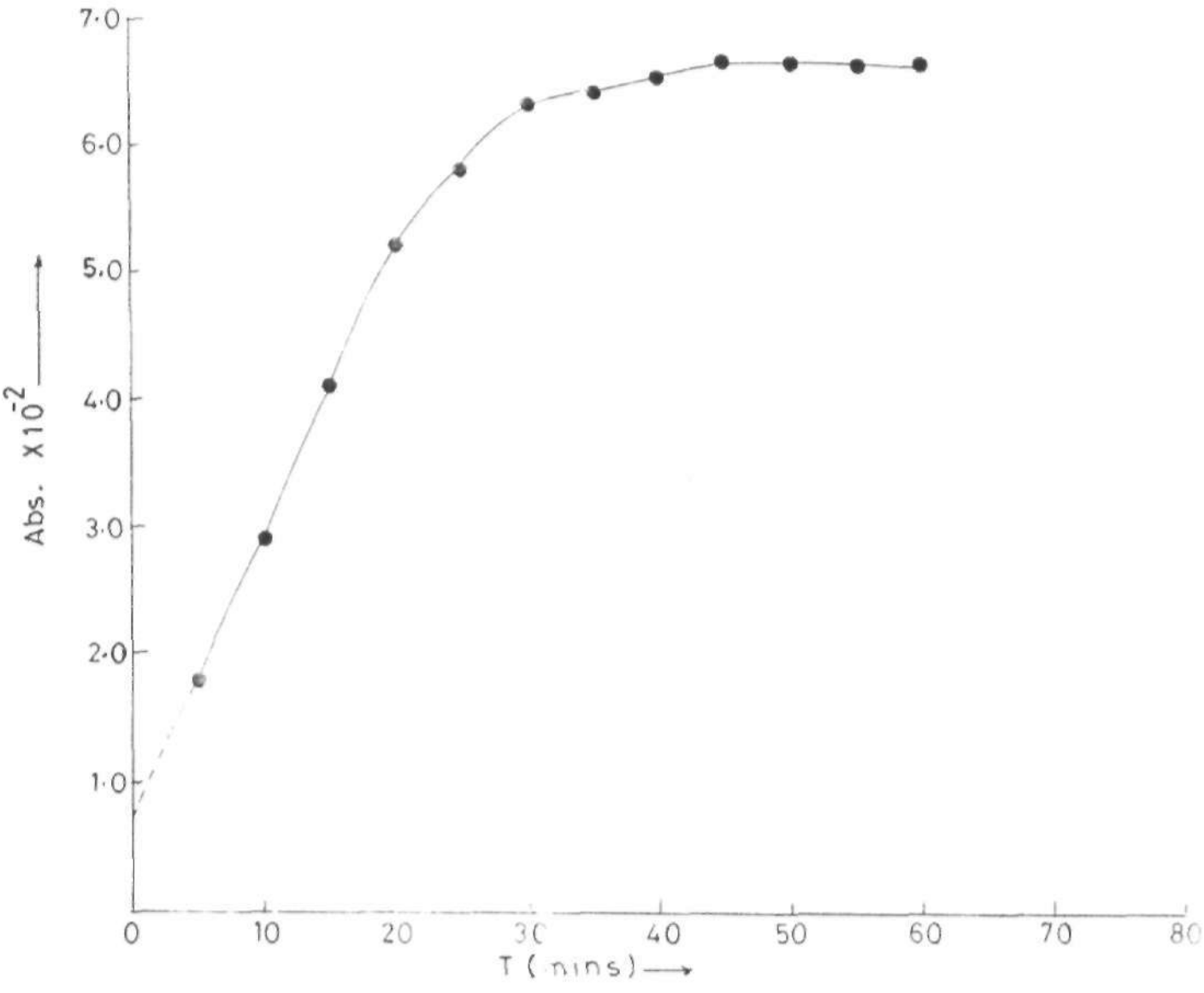


FIG.5; LINEAR PLOT FOR A FIRST ORDER CYCLISATION OF N-CARBO BENZOXY-L-α-PHENY ALANINE UNDER THE INFLUENCE OF ETHANOIC - ANHYDRIDE

