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A T H E S I S

submitted to

THE UNIVERSITY OF GLASGOW

by

JAMES CHILTON

in fulfilment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

SEPTEMBER, 1961

The Department of Pharmacy,
The Royal College of Science
and Technology,
Glasgow.

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STUDIES IN COMPOUNDS

RELATED TO ERGOMETRINE

The author wishes to thank Professor J.B. Stenlake, under whose guidance this work was carried out, for his most helpful suggestions and criticism which have proved invaluable, also Professor J.P. Todd for his help and encouragement.

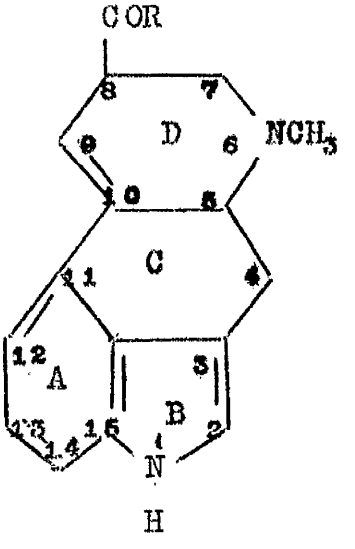
C O N T E N T S

PART I

INTRODUCTION

Synthetic Oxytocics Related to the Ergot Alkaloids

Elucidation of the structure of the ergot alkaloids, which has been reviewed by Glenn[1], has led to the synthesis or partial synthesis of many related compounds in the hope that these would similarly show oxytocic activity. By this means, in addition to the possibility of discovering compounds of value in obstetrics, some general relationship between chemical structure and oxytocic activity may have been demonstrable. Unfortunately, despite the synthesis of numerous compounds with significant oxytocic activity, these aims remain largely unrealised; no completely synthetic compound related to lysergic acid (I, R = OH) has found general clinical acceptance as an oxytocic, nor has any structural feature or combination of features been shown to be consistently associated with oxytocic activity.



I

Oxytocics have been classified by de Jongh[2] into two groups each with a characteristic type of action and corresponding clinical application. The first group produces an accentuation of the normal rhythmic contractions of the uterus at term and has a transient effect - properties which are ideal for the induction of labour. For this purpose oxytocin is normally used, since it combines the desired type of oxytocic action with a minimum of side-effects. Oxytocin was first synthesised by du Vigneaud and co-workers[3], while the synthetic route of Boissonnas and his colleagues[4] has led to the commercial production of a synthetic oxytocin ('Syntocinon', Sandoz Ltd.). The synthesis of peptide analogues of higher potency has recently been reported,[5,6,7] but these investigations are outwith the scope of the present work and will not be discussed further.

More powerful and sustained action on the uterus, leading to tetanic contractions in larger dosage, is shown by the second group of oxytocics of which ergometrine (I; R = $NHCH(CH_3)CH_2OH$) is typical. This action is employed in obstetrics to contract the uterus at the completion of labour. Post-partum haemorrhage is thereby limited by the pressure of the constricted uterine muscle mass on the blood vessels of the evacuated uterus. Small repeated doses may also be given during the puerperium to hasten

involution of the uterus in cases where this would otherwise be delayed.[8] Oxytocic action of this type would be expected from compounds related to or derived from lysergic acid.

Evaluation of potential oxytocics is complicated by the difficulty in assessment of oxytocic activity due to the extreme variability of uterine response. Results may vary widely depending on the choice of technique (isolated tissue preparations giving different responses from uteri in situ), upon the choice of experimental animal or on the endocrine balance of the same animal on the different occasions.[9] In addition the relative potencies of oxytocics on the human uterus have been shown on occasions to bear little relationship to those observed in experimental animals.[2,10] This makes it almost impossible to compare oxytocic activities as determined by different techniques and renders clinical assessment of pharmacological results even more difficult than usual. No attempt will therefore be made to compare quantitatively the oxytocic activities of compounds in the present discussion, but only to indicate those which showed significant activity under the experimental conditions which were employed.

Almost all the structural features which have been shown to be associated with the oxytocic activity of lysergic acid

derivatives (unsaturation in the 9-10 position,[11] configuration at $C_{(8)}$, nature and configuration of the substituent at $C_{(8)}$[12]) are associated with Ring D of the lysergic acid molecule (I, R = OH). It is not surprising therefore that most synthetic oxytocics modelled on lysergic acid have included structures related to this ring or to open-chain analogues of it; that is to substituted ω-amino acids.

Derivatives of Aliphatic ω-Amino Acids.

(a) 2-Substituted 3-Aminopropionic Acid Derivatives. Despite the similarity of such compounds to Ring D of lysergic acid, only one, 2-benzyl-3-methylaminopropionic acid (II) has been reported.[13] This compound was prepared by a Mannich condensation of benzylmalonic acid, formaldehyde and methylamine, followed by decarboxylation.

$$Ph.CH_2CH(COOH)_2 + H.CHO + H_2NMe \longrightarrow Ph.CH_2.C.(COOH)_2CH_2NHMe$$

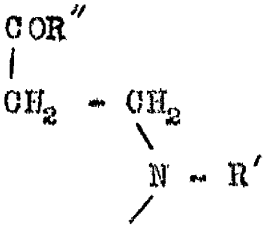
$$\downarrow \text{ heat}$$

$$Ph.CH_2.CH(COOH)CH_2NHMe$$

(II)

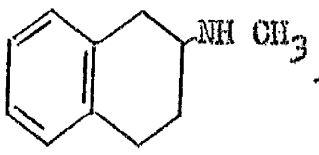
Yields were poor and the product tended to decompose with formation of methylamine and benzylacrylic acid. No oxytocic activity was reported.

(b) Derivatives of N-Substituted Aliphatic ω-Amino Acids (III). A wide variety of carboxyl substituents in compounds of this type has been reported (III; R" = OH, OCH_3 to OC_5H_{11}, NH_2, $NHCH_3$ to NHC_5H_{11}, $N(CH_3)_2$, $N(C_2H_5)_2$ etc). Although such simple esters and amides of lysergic acid have little or no oxytocic activity, many synthetic analogues of this type are active as oxytocics. Only a few alkanolamides (R" = $NHCH_2CH_2OH$ or $NHCH(CH_3)CH_2OH$) have been reported despite the fact that lysergic acid derivative of this type are most potent oxytocics.

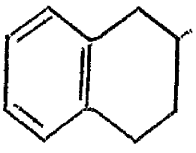


(III)

A direct synthesis of the propanolamide of N-tetrahydronaphthyl-N-methyl-3-aminopropionic acid (IV) has been claimed by Marini-Bettolo and co-workers.[14,16]



$+ BrCH_2.CH_2CONHCH(CH_3)CH_2OH$



$N(CH_3).CH_2CH_2CONH.CH(CH_3)CH_2OH$

(IV)

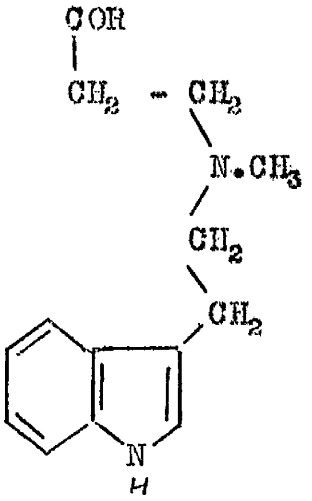
The intermediate, 3-bromopropionic acid propanolamide was stated to have been prepared from the corresponding acid chloride and 2-aminopropanol. Both the intermediate and final products are described as oils, no solid derivatives are described, no yields are quoted and the only analytical figures are for nitrogen. In a later paper[16] a melting point for the hydrochloride of the final product is given (an unusual derivative for amino-alcohols) but no analytical or other data. It is interesting to note that no further alkanolamides have been described in the large subsequent output of this school, despite the marked oxytocic activity claimed for this compound.

Alkanolamides have also been prepared in this series from the corresponding acid azides, the method used by Stoll[12] for his partial synthesis of ergometrine. Baltzly and co-workers[1] prepared the ethanolamides of ring-substituted N-(2'-phenylethyl)-N-methyl-3-aminopropionic acids by reaction of the acid azide with ethanolamine. Yields were poor due to incomplete extraction of the water-soluble azide from the aqueous reaction mixture. The products showed less oxytocic action than the corresponding methyl esters, in marked contrast to the related lysergic acid derivatives.

The ease of addition of amines to acrylic acid derivatives and the ready reactivity of most ω-halogeno aliphatic acids with

amines has led to the synthesis of ω-amino aliphatic acids with a wide range of N-substituents.

Relating these N-substituted compounds to lysergic acid, ring C may be replaced by a fragment consisting of two methylene groups, rings A and B being represented by an indole nucleus (V).