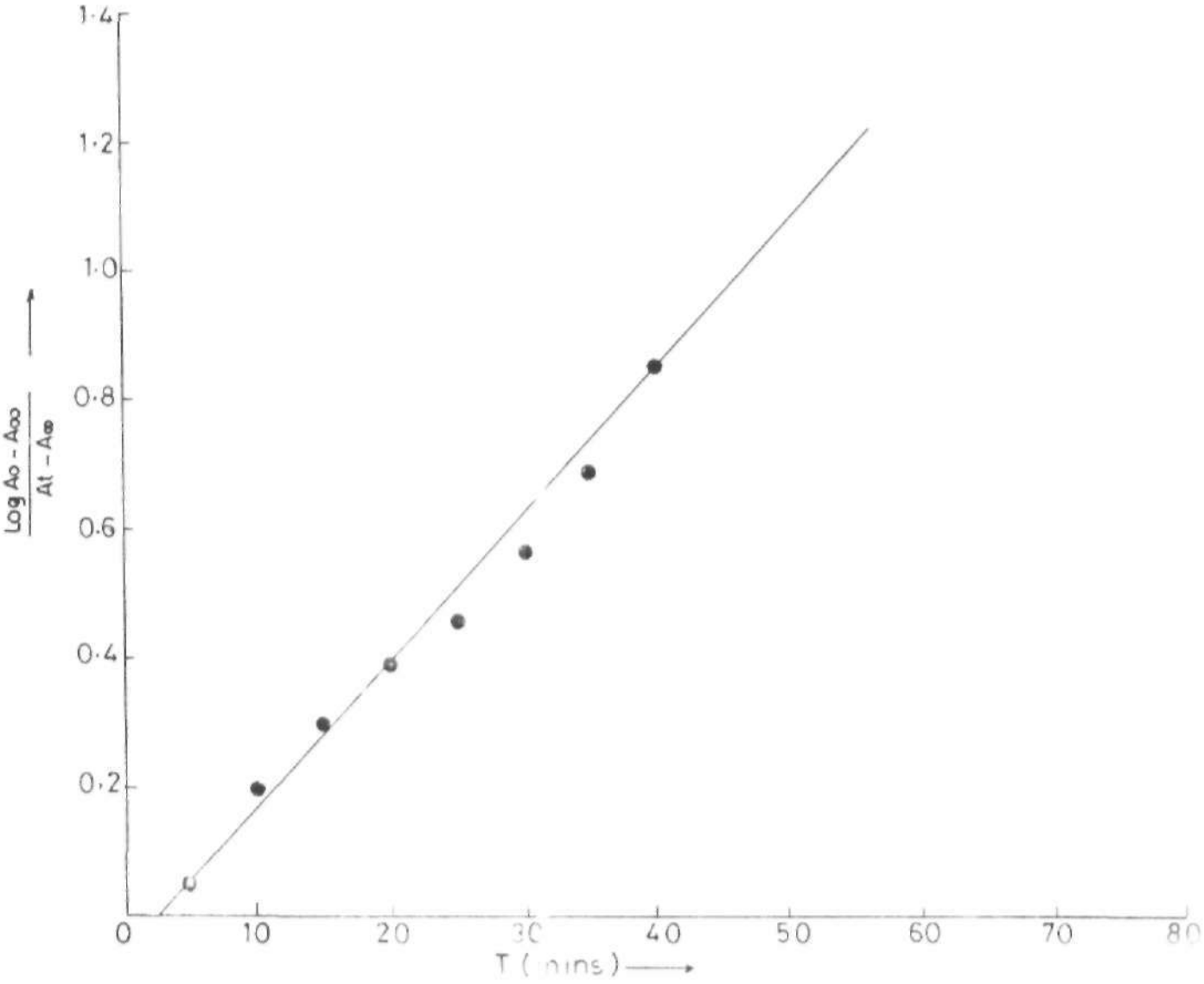


FIG.6 LINEAR PLOT FOR A FIRST ORDER CYCLISATION OF N CARBO-BENZOXY-L-α-ALANINE UNDER THE INFLUENCE OF ETHANOIC-ANHYDRIDE

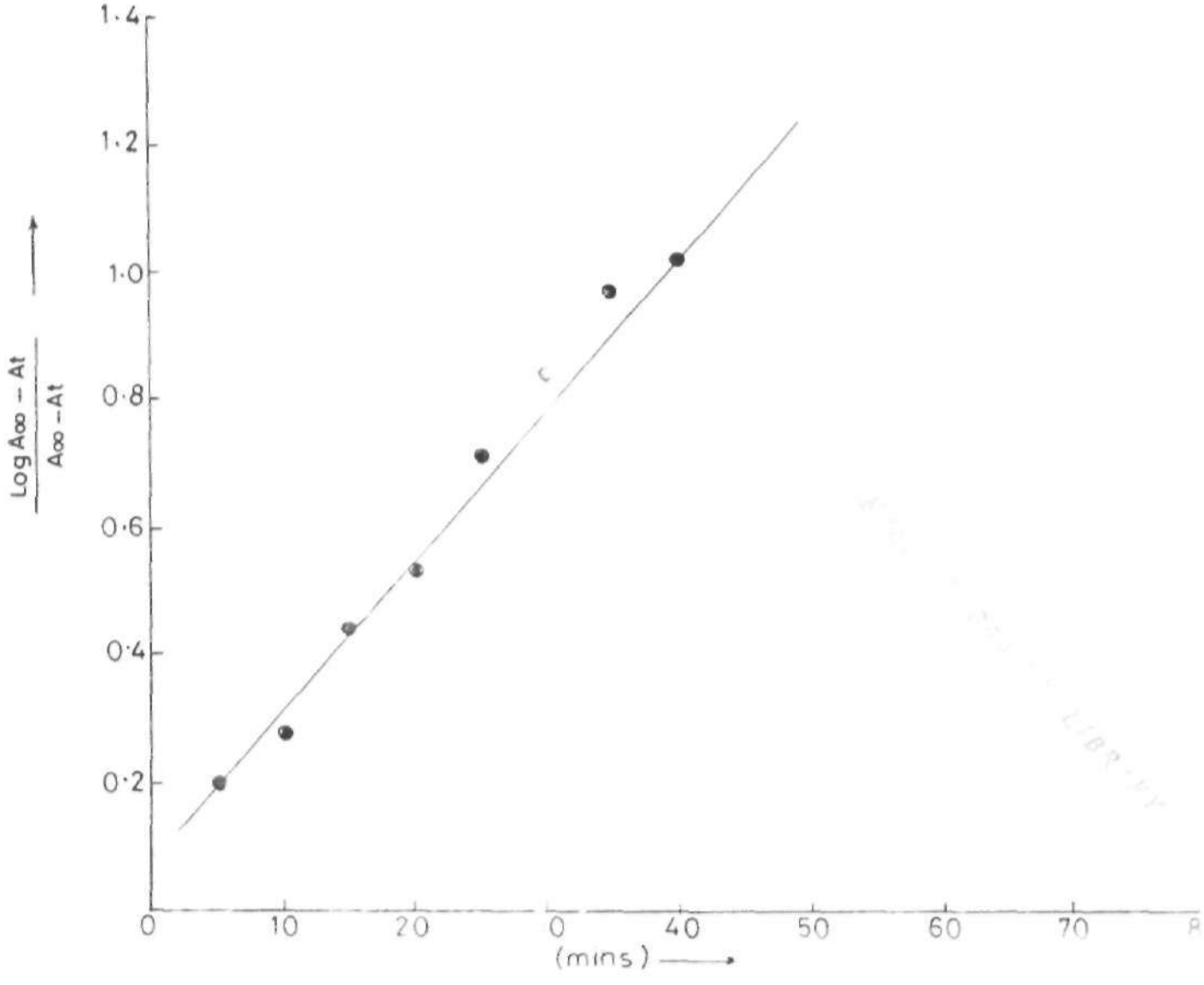


FIG. 7; LINEAR PLOT FOR A FIRST ORDER CYCLISATION OF N-CARBOBENZOXY-L-α-LEUCINE UNDE THE INFLUENCE OF ETHANOIC-ANHYDRIDE