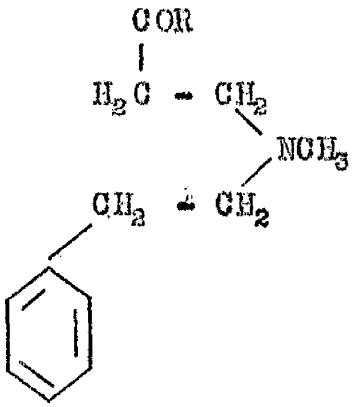


(V)

Compounds of this type (V; R = $N(C_2H_5)_2$ and R = OCH_3) have been prepared[16] and shown to have slight oxytocic action.

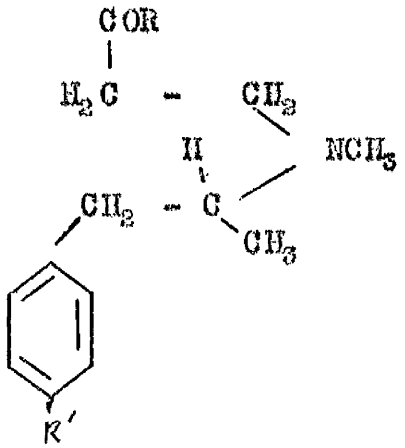
If ring B is omitted and ring C again represented by two methylene groups (VI), some oxytocic activity is again shown, this being greatly potentiated by the inclusion of methoxy ring-substituents.[17] The ethyl ester of the 3,4-dimethoxy-phenyl compound showed a maximum activity amongst the compounds tested. The same workers[18] showed that shortening of the chain

between the carboxyl and amino groups to form aminoacetic acid derivatives resulted in loss of oxytocic action, but that increase in length of this chain up to six methylene groups did not adversely affect activity.



(VI)

Related compounds with a centre of asymmetry corresponding to that at $C_{(5)}$ in lysergic acid (VII) were prepared by Plieninger.[19]

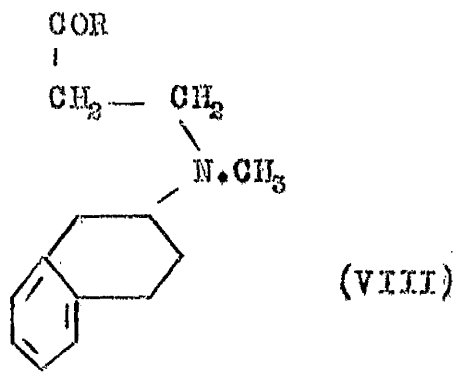


(VII)

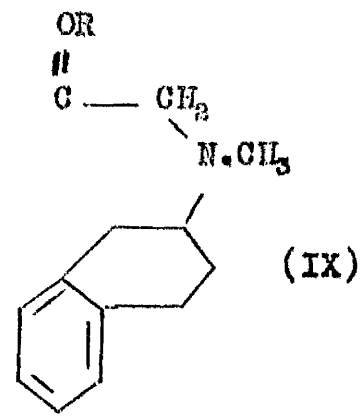
The two stereoisomeric forms differed in their oxytocic action,

maximum activity being shown by the ethyl ester of the laevo form having a paramethoxy ring-substituent (VII, R = OC_2H_5, R' = OCH_3). The usefulness of these compounds was limited by their low stability in aqueous solution.

A series of 3-aminopropionic acid derivatives (VIII) with a saturated ring system corresponding to that of ring C in lysergic acid and an aromatic ring corresponding to ring A has been prepared by Marini-Bettolo and co-workers from 3-halogenopropionic acid derivatives and 2-methylamino-1,2,3,4-tetrahydronaphthalene.



(VIII)



(IX)

Compound (VIII; R = $N(C_2H_5)_2$) was shown to have activity on the isolated uterus of the rat and guinea pig comparable with that of ergometrine.[20] The corresponding glycinamide derivative (IX; R = $N(C_2H_5)_2$) was also shown to have an oxytocic action in animal experiments,[20,21] although independent clinical investiga-

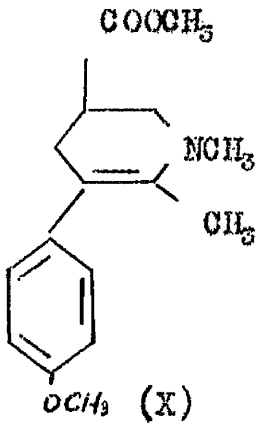
tion[10] showed it to be inactive on the human uterus in doses up to 20 mg. This may be contrasted with the findings of Baltzly[18] for N-(2-phenylethyl)-amino esters and also with the work of Gearien and Liska[22] on similar N-(2-naphthyl)-substituted amino-acid amides, in both of which oxytocic activity in animals was shown only by compounds with two or more methylene groups separating the amino and carboxyl functions.

Confirmation of Marini-Bettolo's findings was obtained when much simpler glycinamides were shown to have oxytocic activity in animal experiments.[9] Even such a simple compound as N,N,N',N'-tetraethylglycinamide was active on cat and rabbit uteri in situ and in vitro.[21,23] The resemblance of these compounds to ergometrine is so remote however that their mode of action may be completely unrelated to that of the ergot alkaloids.

Derivatives of Piperidine.

(a) Tetrahydronicotinic Acid Derivatives. Since dihydrolysergic acid derivatives have little oxytocic action,[11] analogues with a saturated ring system corresponding to ring D of lysergic acid would not be expected to show high activity if their mode of action were the same. It would therefore appear profitable to attempt the synthesis of compounds having a single unsaturated bond in the ring corresponding to ring D of lysergic acid. Few

such compounds have in fact been reported and they have not shown sufficient activity compared with saturated derivatives to stimulate further research in this field. Plieninger[19] prepared a compound (X or XI) of moderate oxytocic action but does not appear to have prepared amides which would have more closely approached ergometrine in structure. Attempts to prepare alkanolamides of N-(3'-indolylmethyl)-1,2,5,6-tetrahydronicotinic acid[24] were unsuccessful.



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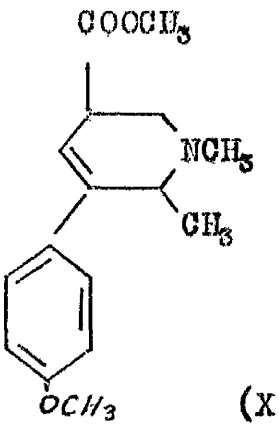
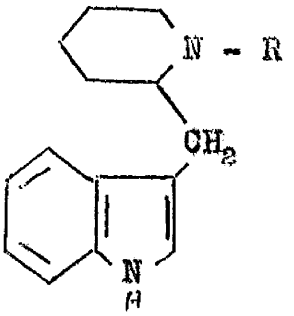


(XI)

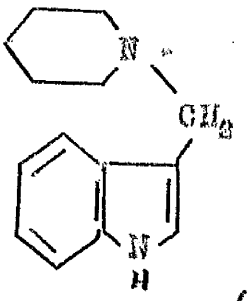
(b) 2-Substituted Piperidine Derivatives. Akkerman and Veldstra[24] prepared compounds of structure (XII) in which the indole nucleus corresponding to rings A and B of lysergic acid is joined by a single methylene group to a piperidine ring corresponding to a saturated ring D.



(XII)

Some compounds of this type, lacking ring C, were shown to have considerable oxytocic action,[26] which was greatest in the N-methyl derivative (XII, R = Me). Compounds with a carboxyl substituent in the piperidine ring corresponding to that at $C_{(8)}$ in lysergic acid were quite inactive. Despite the improved synthetic route of Bader and Oxoshnik,[27] 2-(3'-indolylmethyl)-piperidine derivatives are difficult to prepare and were shown to have lower oxytocic activity than the more readily accessible N-(3-indolylmethyl)-piperidines.

(c) N-Indolylmethylpiperidine Derivatives. The first compounds of this type were described by Hoffmann and Schellenberg[28] who prepared N-(2-indolymethyl)-piperidine, which they stated to have oxytocic activity. More closely related to lysergic acid are the N-(3-indolymethyl)-piperidine derivatives (XIII) prepared by Akkerman, de Jongh and Veldstra.[29]



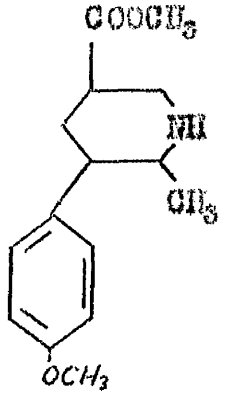
(XIII)

Oxytocic activity of these compounds, was higher than that of the corresponding 2-piperidine substituted compounds, and was similarly lost on inclusion of a 3-carboxyl substituent in the piperidine ring[30] indeed some 3-carboxyl derivatives showed an inhibitory action on uterine contraction. The effect of the configuration of the carboxyl substituent at $C_{(5)}$ in the piperidine ring and of the optical activity of the 2-aminopropanol used in the preparation of propanolamides was investigated, but no conclusions could be reached, since all isomers were virtually inactive as oxytocics. Greatest activity was shown by the 2,4,6-trimethylpiperidine derivative, but this was unstable. The more stable and highly active 2,6-dimethylpiperidine compound has undergone successful clinical trials[2] but does not appear to be available in Britain at the present time.