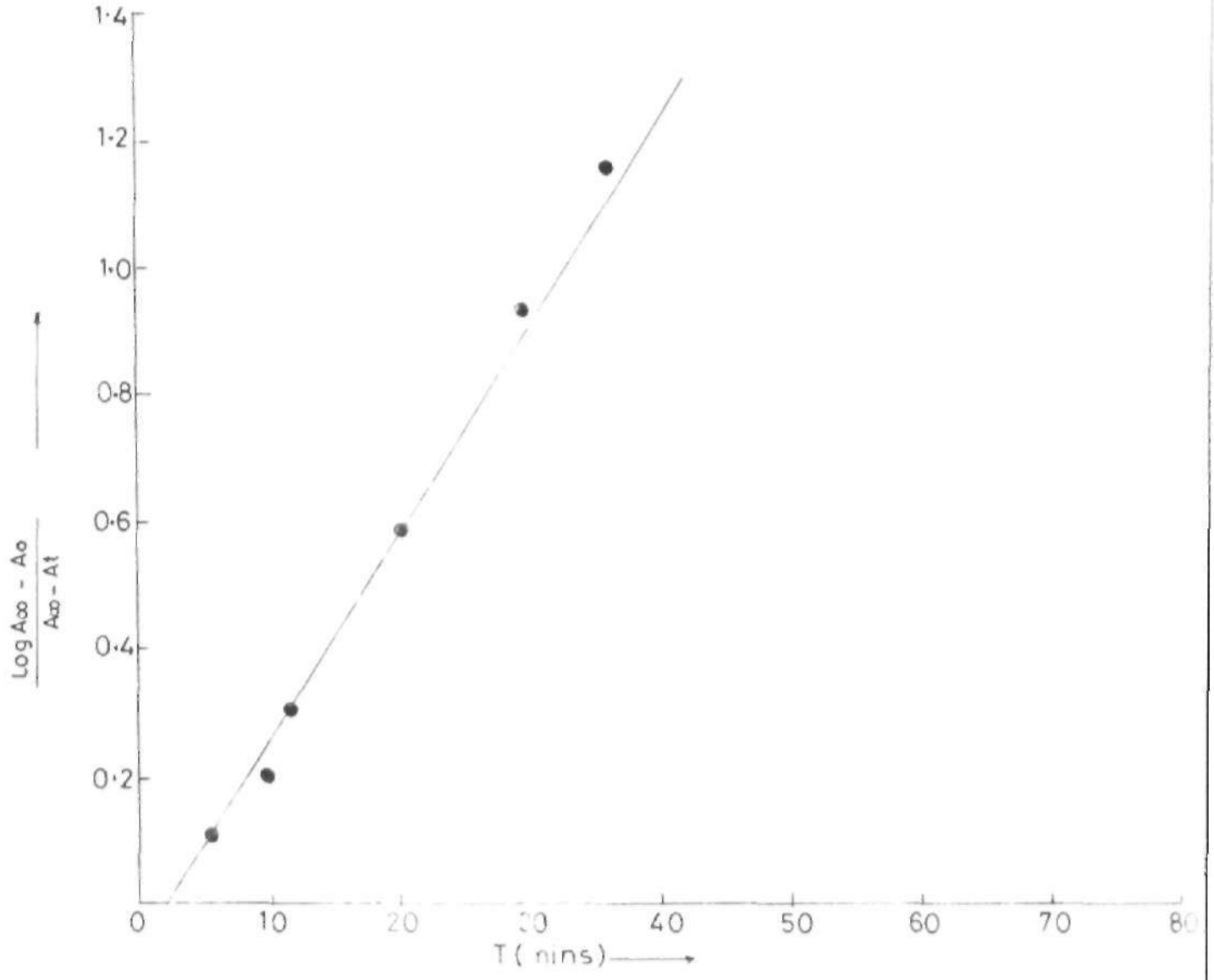


FIG.8: LINEAR PLOT FOR A FIRST ORDER CYCLISATION OF N-CARBOBENZOXY-L-α-VALINE UNDER THE INFLUNCE OF ETHANOIC ANHYDRIDE

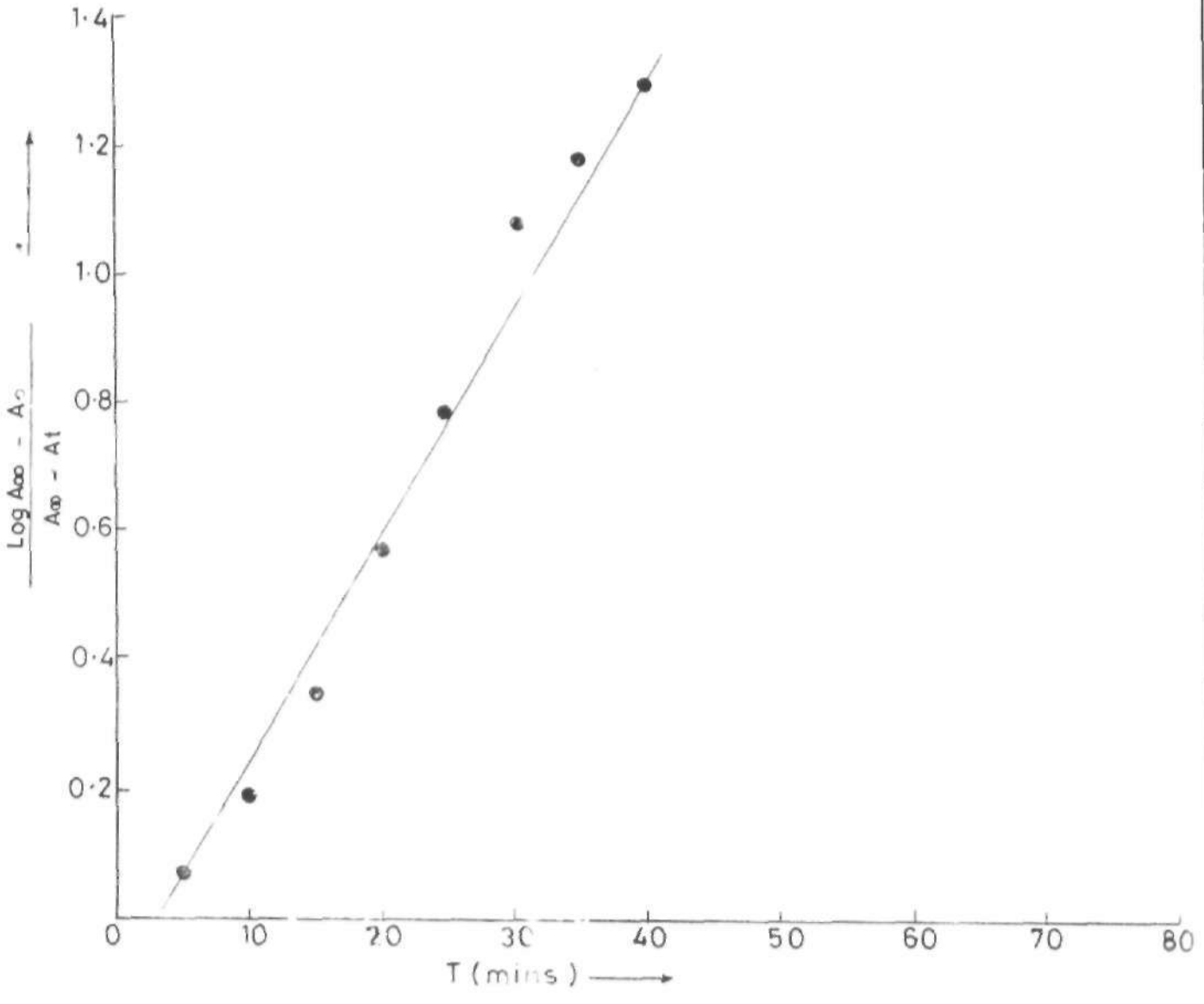


FIG-9: ULTRAVIOLET SPECTRA OF N-CARBOBENZOXY-L-α-PHENYLALANINE IN VARIOUS SOLVENTS

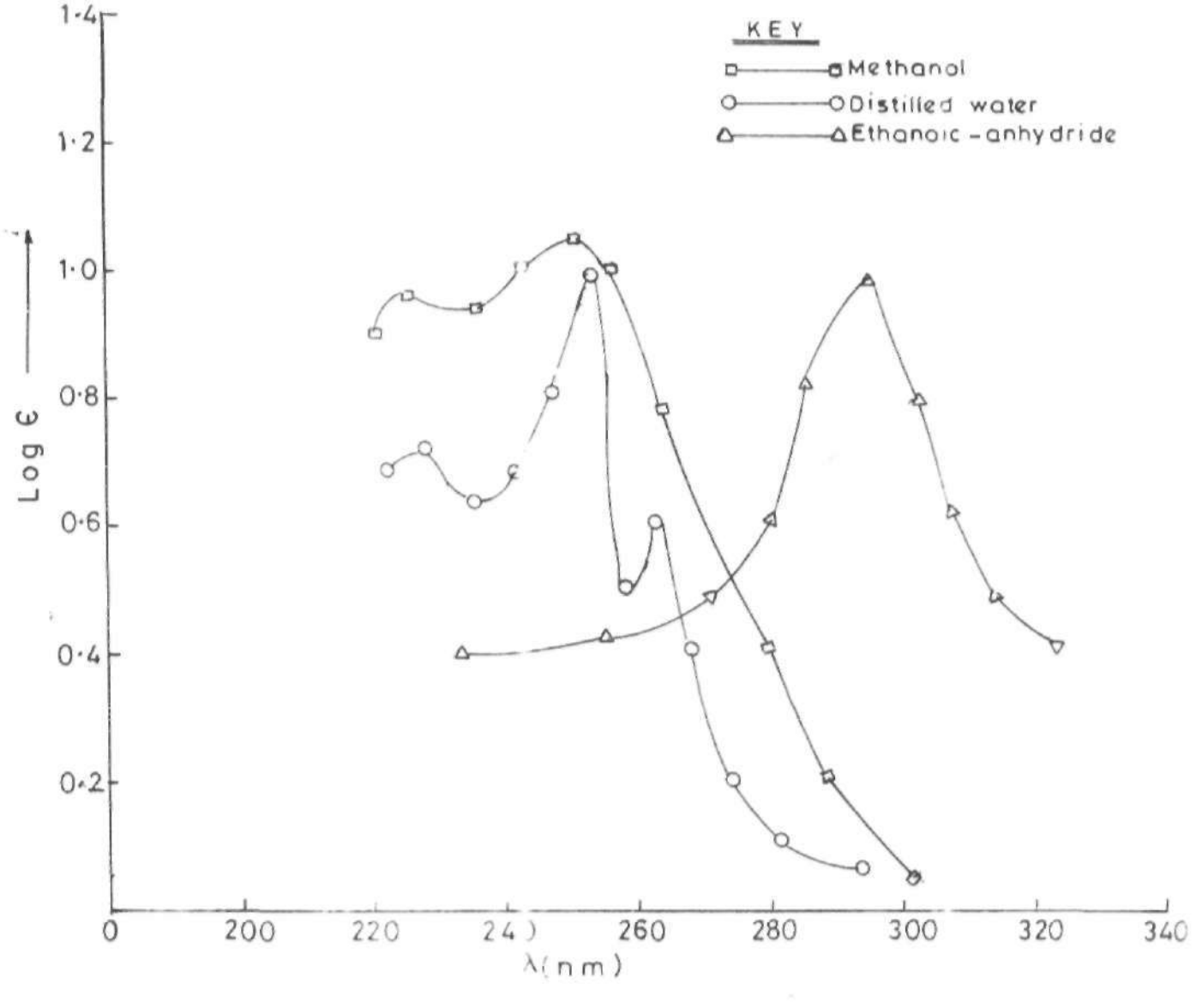