Oxytocic activity has also been reported for N-substituted piperidine derivatives which bear little structural resemblance to lysergic acid. Substituents conferring significant oxytocic

action include tetrahydronaphthylmethyl[31] and substituted benzyl.[32] Votava and Podvalová[33] have investigated a large number of N-benzylpiperidines with methoxy substituents in the aromatic nucleus and shown that N-(3,4-dimethoxybenzyl)-piperidine was the most potent oxytocic (comparable with the findings of Baltzly et al.[17] for N-substituted aliphatic amino acids).

(d) 5-Substituted Nipecotic Acid Derivatives. Only one compound of this class (XIV) has been reported.[10] It had low oxytocic activity. The stereochemistry of this compound, which has three asymmetric centres, was not investigated in detail, although it was observed that distillation yielded a mixture of racemates which differed from that obtained in the initial reaction.

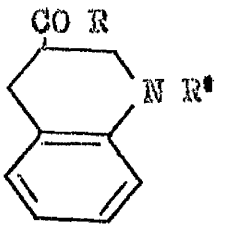


(XIV)

Derivatives of Quinoline.

Quinoline may be considered as a fused ring system related to rings C and D of lysergic acid, a similarity which is increased by the reduction of the heterocyclic ring to form

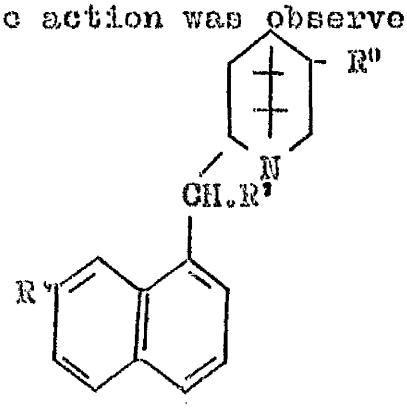
1,2,3,4-tetrahydroquinoline and substitution of a carboxyl group in the 3-position (XV).



(XV)

Tetrahydroquinoline derivatives of this type were prepared by Cain, Plampin and Sam[34] and by Koelle and Kamijo[35]. Considerable oxytocic activity was shown where R = NH C_2H_5 and R' = CH_3. Corresponding derivatives of quinoline were inactive as oxytocics.[36]

The well-known oxytocic action of quinine led Pouwels and Voldstra[36] to investigate the effect of modification of the molecule of quinine (XVI; R = OCH_3, R' = OH, R'' = $CH{:}CH_2$) on its oxytocic action. This action was found to be unchanged in related compounds where R = H and R' = H or Cl, despite the fact that such compounds were inactive as antimalarials. In compounds where R'' = COOH, more closely analogous with lysergic acid, no oxytocic action was observed.



(XVI)

Derivatives of Lysergic Acid.

Stoll's partial synthesis of ergometrine[12] by reaction of lysergic acid azide with 2-aminopropanol opened the way to preparation of a large number of related semi-synthetic amides,[37,38,39] many with oxytocic action greater than that of the naturally occurring alkaloids. A further stimulus to this work was the discovery by Hofmann[40] that the diethylamide was a powerful hallucinogen. It is remarkable that one of the most active oxytocics amongst these compounds, and the only analogue to achieve clinical use in obstetrics is D-lysergic acid (+)-butanolamide(2) or methylergometrine, which was one of the first to be synthesised.[12] This compound has about one and a half times the oxytocic action of ergometrine in the human.

The most potent oxytocics so far reported appear to be the lysergic acid cycloalkyl amides,[39] a homologous series reaching maximum oxytocic activity at C_5, the cyclopentyl amide. This shows about five times the activity of ergometrine on the rabbit uterus *in situ*. The relative activities *in situ* in the rat and guinea pig were similar, although the sensitivity of these animals was lower.

Various attempts to modify the lysergic acid molecule in other ways[41,42,43] do not appear to have led to amides of greater oxytocic activity than those of lysergic acid itself.

Compounds Unrelated to Lysergic Acid.

Oxytocic action has been shown by a number of compounds bearing little or no relationship to either lysergic acid or oxytocin; for example many sympatholytic drugs and anti-histaminics show oxytocic action as a side-effect. Such compounds, which are not directly related to the present work, have been reviewed by de Jongh.[2]

Conclusion.

It is evident that no simple relationship has been shown to exist between chemical structure and oxytocic activity. This has meant that the choice of compounds for synthesis and pharmacological testing has necessarily been empirical. It has been shown in many cases, particularly in the piperidine series, that compounds structurally related to ergometrine have been far less active than similar compounds less closely related. It may well be that the mode of action of such compounds differs fundamentally from that of lysergic acid derivatives; in some cases their action on other smooth muscle has been shown to be the opposite to that of ergometrine.[55]

A new approach to the design of oxytocics is offered by recent detailed studies of the configuration at $C_{(8)}$ and the conformation of lysergic acid, isolysergic acid and their derivatives, which are discussed in the following section.

Since isolysergic acid (with an axial carboxyl substituent at $C_{(8)}$) produces derivatives of negligible oxytocic activity compared with those of lysergic acid (which has an equatorial carboxyl substituent),[12] it appears that the spacial relationship between the carboxyl substituent and neighbouring atoms is of importance in determining the activity of the ergot alkaloids. The synthesis of models of similar molecular shape seems therefore to be worthy of investigation.

A more fundamental study of structure-action relationships in oxytocics is limited by lack of knowledge of the detailed biochemistry of uterine muscular contraction, which appears to be different from that of other smooth muscle.[2] As far as the production of clinically useful oxytocics is concerned, the total synthesis of lysergic acid by Woodward's group[44] may lead to the commercial production of synthetic lysergic acid, the amides of which are still the most powerful known oxytocics, and which are known to have a satisfactorily low toxicity.

The Stereochemistry of Lysergic and Isolysergic Acids and their Derivatives.

Early work on the stereochemistry of the lysergic acids has been reviewed by Glenn.[1] The original supposition that lysergic acid (I, R = OH) and isolysergic acid were double-bond tautomers was disproved by the work of Stoll and his collaborators and it was clear by 1949 that the two acids differed only by the configuration at $C_{(8)}$. It was not possible however at this point to decide which of the two possible epimeric forms was represented by lysergic and which by isolysergic acid.

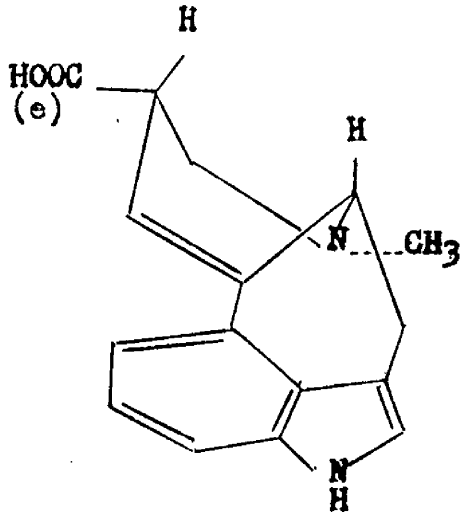
The stereoisomeric pairs of lysergic or isolysergic acids must differ in their configurations at $C_{(5)}$ since this is the only remaining centre of asymmetry. The following discussion relates to the D series, which occurs in the alkaloids of ergot, and the configuration at $C_{(5)}$ is therefore constant.

The methods of conformational analysis, developed by Barton[45] in studies of the steroids and triterpenes, were first applied by Cookson[46] to compounds containing six-membered heterocyclic rings, including ring D of lysergic and isolysergic acids and their derivatives. Postulating a pseudo-boat conformation for ring D and arbitrarily assigning a position

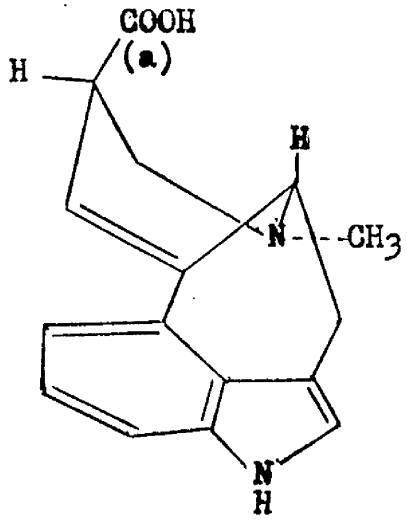
above the ring for the hydrogen substituent at $C_{(8)}$, two configurations (XVII and XVIII) at $C_{(8)}$ are possible. The theoretical thermodynamic stabilities of both of these epimeric forms were considered by Cookson to be similar, since examination of accurate models indicates that distance between the -COOH group and neighbouring atoms are comparable in each case. The findings of Smith and Timmis[47] that equilibration of lysergic acid with boiling water produces approximately equal proportions of each epimer, were quoted as showing that the two epimeric forms were in fact of comparable stability.

The greater basic strengths of isolysergic acid and ergometrinine as compared with lysergic acid and ergometrine were considered by Cookson to indicate that the carboxyl and amino groups were spacially more separated in isolysergic than in lysergic acid, indicating that lysergic acid would have structure (XVIII) and isolysergic acid structure (XVII).

A different interpretation of the differences in pK values between lysergic and isolysergic acids was made by Stenlake,[48] who reasoned that inductive effects through the carbon chain must be identical in both epimers, and that variation in basic strength can only be attributed to differences in the electrostatic interaction of charged groups, the



XVII



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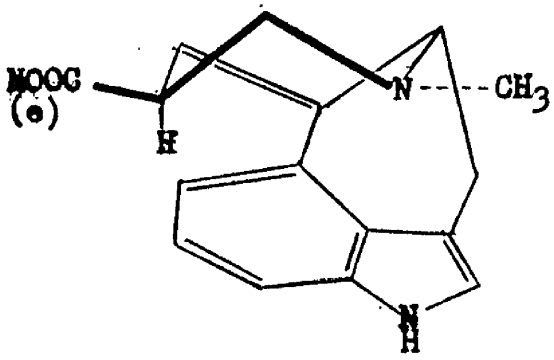
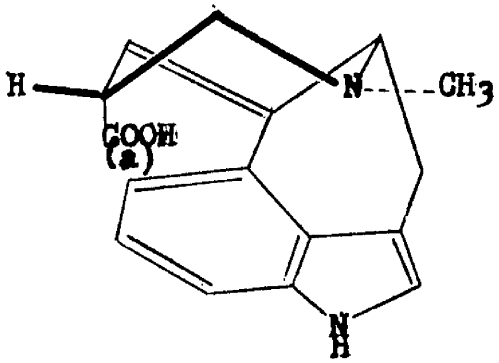
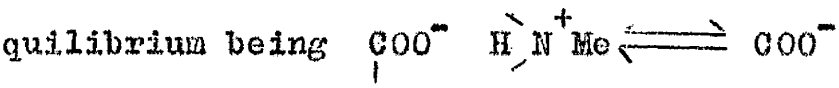


XIX



XX

equilibrium being  Considering the base-conjugate acids as dibasic acids and reasoning by analogy with cis and trans isomers of other dibasic acids, Stenlake concluded that closer proximity of the amino and carboxyl groups should have a base-strengthening effect, a directly opposite conclusion from that of Cookson. This would mean that Cookson's assumptions regarding the configuration at $C_{(8)}$ for lysergic and isolysergic acids would also be incorrect, lysergic acid now being considered as having the equatorial carboxyl group (XVII) and isolysergic the axial (XVIII).

The pseudo-boat conformation for ring D, propounded by Cookson, was also criticised, since its only experimental basis lay in the report that neither lysergic nor isolysergic acid was preferentially formed on equilibration. Against this, Stenlake quoted evidence that equilibration of the ergot alkaloids with ethanolic potassium hydroxide produced always a preponderance of lysergic acid types. Lysergic acid is also much more reactive with diazomethane than is isolysergic acid. This greater stability and reactivity of lysergic acid and its derivatives would indicate that repulsive non-bonded interactions between the carboxyl group and neighbouring atoms should be less for this acid than for isolysergic acid. Assuming a pseudo-boat

conformation for ring D however, Cookson considered that such interactions would be equally favoured in both epimers, whereas Stenlake indicated that isolysergic acid having a chair form for ring D (XX) would show greater steric hindrance of the carboxyl group than would the chair form of lysergic acid (XIX).

The postulated chair structure for ring D, associated with an equatorial carboxyl at $C_{(8)}$ for lysergic acid and an axial carboxyl for isolysergic acid received strong support from Stoll and co-workers.[49] Accurate determinations of epimeric equilibria were made by observing mutarotation of a number of lysergic acid derivatives in dilute methanolic potassium hydroxide. It was found that equilibrium favoured the lysergic acid forms of secondary amine derivatives such as the dimethyl- and diethylamides, indicating the greater thermodynamic stability of lysergic acid dialkylamides as compared with those of isolysergic acid. The observation that lysergic acid derivatives of primary amines gave equilibrium mixtures containing comparable amounts of both epimeric forms was explained by the fact that hydrogen bonding could occur in the isolysergic acid amides where the carboxylamido group is near to the amino group. The resultant six-membered ring could well confer on this molecule a stability comparable with thatof lysergic acid derivatives, as

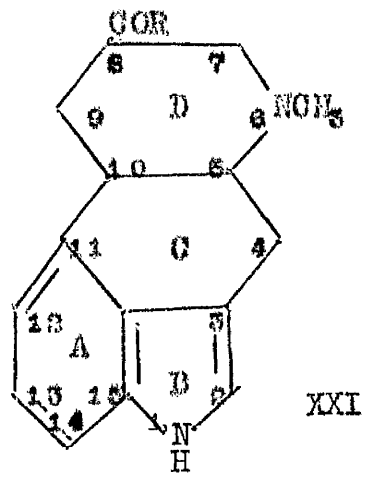
was the case. The equilibria of the dialkylamides in which no such hydrogen bonding could occur were therefore considered to be more significant.

Hydrogen-bonding in monoalkyl amides of isolysergic acid was also considered to be responsible for the difference in pK value between these and the corresponding amides of lysergic acid (Δ pK between lysergic acid monoethyl amide and isolysergic acid monoethyl amide = 0.26). The comparatively large differences in pK value between the dialkylamides (ΔpK between the diethylamides of lysergic and isolysergic acids = 1.15) obtained by Stoll seem to be anomalous, since the proximity or otherwise to the ring nitrogen of the completely substituted dialkylamido group would not be expected to have any appreciable effect on the ionisation of the former. Stoll was in agreement with Stenlake regarding the interpretation of differences in pK value for lysergic and isolysergic acids.

The observation that lysergic acid derivatives are more slowly eluted from alumina chromatograms than are the corresponding isolysergic acid derivatives was interpreted by Stoll as offering additional evidence for the existence of an equatorial carboxyl group in lysergic and an axial carboxyl in isolysergic acids.

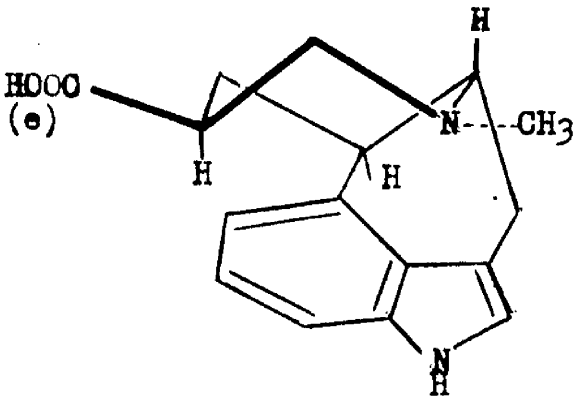
The stereochemistry of the dihydrolysergic acids, although not directly related to the present work, is of interest since there is little doubt that their saturated, piperidine-like ring D has a chair conformation. Comparison of the properties of members of this series with those of corresponding epimers in the lysergic acid series should therefore offer valuable evidence as to the conformation of ring D in the latter.

The dihydrolysergic acids present a more complex problem, since saturation of the ethylenic bond in ring D produces a further asymmetric centre, at $C_{(10)}$ (Formula XXI). This means that dihydrolysergic and dihydroisolysergic acids can each exist in two forms, one having a <u>cis</u> fusion of rings C and D (structures XXIV and XXV), the other a <u>trans</u> fusion (XXII and XXIII).