FIG.10: ULTRAVIOLET SPECTRA OF N-CARBOBENZOXY-L-∝- LEUCINE IN VARIOUS SOLVENTS

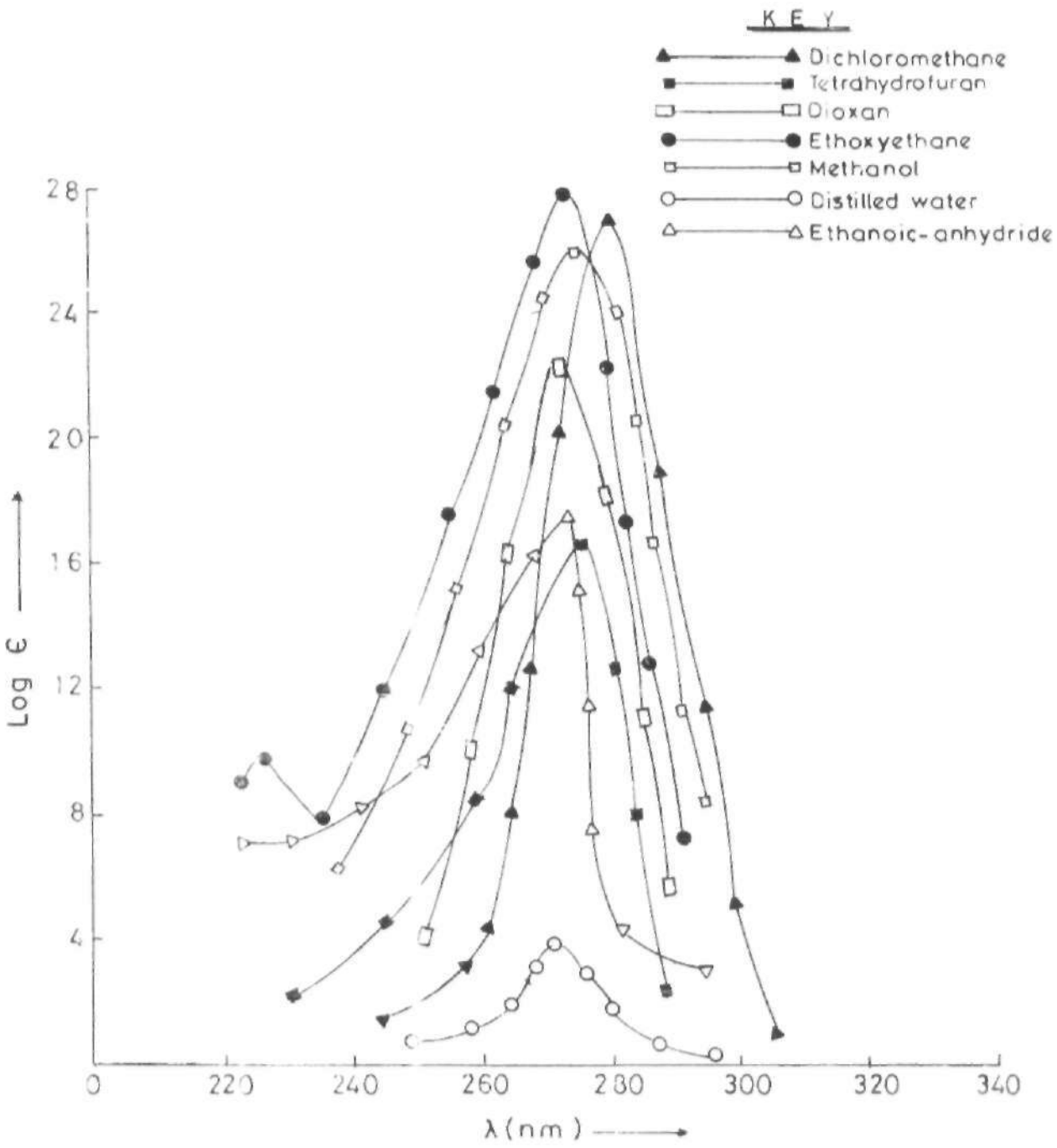


FIG-11:ULTRAVIOLET SPECTRA OF N-CARBOBENZOXY-L-∝- ALANINE IN VARIOUS SOLVENTS

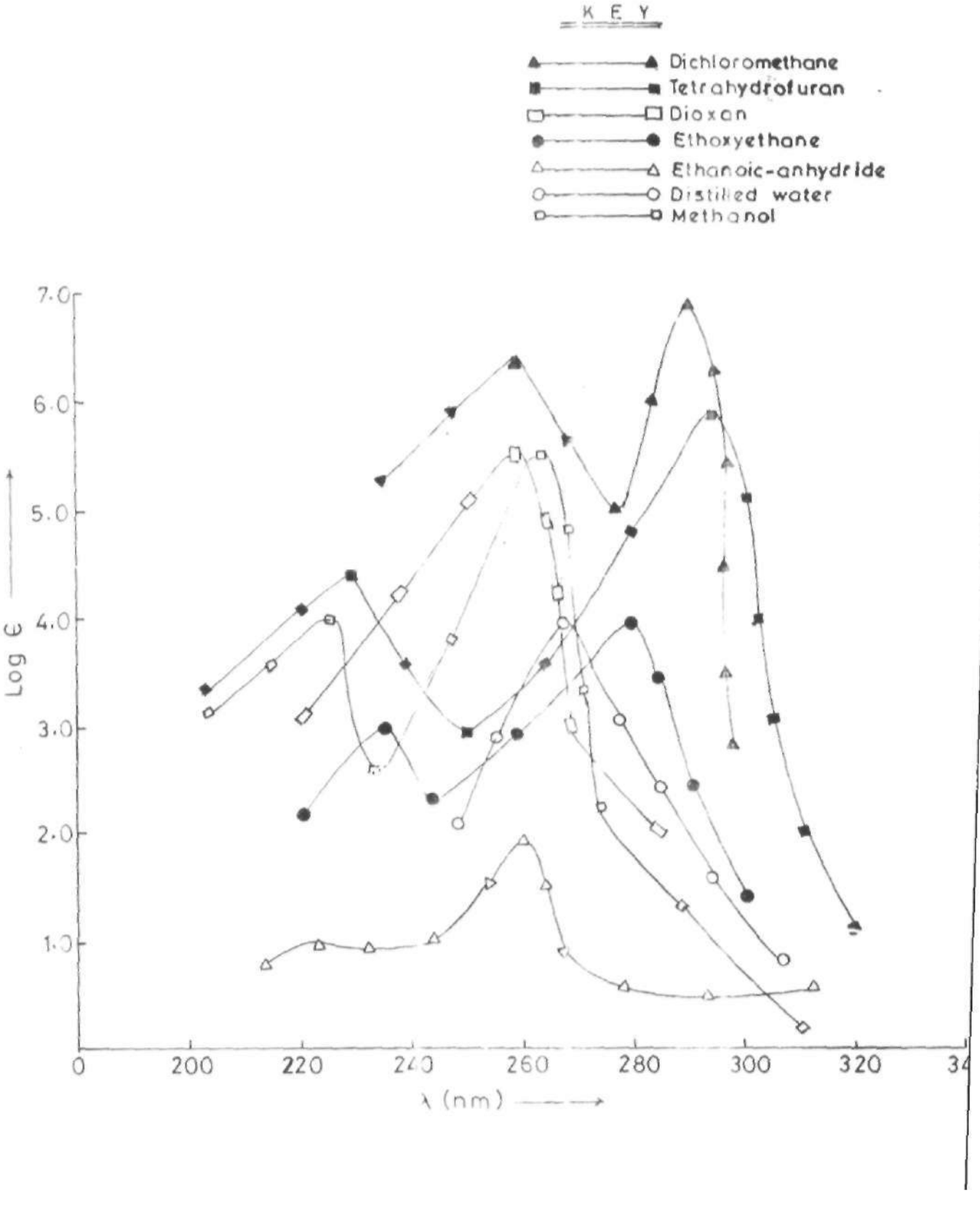


FIG.12: ULTRAVIOLET SPECTRA OF N-CARBOBENZOXY-L-α-VALINE IN VARIOUS SOLVENTS