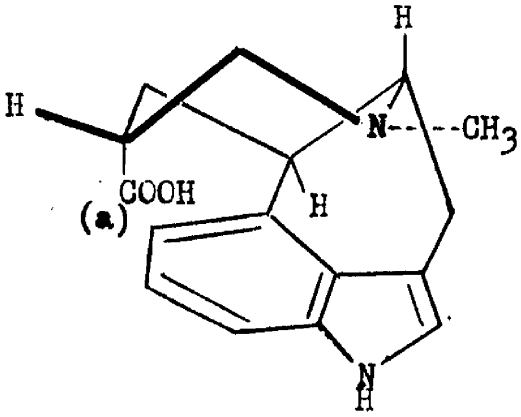


XXI

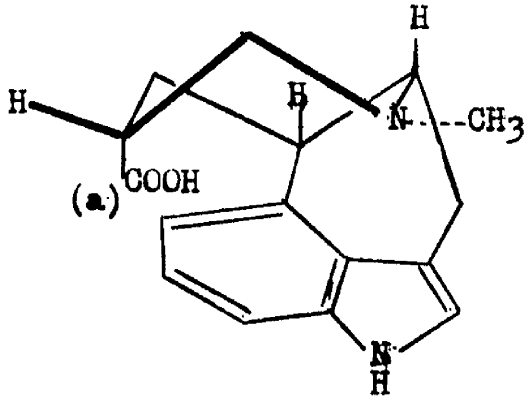
Catalytic hydrogenation of lysergic acid produces only one of the two possible dihydro derivatives (dihydrolysergic



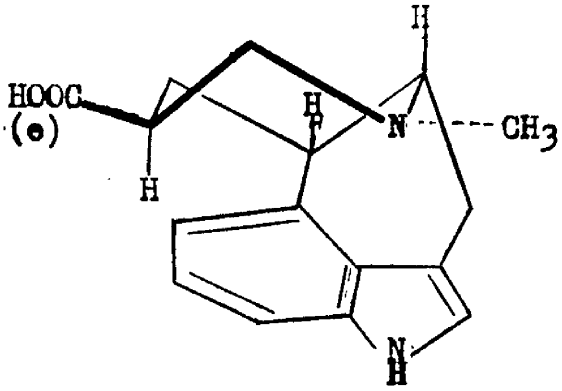
XXII



XXIII



XXIV



XXV

acid I) whereas isolysergic acid yields two dihydro acids. One of these, produced mainly on slow hydrogenation in the presence of palladium catalyst, gives dihydrolysergic acid I on epimerisation and is therefore designated dihydroisolysergic acid I. The other, dihydroisolysergic acid II, is favoured on rapid hydrogenation in the presence of a platinum catalyst and yielded on epimerisation the previously unknown dihydrolysergic acid II.[50]

The configuration of these compounds was first discussed by Cookson[46] who considered, by comparison of the theoretical relative stabilities of the possible forms, that dihydrolysergic acid I should have an equatorial carboxyl and trans ring fusion (structure XXII), dihydroisolysergic acid I an axial carboxyl and trans ring fusion (XXIII) and dihydroisolysergic acid II an axial carboxyl and cis ring fusion (XXIV). Dihydrolysergic acid II had not at that time been isolated. Cookson's ingenious explanation of the formation of only dihydrolysergic acid I on hydrogenation of lysergic acid, in terms of catalyst hindrance, was unfortunately based on a false premise concerning the configuration of lysergic acid, although the configuration which he ascribed to dihydrolysergic acid I proved to be in agreement with the findings of later workers.

Comparison of the dissociation constants of the three

available dihydro acids by Stenlake[61] showed that dihydrolysergic acid I and dihydroisolysergic acid II had very similar pKa values, significantly lower than that of dihydroisolysergic acid I. This was interpreted as showing that dihydroisolysergic acid II has an equatorial carboxyl (XXV) at $C_{(8)}$, contrary to Cookson's findings, while the conclusions regarding configurations at $C_{(8)}$ for the other two available dihydro acids were in agreement with those of Cookson.

The isolation of dihydrolysergic acid II allowed Stoll and his co-workers[49] to carry out a very thorough investigation into the stereochemistry of the dihydro acids. Stenlake's conclusions from the pK values of the acids were confirmed and completed by the publication of values for dihydrolysergic acid II and its derivatives, which indicated that this epimer has a basic strength comparable with that of dihydroisolysergic acid I indicating an axial carboxyl substituent at $C_{(8)}$ structure (XXIV).

Many other experimental findings were adduced by Stoll all leading to the conclusion that the carboxyl at $C_{(8)}$ has an equatorial configuration in dihydrolysergic acid I and dihydroisolysergic acid II, and an axial configuration in dihydrolysergic acid II and dihydroisolysergic acid I. The former pair of acids are more reactive (i.e. show less steric hindrance) their formation is favoured on equilibration, their

8-amino analogues are more resistant to deamination, they have a slower rate of elution from alumina chromatograms and their esters show infrared absorption spectra which by analogy with comparable steroids were considered to be characteristic of an equatorial carboxyl substituent. Corresponding 8-hydroxy-ergolines similarly showed infrared absorption spectra characteristic of an equatorial hydroxyl substituent.

Only in the case of the nor series of dihydroacids were anomalous results observed, possibly due to hydrogen-bonding of the secondary amino group producing a distortion of ring D into a pseudo-boat conformation.

The assignment of a trans C-D ring-junction to dihydro lysergic acid I and dihydroisolysergic acid I and a cis junction to dihydroisolysergic acid II, which Cookson had deduced from theoretical considerations of conformational analysis was given experimental support by Stoll, who considered that the formation of mainly dihydroisolysergic acid I on slow hydrogenation of isolysergic acid and of dihydroisolysergic acid II on rapid hydrogenation was evidence of trans addition in the former and cis addition in the latter case. Dihydrolysergic acid II would be expected to have structure (XXIV) i.e. with a cis C-D ring junction as in the epimeric dihydroisolysergic acid II (XXV).

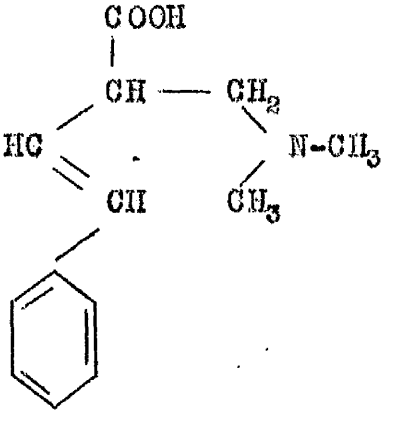
The properties of lysergic acid and isolysergic acids and their derivatives are seen to be comparable in all cases with those of corresponding epimers in the dihydro series. The relationship between pK values, the stability of derivatives, the relative rates of reaction and the chromatographic characteristics in both series show a related and distinctive pattern. This offers strong evidence that ring D exists in the same conformation (i.e. a chair) in both the lysergic acid and the dihydrolysergic acid series.

PART II

DISCUSSION

Styryl Substituted Aliphatic ω-Amino-Acids

The first part of the present investigation was concerned with the preparation of N-substituted 2-styryl-ω-amino aliphatic acids as possible oxytocic agents. Formula (XXVI) shows how the dimethylaminopropionic acid derivative may be considered as an open-chain analogue of ring D of lysergic acid (I, R = OH), including an ethylenic bond corresponding to the 9-10 double-bond in lysergic acid. The phenyl group is in a position corresponding to that of ring A of lysergic acid.



(XXVI)

The only 2-substituted 3-dimethylaminopropionic acids previously reported,[13] which had a benzyl or naphthylmethyl substituent in the 2-position, bear less resemblance to lysergic acid.

Preparation of 2-styryl-3-dimethylaminopropionic acid should be possible by a Mannich reaction of styrylacetic acid

(or ester), formaldehyde and dimethylamine:

$Ph.CH{:}CH\ CH_2COOH + HCHO + HN(Me)_2 \rightarrow Ph.CH{:}CH\ CH(COOH)CH_2N(Me)_2$,

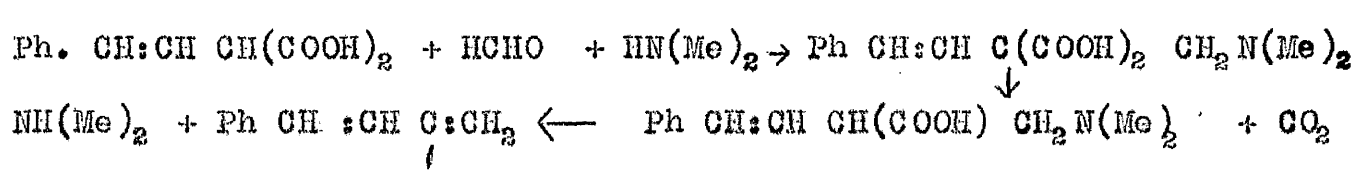
if the 2-methylene group of styryl acetic acid were sufficiently reactive. No analogous reactions with styrylacetic acid were found in the literature, the only observations on the reactivity of the 2-methylene group being those of Ivanov and Pshenichnii[52] who considered that it had the same order of reactivity as the corresponding group in phenylacetic acid. The latter is known not to react with formaldehyde and primary or secondary amines although paranitro- and 2,4-dinitrophenylacetic acids do so react.[53]

Despite this evidence it might be expected that the presence of a vinyl group in conjugation with the phenyl nucleus would increase its electronegative character and hence the reactivity of the adjacent methylene group. A Mannich reaction was accordingly attempted.

Reactions using ethyl styrylacetate dissolved in a series of aliphatic alcohols yielded only unchanged starting materials, even after heating under reflux in n-amyl alcohol for three days. Heating at 206° for 6 hr. in a sealed tube in the absence of solvent produced slight decomposition but no appreciable yield of high-boiling base. An aqueous solution of the dimethylamine salt of styrylacetic acid heated under reflux

with formaldehyde solution gave almost quantitative recovery of unchanged acid. Similarly unsuccessful was an attempted reaction of ethyl styrylacetate, dimethylamine and paraformaldehyd heated under reflux in an anhydrous system consisting of glacial aceti acid with a trace of acetic anhydride.

Since it appeared that the reactivity of the 2-methylene group in styrylacetic acid was too low to permit reaction, an additional activating group was required. Norris and Blicke[13] had successfully carried out Mannich reactions of the same type with phenylmalonic acid, the vinologue of which, styrylmalonic acid was known.[52] Treatment of styrylmalonic acid with formaldehyde and dimethylamine in aqueous solution at 0° resulted in an immediate reaction with spontaneous decarboxylation. The required amino-acid could be recovered only in small yield however (as its picrate), the major product produced after overnight standing at 0° being an amorphous acid. This acid forme with para-bromophenacyl bromide a crystalline derivative, analysis of which indicated that styrylacrylic acid (XXVII) had been produced by deamination of the required product.



(XXVII)

Such decomposition of 2-substituted dialkylaminopropionic acids is well known[54] and is a commonly used method for the preparation of substituted acrylic acids. The 2-styryl-substituted dimethylaminopropionic acid appears however to be much more labile than the benzyl- and naphthylmethyl-substituted compounds described by Norris and Blicke, since the latter compounds did not decarboxylate spontaneously and withstood heating at 125°-130° for 15 min. followed by esterification in ethanolic hydrogen chloride.[13]

A similar reaction using methylamine in place of dimethylamine gave only a trace of amino-acid, which formed a crystalline picrate, but in quantities too small to allow characterisation. The main product was an acid identical with that obtained in the previous preparation.

The unexpectedly low stability of 2-styryl-3-aminopropionic acids made further investigation of these compounds pointless and the homologous 2-styryl-4-dimethylaminobutyric acid was next investigated. The increase in chain length between the dimethylamino and carboxyl groups would be expected to reduce the tendency to deamination, while the oxytocic action of derivatives of comparable N-substituted aminoacids was found by Phillips and Baltzly[10] to be largely unaffected by increase in this chain-length.

Reaction of the sodio derivative of ethyl styrylacetate

with dimethylaminoethyl chloride produced the required ethyl 2-styryl-4-dimethylaminobutyrate (XXVIII):

$$Ph.CH{:}CH\ CHNa\ COOEt + Cl\ CH_2CH_2N(Me)_2 \rightarrow Ph.CH{:}CH\ CH(COOEt)CH_2CH_2N(Me)_2$$

(XXVIII)

- contrary to the findings of Ivanov and Pshenichnii[52] that styrylacetic acid does not form a sodio derivative. The product was an oil of the required equivalent weight which did not form crystalline salts; the picrate of the corresponding acid was however crystalline and suitable for characterisation.

Yields were poor when the reaction was carried out in dry ethanol at reflux temperature for 2 hr. To ensure complete formation of the sodio derivative in a further attempt, the ester and sodium ethoxide in ethanol were heated initally under reflux for 2 hr. before addition of the dimethylaminoethylchloride. The yield was not changed appreciably. On increasing the time of initial refluxing to 18 hr., none of the required base was obtained.

The non-basic product of the above reactions was found to consist predominantly of a high-boiling, very viscous liquid. This yielded on saponification an acid much higher in melting poin than styrylacetic acid, but having the same elemental analysis and equivalent weight. Since this acid had an approximate molecular weight about twice that of styrylacetic acid, it was considered

that dimerisation must have occurred in preference to the desired condensation.

An attempt to limit dimerisation by heating the ester with 'molecular' sodium in xylene resulted in the rapid formation of a red resin, presumably by polymerisation of the ethyl styrylacetate, without any evolution of hydrogen which would have indicated formation of a sodio derivative.

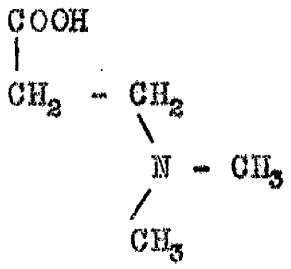
When the sodio derivative was prepared in ethanol, dried *in vacuo* then heated with a solution of dimethylaminoethyl chloride in xylene, the total yield of high-boiling base was greatly increased, but only a minor proportion of this was the required product, the yield of which was about the same as previously. The remainder of the basic fraction could not be distilled without decomposition and did not form any crystalline salts. It may have contained condensation products of ethyl styrylacetate polymers with dimethylaminoethyl chloride.

The stability of 2-styryl-4-dimethylaminobutyric acid was low, although much greater than that of the homologous propionic acid derivative. The ester base was seen to darken in a few days on storage at room temperature in a sealed container, and resinified completely within weeks. The nature of this decomposition was not clear since the resinous product did not form crystallisable derivatives and could not readily be characterised.

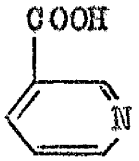
Because of the apparently inherent lack of stability of N-substituted 2-styryl-ω-amino-acids, other possible synthetic routes to these compounds were not explored and work in this direction was discontinued.

Alkanolamides of Amino-Acids.

The ethanolamides and propanolamides of 3-dimethylamino-propionic acid (XXIX), nicotinic acid (XXX), 1-methylhexahydro-nicotinic acid (XXXI) and 1-methyl-1,2,5,6-tetrahydronicotinic acid (arecaidine; XXXII) were prepared because of the structural relationship between these acids and ring D of lysergic acid (I) and hence between their propanolamides and the corresponding fragment of ergometrine.



(XXIX)



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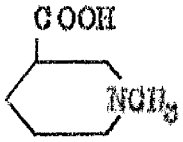
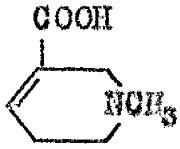


(XXXI)



(XXXII)

This relationship was considered sufficient to make the compounds of potential value as oxytocics, especially since other amides of simple N-substituted 3-aminopropionic acid derivatives had been shown by Rosen and co-workers[•] to have oxytocic activity. It has also been shown in the course of the present study that the propanolamides of (XXIX), (XXXI) and (XXXII) have their carboxylamido and their amino groups in a steric configuration related to that of ergometrine and different from that of the pharmacologically inert ergometrinine.

These amino-acids and their alkanolamides were

additionally of use as model substances of known conformation, the physical properties of which could be compared with those of lysergic and isolysergic acids in order to elicit information about the detailed stereochemistry of the latter. This aspect will be dealt with in more detail in the third part of this discussion.

Synthesis of alkanolamides is complicated by the fact that acylation of amino-alcohols may take place in three ways:- N-acylation, giving the required alkanolamide, R $CONH(CH_2)_nOH$, O-acylation, giving an alkylamino ester; R $COO(CH_2)_nNH_2$ and O,N-diacylation to give an O,N-bis-acyl structure; R $COO(CH_2)_nNH\overset{|}{\underset{CO\ R.}{}}$

No practical problem is presented by the formation of amino-esters, since amino-esters and alkanolamides are generally readily interconvertible. The acyl migration involved has long been known[55,56] and the mechanism of the reaction was discussed by Phillips and Baltzly[57] and Welsh,[58] all of whom postulated an intermediate cyclisation to form hydroxyoxazolidine derivatives. Since the formation of such a cyclic intermediate would be favoured in amino-alcohols with amino and hydroxyl groups in close proximity, Fodor and co-workers[59,60,61,62] have studied the kinetics of N⇌O acyl migration as an aid to the elucidation of the stereochemistry of diastereomeric amino-alcohols.

Koczka and Fodor[65] were able to confirm the previously postulated reaction mechanism by isolation and characterisation of the cyclic intermediate. Fodor's investigations were concerned with fairly complex amino-alcohols and simple acylating agents such as benzoyl chloride, whereas the present work involved simple amino-alcohols and more complex acylating agents, but the general principles should apply to both.

In most cases amide is formed rapidly and completely in cold aqueous alkaline solution, while ester formation occurs rather more slowly and less completely in ethanolic hydrogen chloride. Formation of O,N-bis-acyl compounds is to be avoided, since these are not readily convertible to either of the monoacyl forms.

Aminolysis.