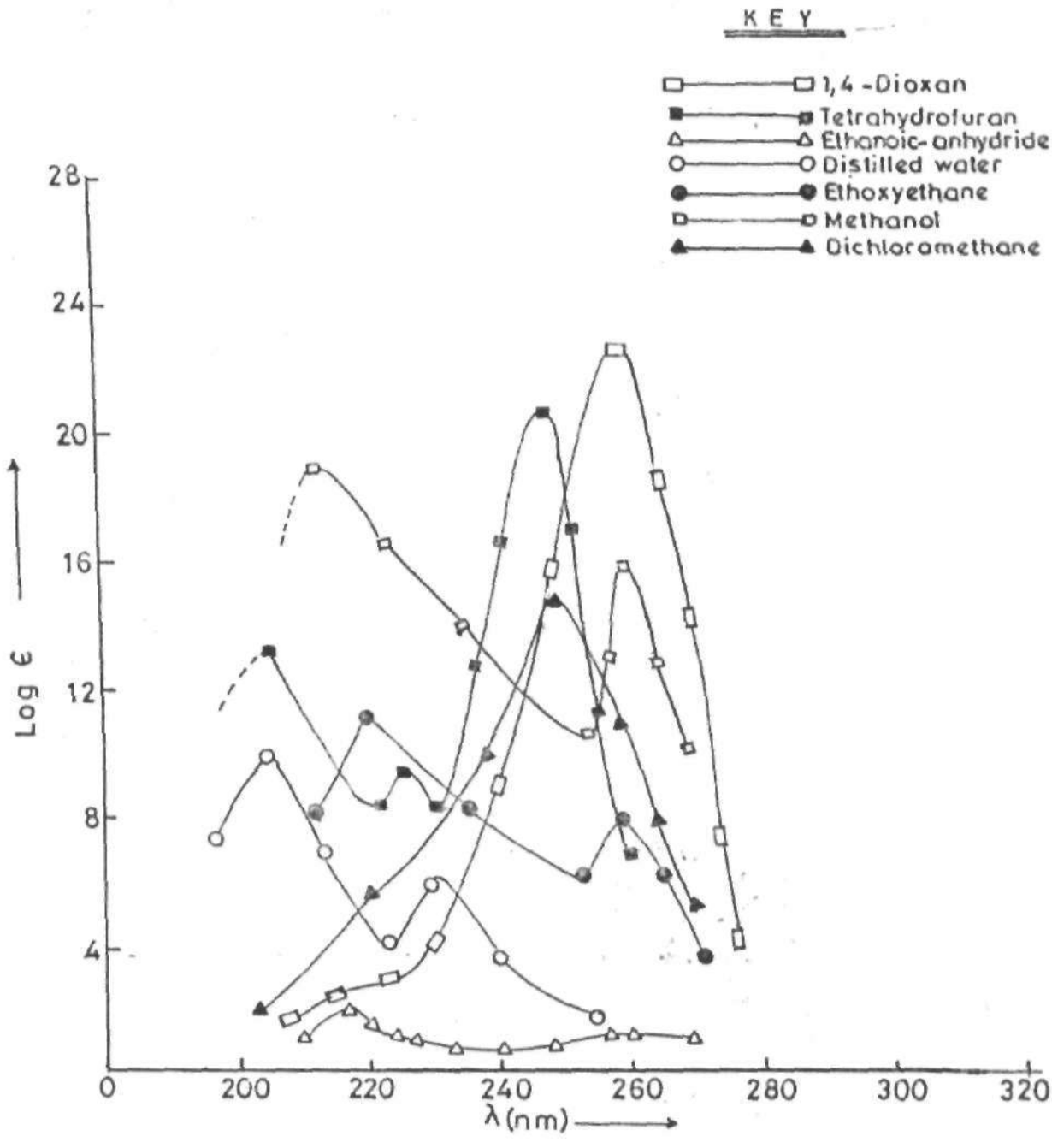


FIG.13: A PLOT OF LOG(k/k_o) AGAINST TAFT σ^* VALUES FOR CYCLISATION OF N-CARBOBENZOXY-L-α-AMINO ACID DERIVATIVES

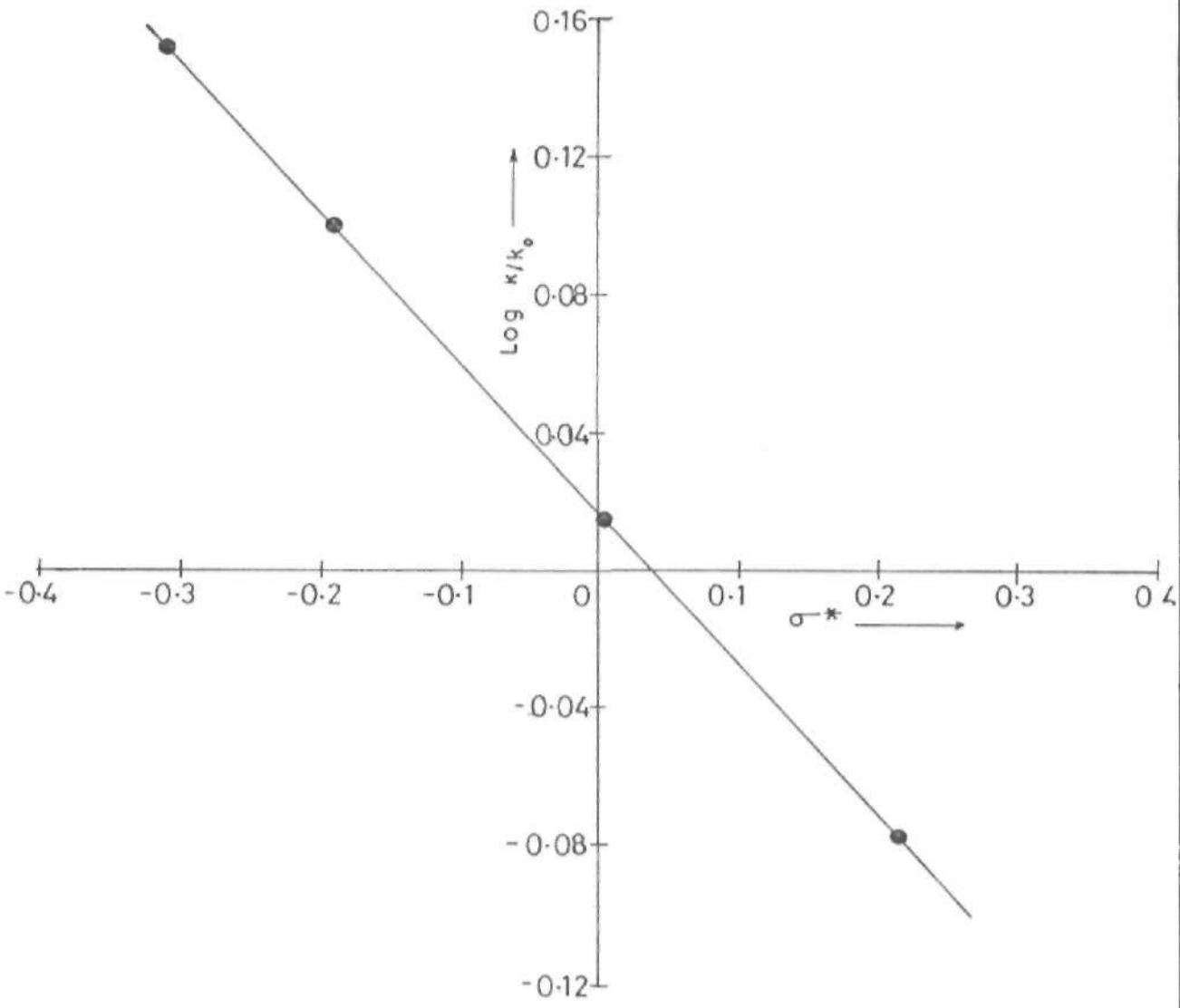


FIG.14 I R SPECTRUM OF N-CARBOBENZOXY-L- LEUCINE

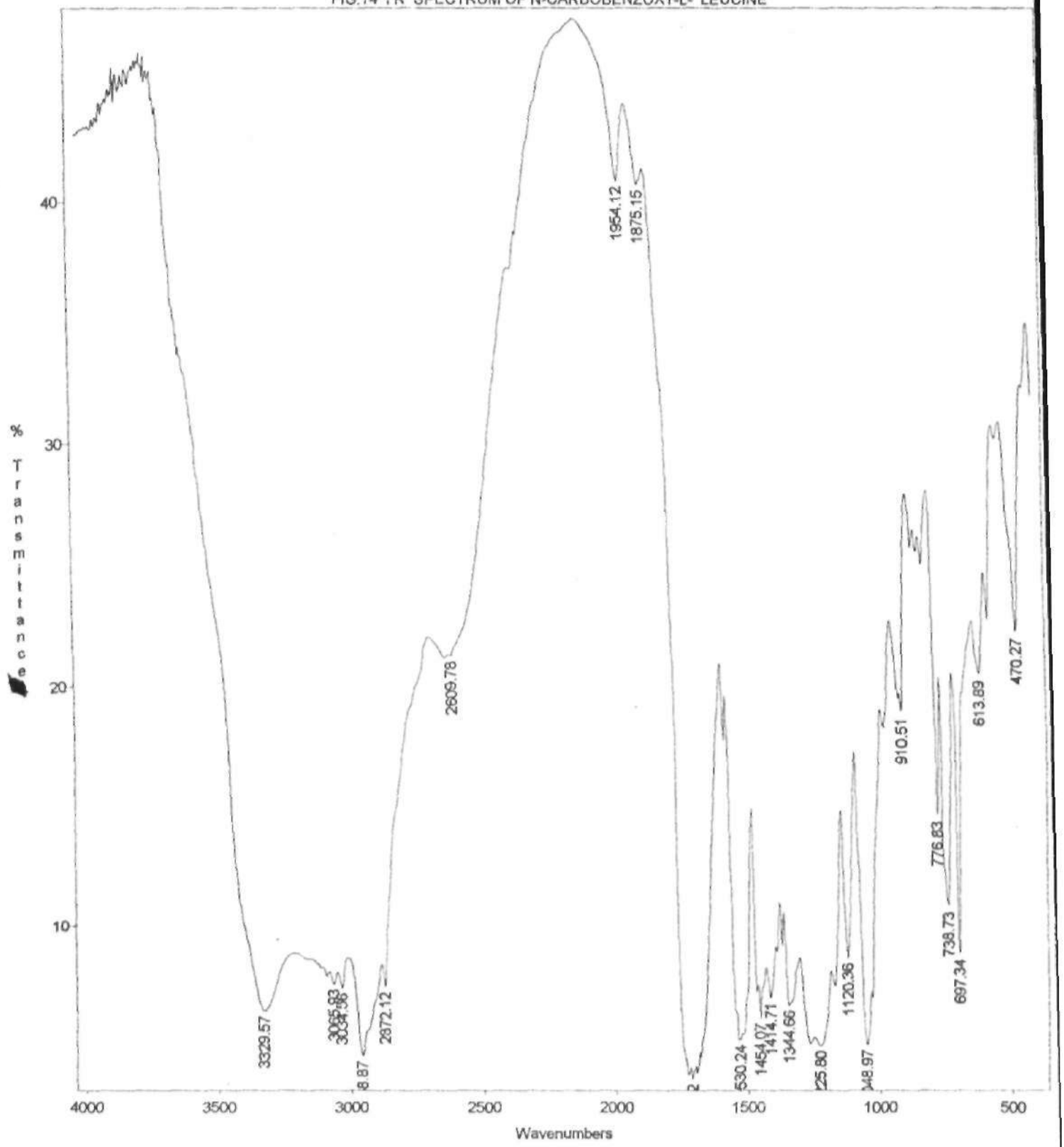
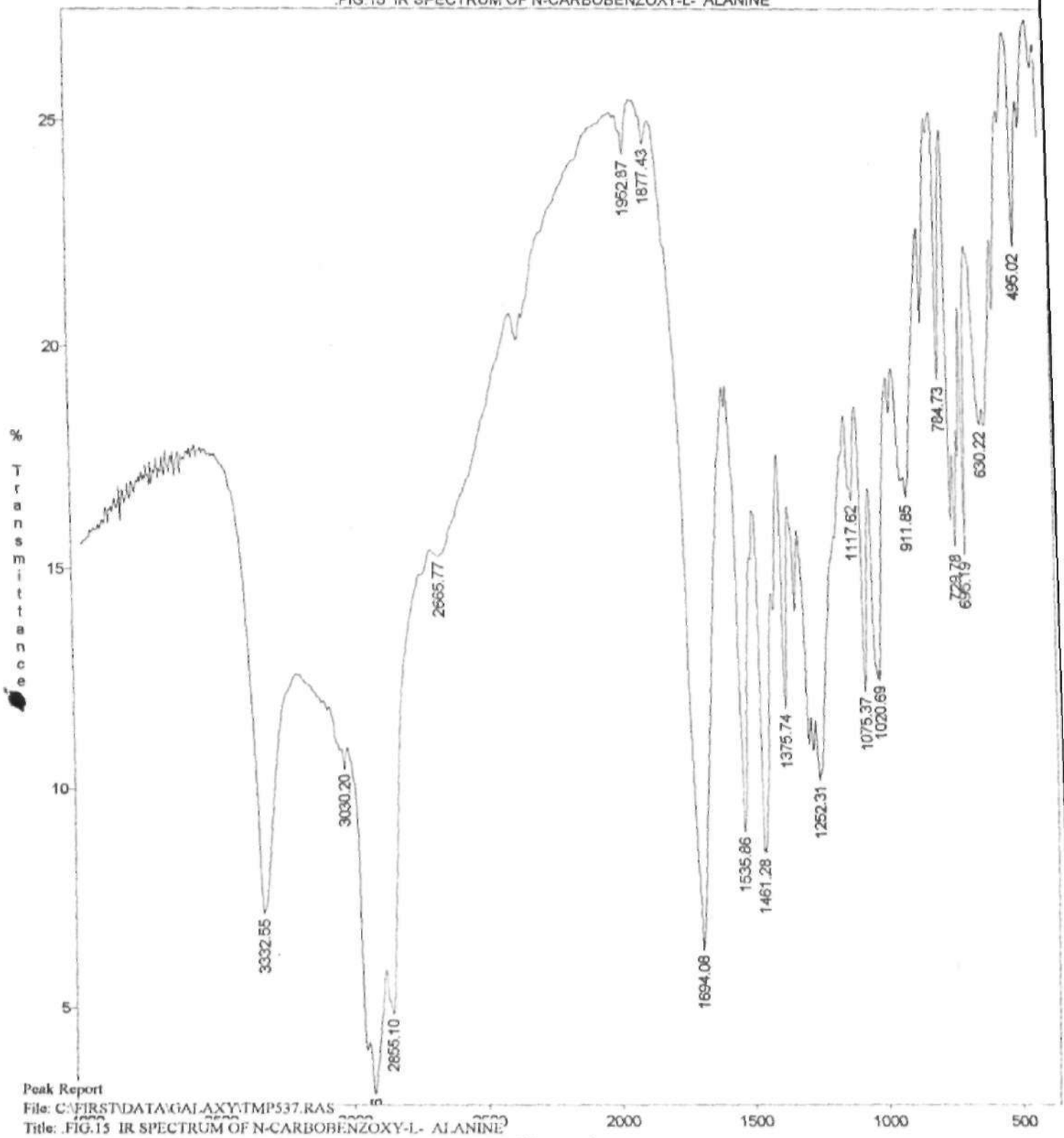