The simplest method of amide formation is the aminolysis of an ester by heating under reflux with a primary or secondary amine.

$$R\,COOCH_3 + R'NH_2 \xrightarrow{heat} R\,CONH\,R' + CH_3OH$$

This method has been widely used for the preparation of alkanolamides of long-chain fatty acids which are of commercial importance as foam-stabilisers.[64] N-Acylation occurs predominantly if there is an excess of amino-alcohol present or if the

reaction is carried out in the presence of a base-catalyst such as sodium methoxide, otherwise some O-acylation also takes place to give the unwanted O,N-bis acyl compound.[65]

The major disadvantage of this method is that amino-alcohols react with esters only at temperatures which tend to cause decomposition or racemisation of thermolabile products. Attempts to prepare (methyl)ergometrine, for example, by heating D-lysergic acid methyl ester with (+)-2-aminobutanol were carried out by Semonský, Černý and Zikán,[66] who obtained a 55% yield of a mixture of the (+)-2-butanolamides of D and L-lysergic and D and L-isolysergic acids. More thermostable esters, such as those of nipecotic[29] and 1-methylnipecotic acids[57] however gave good yields of alkanolamides by this route.

The technique used in the present work was to heat the methyl or ethyl ester with a large excess of ethanolamine or (±)-2-aminopropanol in a flask fitted with a fractionating column capable of separating ethanol or methanol from the less-volatile amino-alcohol. By this means the alcohol produced during aminolysis was continually removed from the system, thereby shifting the reaction equilibrium in favour of the required amide. A further advantage is that the course of the reaction may be followed by measurement of the amount of alcohol produced, the end being marked by a rise in the temperature at the still-head

to the boiling point of the amino-alcohol. After removal of the excess of amino-alcohol by distillation *in vacuo*, the syrupy reaction product was purified where possible by crystallisation of its acid oxalate.

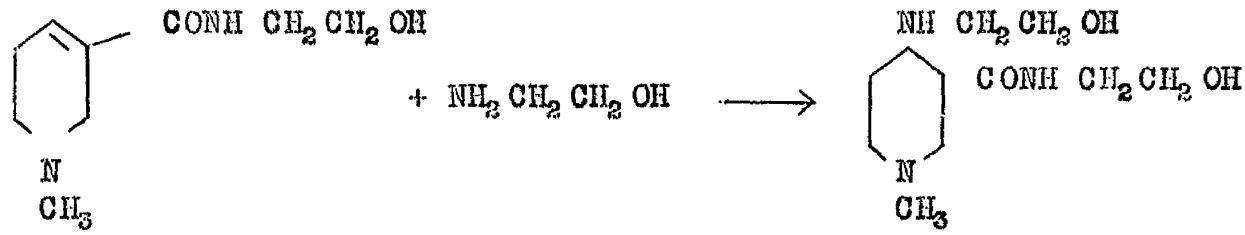
The propanolamide of nicotinic acid and the previously known[57] ethanolamides of 1-methylhexahydronicotinic and nicotinic acids were prepared by aminolysis as described, and characterised as their crystalline acid oxalates.

The propanolamide of N-methylhexahydronicotinic acid was probably formed, since the required amount of pure alcohol was recovered during the reaction. The product, a hygroscopic amorphous semi-solid, could not however be crystallised, nor did it appear to form any crystalline salts. Acyl migration by treatment with ethanolic hydrogen chloride, which yields a crystalline aminoester dihydrochloride with 1-methylhexahydronicotinic acid ethanolamide,[57] failed to give a crystalline product in the case of the propanolamide. Attempted purification of the base by short-path distillation under high vacuum yielded an amorphous product with an equivalent weight on potentiometric titration 9 per cent above the calculated value for the propanolamide.

The ethanolamide of 3-dimethylaminopropionic acid could

not be prepared by aminolysis of its ester, since the temperature of 140-180° required for reaction caused deamination of the product, shown by evolution of dimethylamine along with ethanol. The other reaction product was a red resin which was not characterised since it formed no crystalline derivatives and was not distillable; it may well have been a polymerised acrylic acid amide. A similar reaction reported by Baltzly and co-workers[17] using ethyl N-(2'-phenylethyl)-N-methyl-3-amino-propionate and ethanolamine produced the required amide 'in poor yield with difficulty', but details were not given. A repeat attempt with prolonged heating at 100° (at which temperature deamination does not occur) produced no reaction whatever.

Arecaidine methyl ester (arecoline) heated under reflux with ethanolamine resulted in acylation, as shown by evolution of methanol. The product yielded a crystalline oxalate which, however, did not have the required elemental analysis for arecaidine ethanolamide acid oxalate, had a melting point much higher than that of other oxalates in this series and was, atypically, sparingly soluble in absolute ethanol. The analysis indicated that addition of ethanolamine to the α,β-double bond of arecaidine may have occurred.



This is supported by the finding that the ultraviolet absorption of a hydrochloride prepared from the oxalate (Fig.1) differed from that of arecoline hydrobromide in not having a maximum at 205 mμ characteristic of α,β-unsaturated carboxylic acid derivatives. Addition of ammonia to arecaidine under similar conditions has been reported by Karrer and Ruckstul.[67]

A related reaction attempted by Wheeler and co-workers,[24] in which the ethyl ester of 1-(3′-indolylmethyl)-guvacine was heated with ethanolamine, resulted in the formation of a red resin from which no crystallisable product could be isolated.

Aminolysis of arecoline and esters of 3-dimethylaminopropionic acid with (±)-2-aminopropanol was not attempted, since this amino-alcohol is not easily obtained and there was no evidence to suggest that it would react satisfactorily where ethanolamine did not.

An attempt to prepare arecaidine 2-aminoethyl ester by ester interchange under acid conditions, melting together arecaidine methyl ester hydrobromide and ethanolamine hydrobromide,

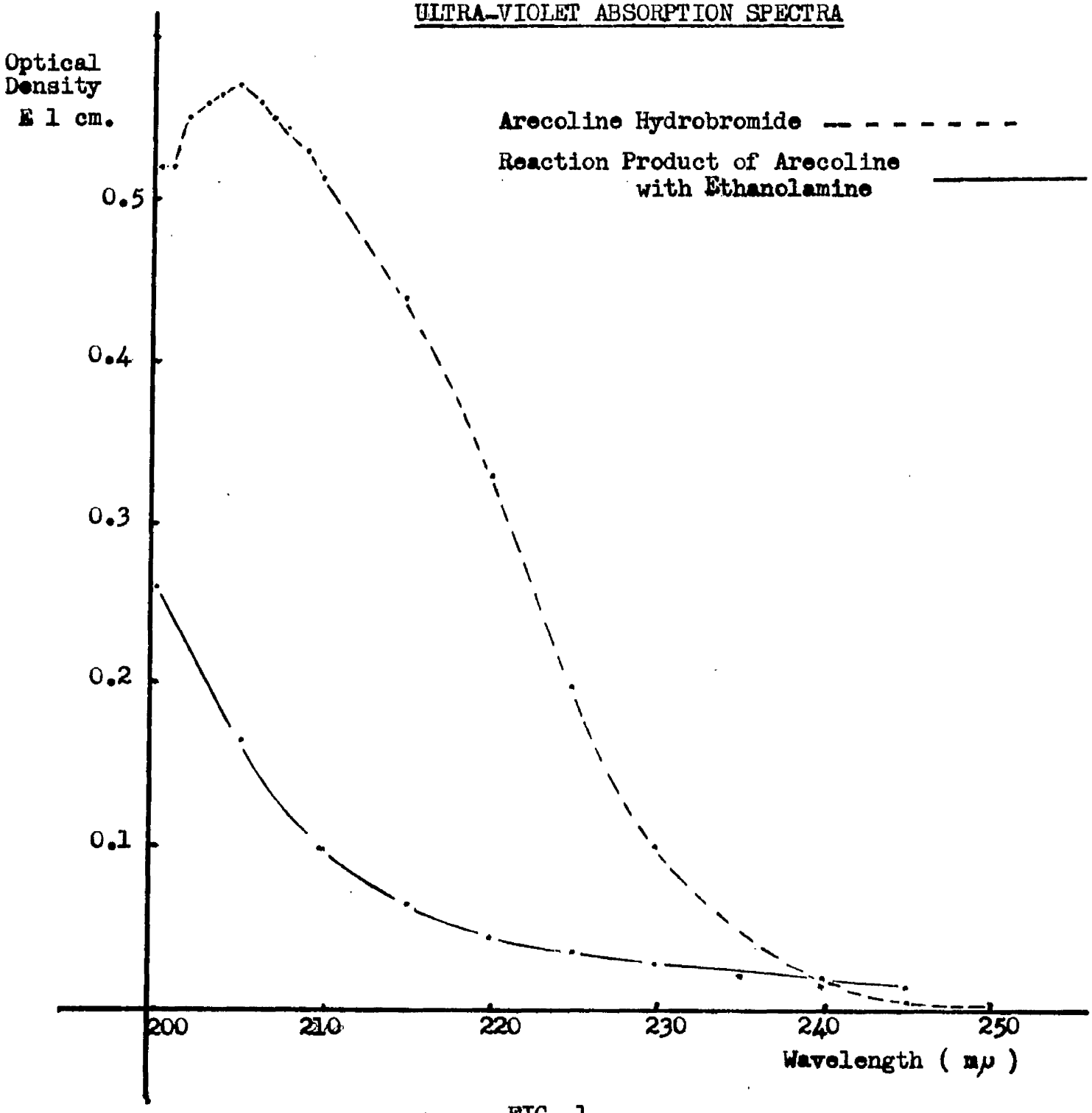

ULTRA-VIOLET ABSORPTION SPECTRA
Optical Density E 1 cm.
Arecoline Hydrobromide
Reaction Product of Arecoline with Ethanolamine
0.5
0.4
0.3
0.2
0.1
200
210
220
230
240
250
Wavelength (mμ)

FIG. 1.

was unsuccessful, decomposition occurring before any evolution of methanol which would have indicated reaction.