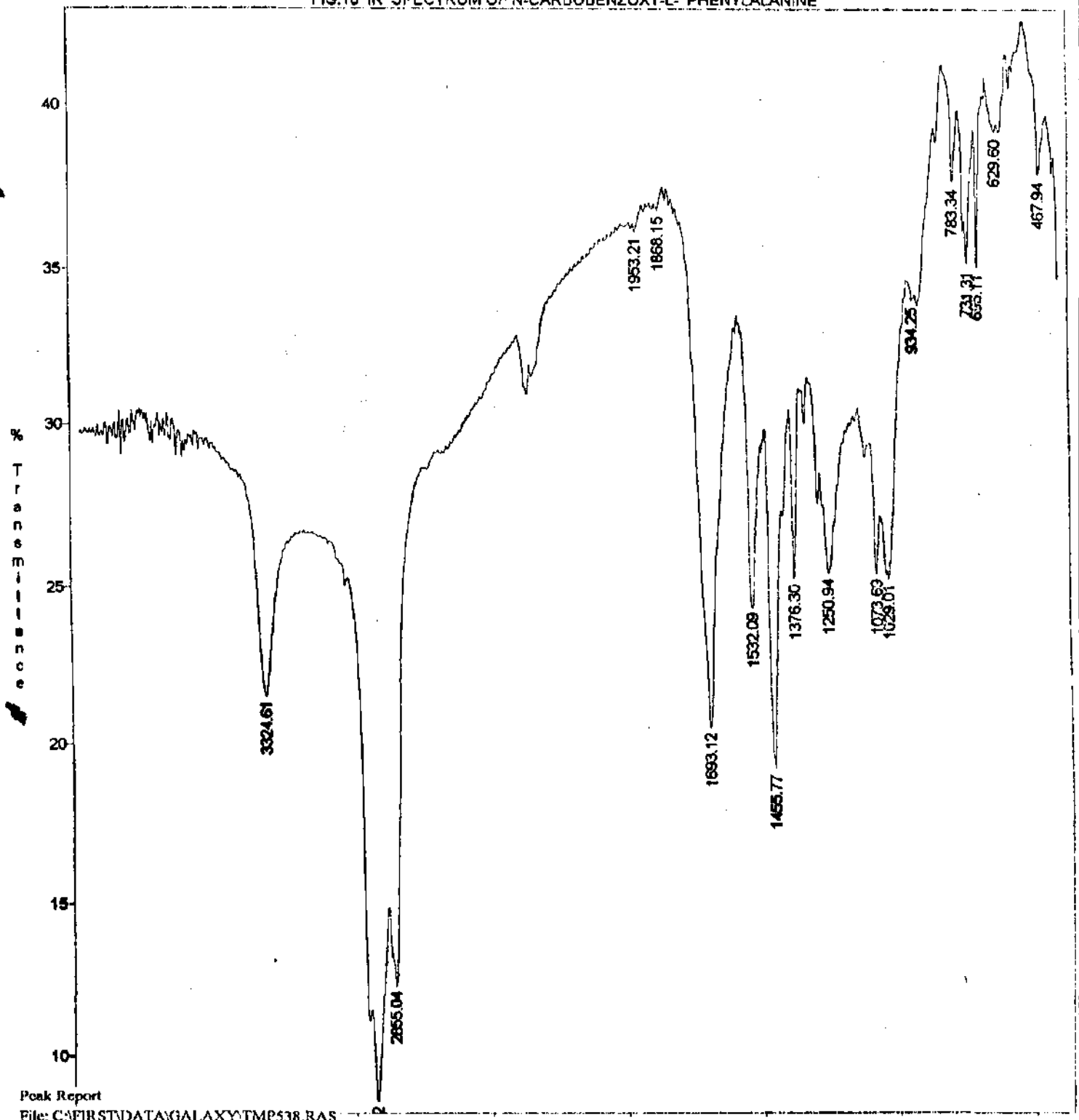
FIG.15 IR SPECTRUM OF N-CARBOBENZOXY-L- ALANINE



Peak Report
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Title: .FIG.15 IR SPECTRUM OF N-CARBOBENZOXY-L- ALANINE
Filter: Three Point Center of Gravity

cm-1	%T	cm-1	%T	cm-1	%T
3332.55	7.11	3030.20	10.40	2923.06	2.92
2855.10	4.81	2665.77	15.19	1952.87	24.18
1877.43	24.44	1694.08	6.26	1535.86	8.90
1461.28	8.41	1375.74	11.72	1252.31	10.08
1117.62	16.39	1075.37	12.10	1020.69	12.36
911.85	16.43	784.73	19.12	729.78	15.33

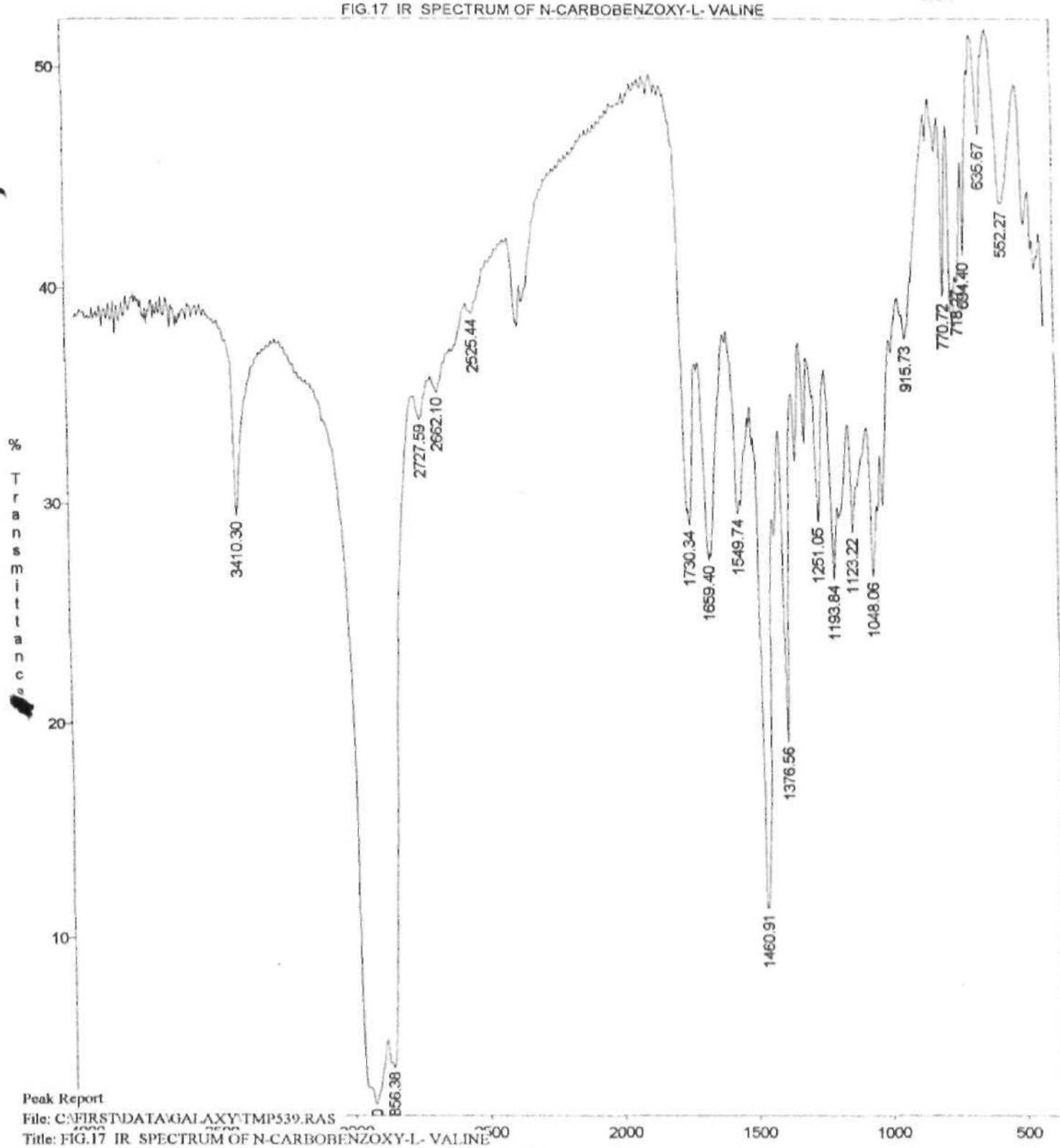
FIG.16 IR SPECTRUM OF N-CARBOBENZOXY-L- PHENYLALANINE



Peak Report
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Title: FIG.16 IR SPECTRUM OF N-CARBOBENZOXY-L- PHENYLALANINE
Filter: Three Point Center of Gravity

cm-1	%T	cm-1	%T	cm-1	%T
3324.61	21.53	2923.22	8.52	2855.04	12.32
1953.21	36.12	1868.15	36.77	1693.12	20.50
1532.09	24.32	1455.77	19.19	1376.30	25.20
1250.94	25.37	1073.69	25.36	1029.01	25.21
934.25	33.93	783.34	37.66	731.31	35.09
695.11	35.01	629.60	39.21	467.94	37.89

FIG.17 IR SPECTRUM OF N-CARBOBENZOXY-L- VALINE



Peak Report
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Title: FIG.17 IR SPECTRUM OF N-CARBOBENZOXY-L- VALINE
Filter: Three Point Center of Gravity

cm-1	%T	cm-1	%T	cm-1	%T
3410.30	29.37	2923.60	1.97	2856.38	3.75
2727.59	33.82	2662.10	35.07	2525.44	38.73
1730.34	28.86	1659.40	27.30	1549.74	29.37
1460.91	11.20	1376.56	18.91	1251.05	29.00
1193.84	26.40	1123.22	28.53	1048.06	26.52
915.73	37.47	770.72	39.43	718.27	40.08

FIG. 18 IR SPECTRUM OF 2-BENZYLOXY-4-ISOPROPYLOXALIN- 5 - ONE

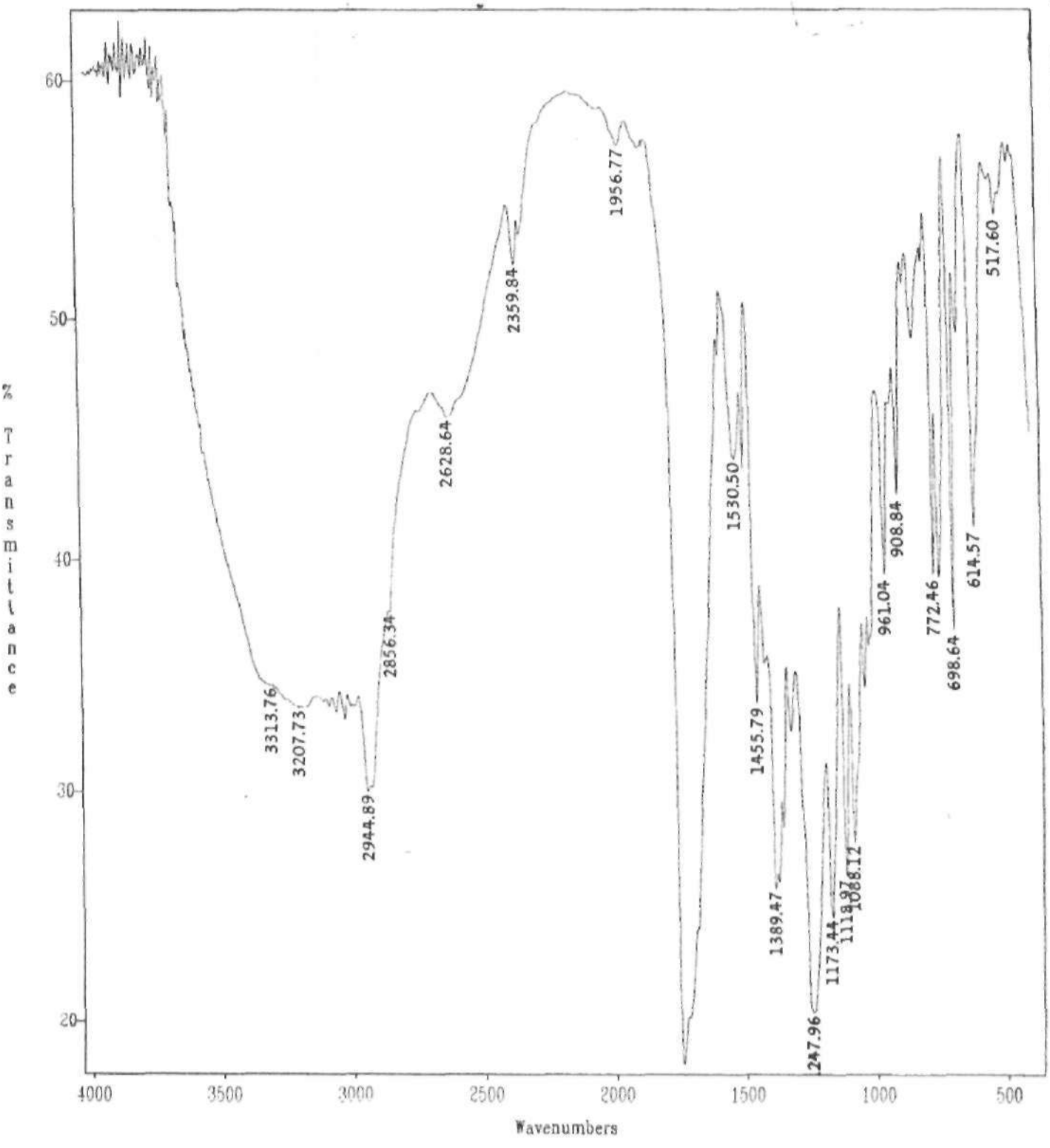


FIG. 19 IR SPECTRUM OF 2-BENZYLOXY - 4 - METHYLOXAZOLIN -5 - ON

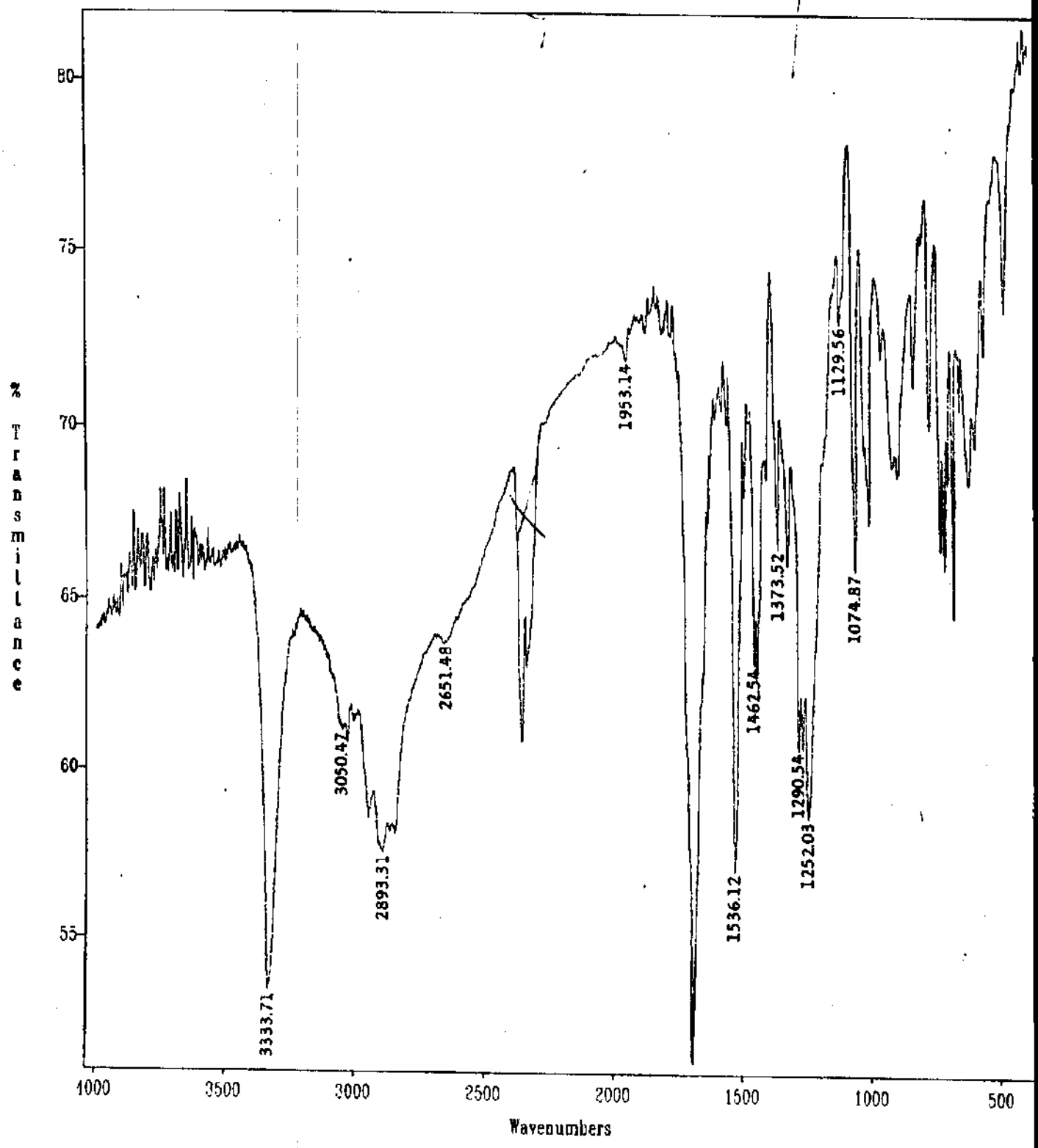


FIG.20. IR SPECTRUM OF 2 - BENZYLOXY - 4 - BENZYLOXAZOLIN-5-ON

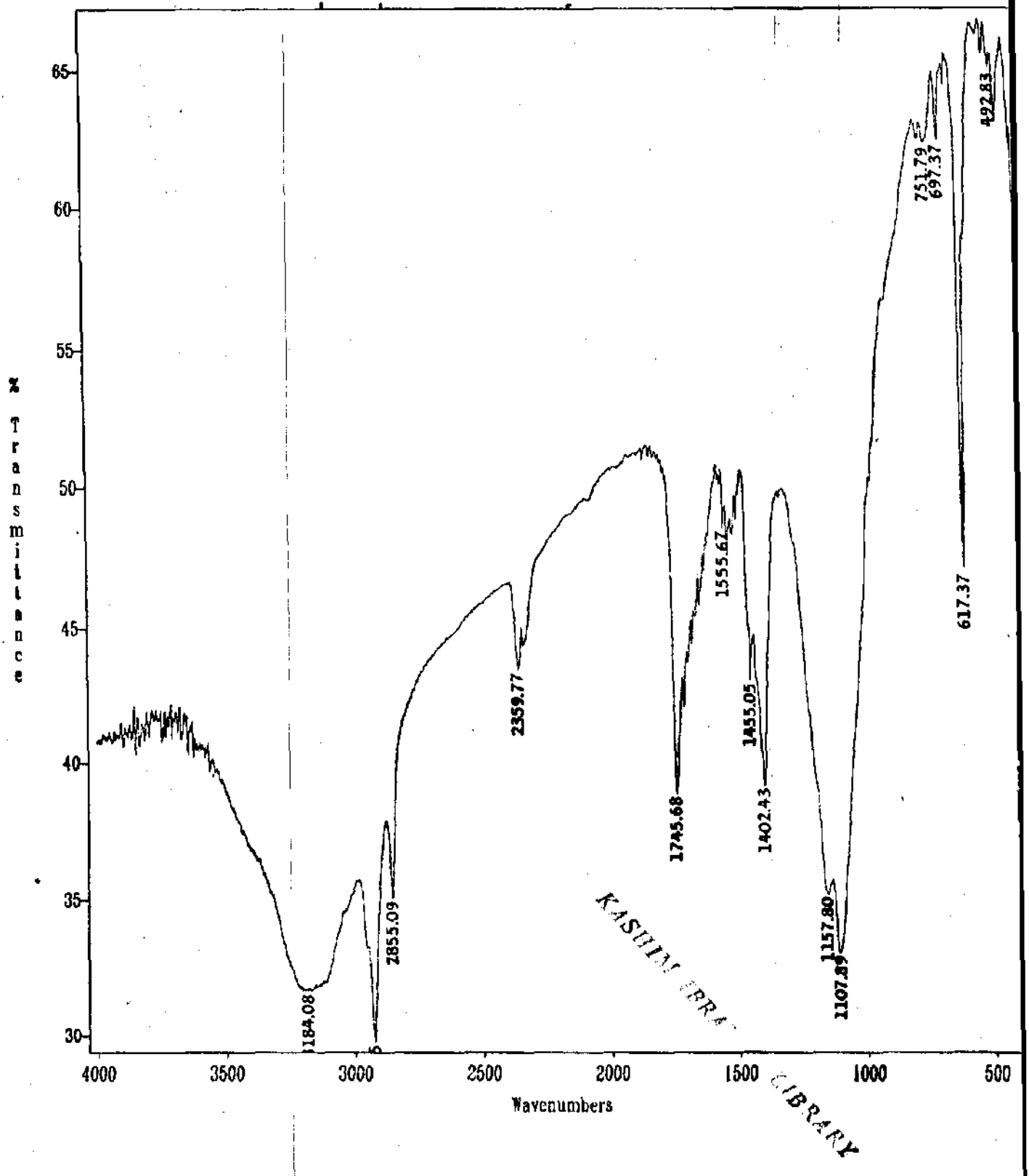
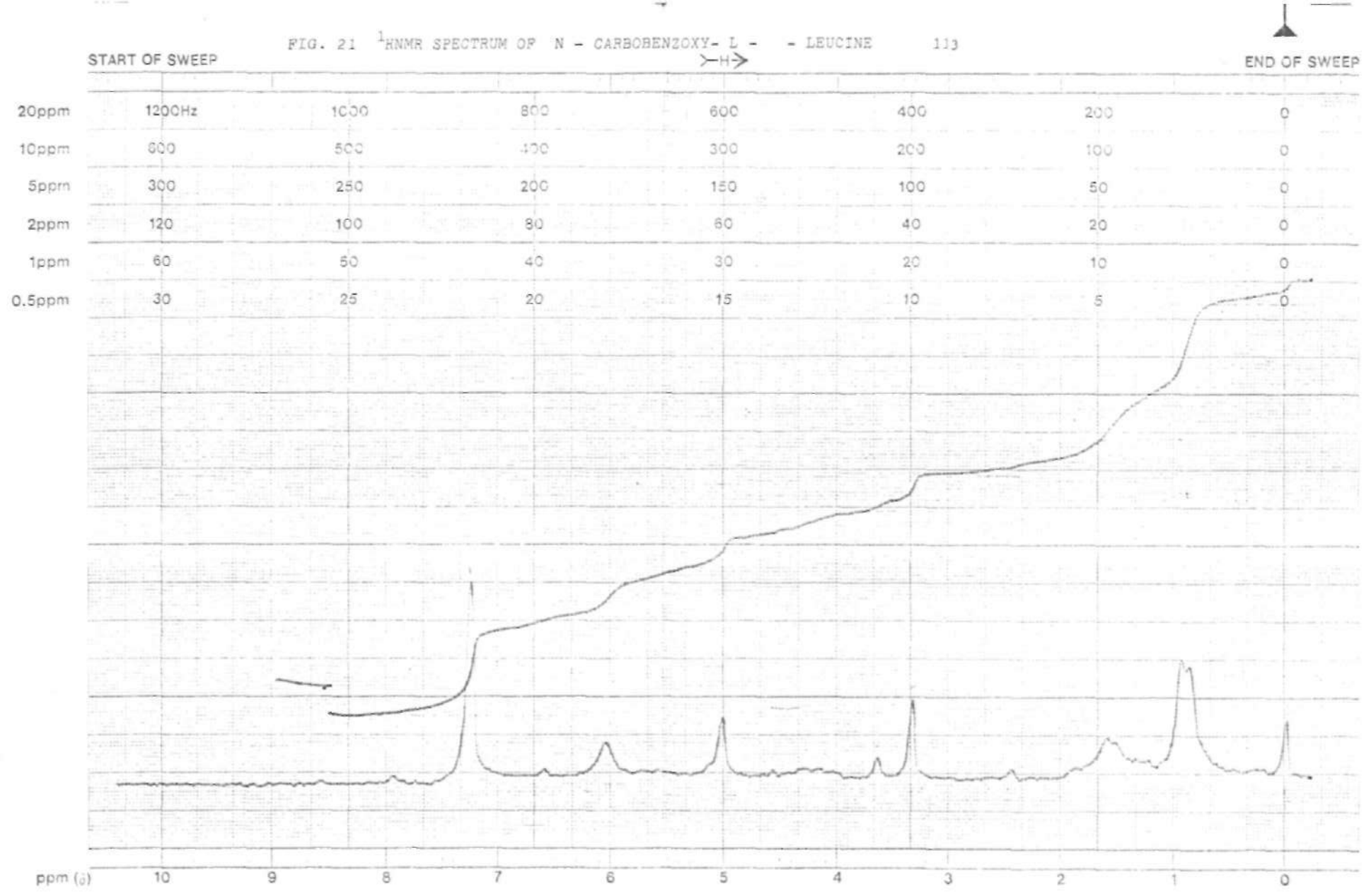


FIG. 21 ^{1}HNMR SPECTRUM OF N - CARBOBENZOXY- L - - LEUCINE 113



LOCK POS. _____ ppm
LOCK POWER _____ mG
DECOUPLE POS. _____ ppm
DECOUPLING POWER _____ mG

SPECTRUM AMPL. 100 IC
FILTER _____ sec
RF POWER 0.05 mG

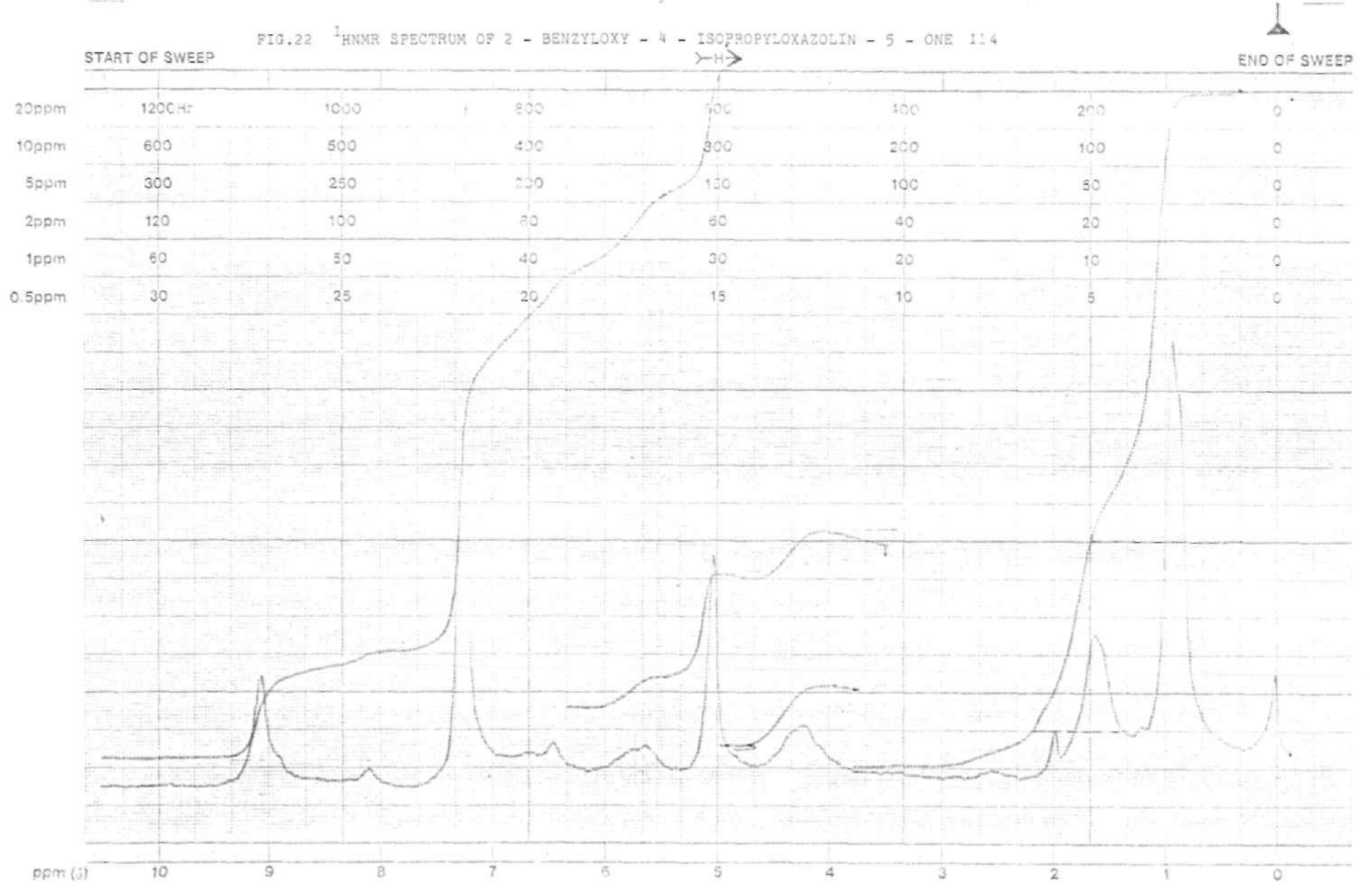
SWEEP TIME 1' 0 min
SWEEP WIDTH _____ ppm
END OF SWEEP 0 - 10 ppm

NUCLEUS ^{1}H
ZERO REF. _____
SAMPLE TEMP. _____ C

SAMPLE:
SOLVENT: CCl_4

OPERATOR Sajadi
DATE 14-05-98
SPECTRUM NO. _____

FIG.22 ^{1}HNMR SPECTRUM OF 2 - BENZYLOXY - 4 - ISOPROPYLOXAZOLIN - 5 - ONE 114



varian instrument division palo alto, california

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PART NO. 950209-05

EM-360, 60 MHz NMR SPECTROMETER

LOCK POS. _____ ppm
LOCK POWER _____ mG
DECOUPLE POS. _____ ppm
DECOUPLE POWER _____ mG

SPECTRUM AMPL. 0.82
FILTER _____ sec
RF POWER 0.05 mG

SWEEP TIME 1.0 min
SWEEP WIDTH _____ ppm
END OF SWEEP _____ ppm

NUCLEUS _____
ZERO REF. _____
SAMPLE TEMP. _____ C

SAMPLE:
SOLVENT: CCl4

OPERATOR Lychiba
DATE 04-05-85
SPECTRUM NO. _____

FIG. 23 ^{1}HNMR SPECTRUM OF N - CARBOBENZOXY - L - ALANINE

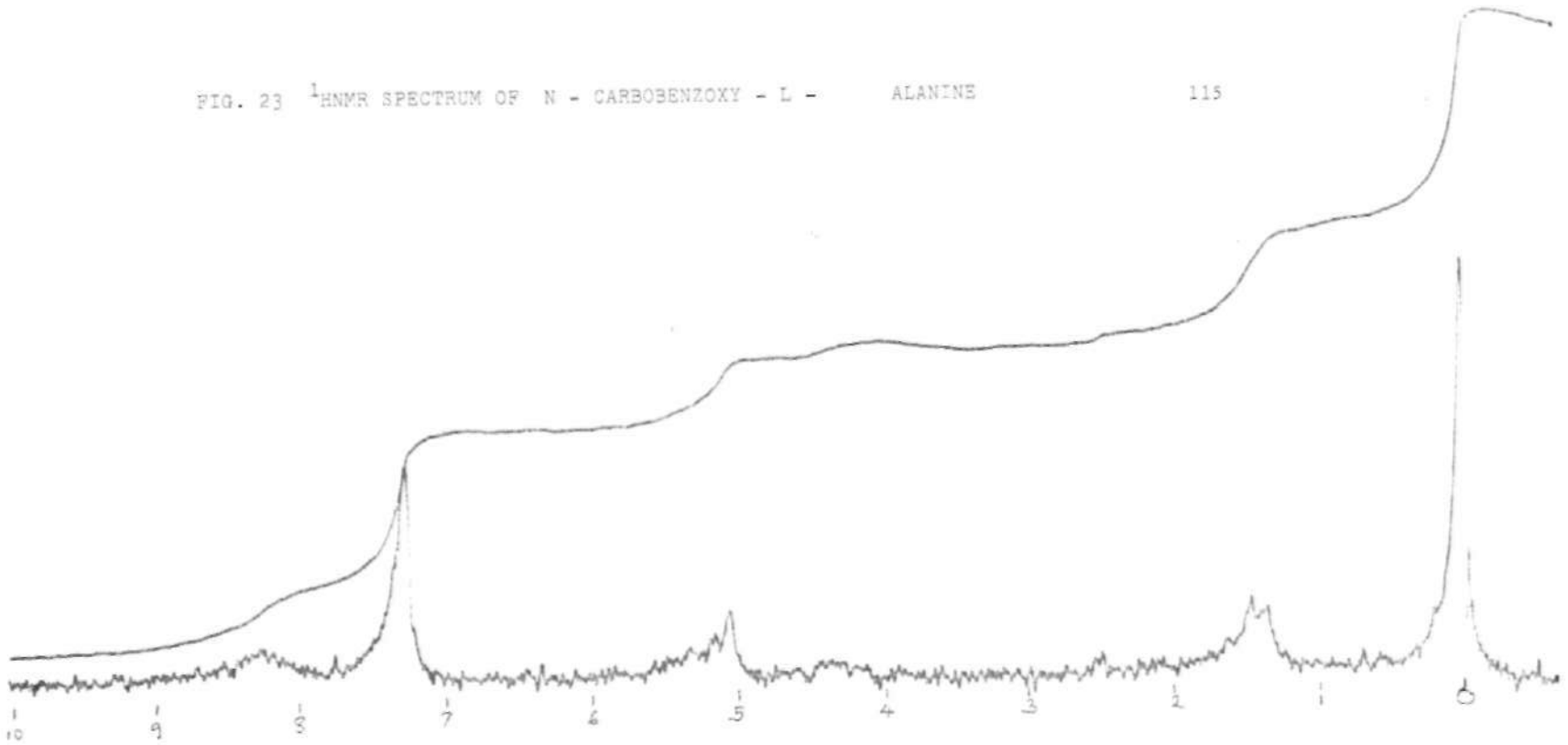
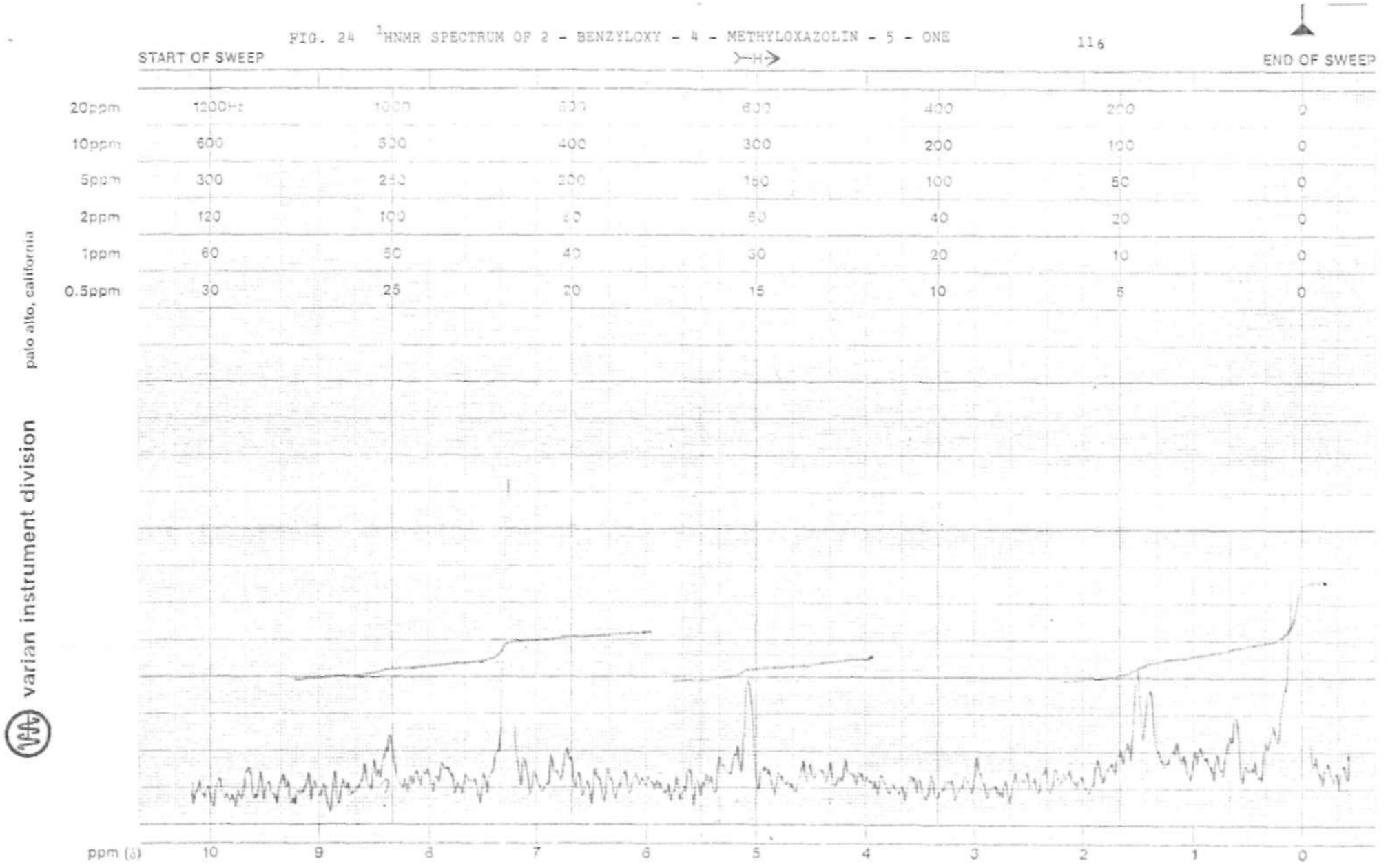


FIG. 24 ^{1}HNMR SPECTRUM OF 2 - BENZYLOXY - 4 - METHYLOXAZOLIN - 5 - ONE



varian instrument division palo alto, california
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PART NO. 950269-00

LOCK POS. _____ ppm
LOCK POWER _____ mG
DECOUPLE POS. _____ ppm
DECOUPLE POWER _____ mG

SPECTRUM AMP 100 RF
FILTER _____ sec
RF POWER 0.04 mG

SWEEP TIME 1.0 min
SWEEP WIDTH _____ ppm
END OF SWEEP _____ ppm

NUCLEUS H
ZERO REF. _____
SAMPLE TEMP. _____ C

SAMPLE:
SOLVENT: [redacted]

OPERATOR [redacted]
DATE 12-10-98
SPECTRUM NO. _____

FIG. 26 ^{1}HNMR SPECTRUM OF 2 - BENZYLOXY - 4- BENZYLOXAZOLIN-5-ONE

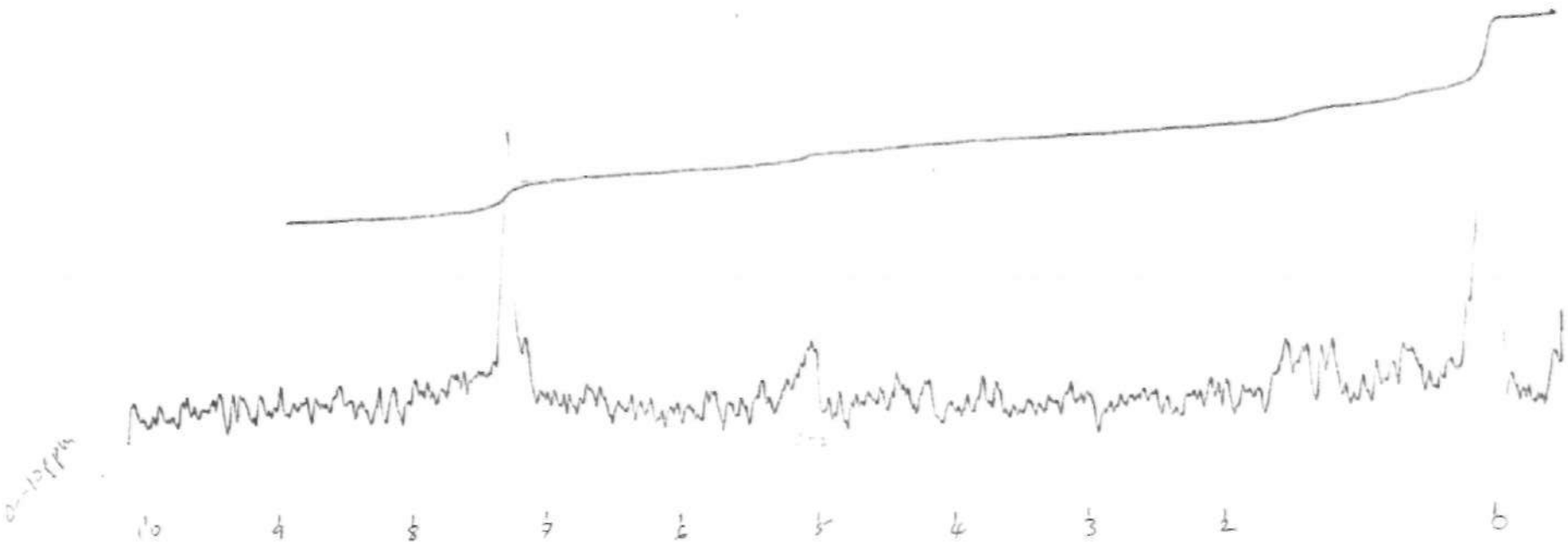


FIG. 25 ^{1}HNMR SPECTRUM OF N - CARBOBENZOXY - L - - PHENYLALANINE