Acid Anhydrides

Amides are formed readily by the reaction of acid anhydrides (including mixed anhydrides)with ammonia or primary or secondary amines

$$R\,CO\,X + HN\begin{matrix}R'\\R''\end{matrix} \longrightarrow R\,CON\begin{matrix}R'\\R''\end{matrix} + HX$$

This method is generally satisfactory for the preparation of amides of labile substances (assuming that the acid anhydride is available) since reaction occurs at moderate temperatures.

Reaction of acid anhydrides and amino-alcohols may produce O, N, or O,N-bis acyl derivatives as described in the previous section, and one of the main problems in the present work has been this non-specificity, particularly the formation of O,N-bis-acyl compounds.

The mixed anhydride which has been longest and most widely used is the acid chloride, one of the first ethanolamides reported[68] being prepared from benzoyl chloride and ethanolamine. Acid chlorides appear to have found little favour however in the preparation of alkanolamides from amino-acids. Attempts to prepare ergometrine from the acid chloride of lysergic acid and 2-aminopropanol have been described, but led only to resinous products.[69]

The acid chloride hydrochloride of 3-dimethylaminopropionic acid was formed readily by heating the amino-acid hydrochloride with a large excess of thionyl chloride at a temperature of 60-70°. (Higher temperatures caused discolouration and ultimate resinification of the product). A satisfactorily pure sample of the acid chloride hydrochloride was obtained by evaporation of excess thionyl chloride *in vacuo* followed by washing of the crystalline residue with dry light petroleum until the washings were colourless. The product reacted vigorously with dry ethanol to form almost pure ethyl 3-dimethylaminopropionate hydrochloride. Heating the acid chloride hydrochloride at 100° produced immediate darkening and decomposition. The acid chloride base, produced by treating a solution of the hydrochloride in chloroform with an equimolecular proportion of triethylamine, was even more labile than the hydrochloride and resinified under the heat of neutralisation unless the mixing was done with very efficient stirring and cooling. One attempted reaction of the acid chloride base with ethanolamine at low temperatures resulted in the formation of 3-dimethylaminopropionic acid dimethylamide; presumably dimethylamine produced by deamination of 3-dimethylaminopropionyl chloride base had reacted more readily with unchanged acid chloride than had the ethanolamine.

Treatment of 3-dimethylaminopropionyl chloride hydrochloride in dry chloroform with an equimolar proportion of ethanolamine yielded mainly the dihydrochloride of the O,N-bis-acyl derivative (XXXIII) with a small yield of the aminoester dihydrochloride (XXXIV).

$$2N(CH_3)_2CH_2CH_2COCl.HCl + NH_2CH_2CH_2OH \rightarrow N(CH_3)_2(CH_2)_2CONHCH_2CH_2OCO(CH_2)_2N(CH_3)_2.2HCl.$$

(XXXIII)

$$N(CH_3)_2CH_2CH_2COCl.HCl + NH_2CH_2CH_2OH \rightarrow N(CH_3)_2CH_2CH_2COOCH_2CH_2NH_2.2HCl.$$

(XXXIV)

The two reaction products were easily separable, since the dihydrochloride of the bis-acyl compound (XXXIII) is readily soluble in chloroform, whereas the amino-ester dihydrochloride (XXXIV) is almost insoluble. The bis-acyl compound was characterised as its crystalline picrate, since its dihydrochloride was exceedingly deliquescent.

Yields of amino-ester (XXXIV) varied inexplicably between 10 and 20% of theory and on one occasion reached 50 per cent. Recovery of bis compound (XXXIII) varied inversely as that of amino-ester.

On no occasion was alkanolamide identified as a reaction product. This may be contrasted with the findings of previous workers[68,70,71] who isolated alkanolamides as the major products from acid chloride - amino-alcohol reactions under similar

conditions, though using acids other than amino-acids. This raises the question whether the amino-ester in the present reactions was produced by an initial O-acylation or by N-acylation followed by acyl migration, a matter which will be discussed later in this section.

Treatment of the amino-ester dihydrochloride (XXXIV) with aqueous alkali produced O→N acyl migration to yield the required ethanolamide (XXXV).

$$N(CH_3)_2CH_2CH_2COOCH_2CH_2NH_2.2HCl \xrightarrow{\text{aqueous NaOH}} N(CH_3)_2CH_2CH_2CONHCH_2CH_2OH$$

(XXXV)

This was characterised as its acid oxalate and succinate, the only crystalline derivatives which could be prepared.

Attempts to purify the ethanolamide base (a viscous oil) by short-path distillation under high vacuum produced partial deamination, shown by loss of dimethylamine and production of a distillate deficient in basic nitrogen. This decomposition was found to be reversible, treatment of the distillate with excess dimethylamine resulting in a compound of the required equivalent weight.

$$N(CH_3)_2CH_2CH_2CONHCH_2CH_2OH \rightleftharpoons NH(CH_3)_2 + CH_2:CHCONHCH_2CH_2OH$$

Treatment of the ethanolamide with an excess of ethanolic hydrogen chloride did not regenerate amino-ester dihydrochloride.

This indicates that the amino-ester dihydrochloride formed initially was unlikely to have been a secondary product of N $\longrightarrow$ O acyl migration, but was probably a primary reaction product. If this were the case, it would follow that the O,N-bis-acyl compound must have been formed by N-acylation of an initially produced O-acyl compound.

In an attempt to suppress the formation of bis compound in a further reaction, the amount of ethanolamine was doubled. The effect was the reverse of that intended, since bis compound was now formed exclusively and no amino-ester could be recovered. This unexpected result led to an investigation of the effect of alkalinity and acidity on the course of the reaction, since the excess ethanolamine had been in the form of its free base.

Addition of an inert base to benzoyl chloride and ethanolamine was shown by Brinzinger and Koddebusch[71] to promote O,N-bis acylation and it was thought possible that the excess of ethanolamine base may have had a similar base-catalysing effect in the present case. To test this hypothesis, a reaction was carried out using equimolecular proportions of an inert base (triethylamine), 3-dimethylaminopropionyl chloride hydrochloride and ethanolamine in chloroform. This yielded only bis compound indicating that O,N-bis acylation was indeed favoured under basic

conditions in this reaction, and that the basifying effect of the excess ethanolamine used in the previous attempt had had a greater influence in potentiating bis compound formation than the concentration effect had had in suppressing it.

Since O-acylation appeared to be the initial reaction, increased formation of bis compound under basic conditions would indicate that it was N-acylation which was being base-catalysed. This is analogous with the acylating action of carboxylic acids, amide formation with amines being base-catalysed while ester formation with alcohols is acid-catalysed.

It did not seem possible to prevent O-acylation by any means short of blocking the hydroxyl group of the amino-alcohol, so it was decided to concentrate on the production in good yield of the amino-ester, (XXXIV) which could easily be converted to alkanolamide by treatment with alkali. Since N-acylation, which was now to be avoided, appeared to be base-catalysed, reaction was attempted under acid conditions, using ethanolamine hydrochloride and 3-dimethylaminopropionyl chloride hydrochloride in dry chloroform.

Despite the insolubility in chloroform of ethanolamine hydrochloride, it was found that a suspension of the finely powdered amino-alcohol hydrochloride reacted on heating under reflux with a chloroform solution of the acid chloride hydrochloride. Reaction, which could be followed by observing the

evolution of hydrogen chloride, was complete in about two hours and resulted in a 75% yield of the required amino-ester dihydrochloride, with little decomposition. Conversion to alkanolamide (XXXV) was carried out as described previously.

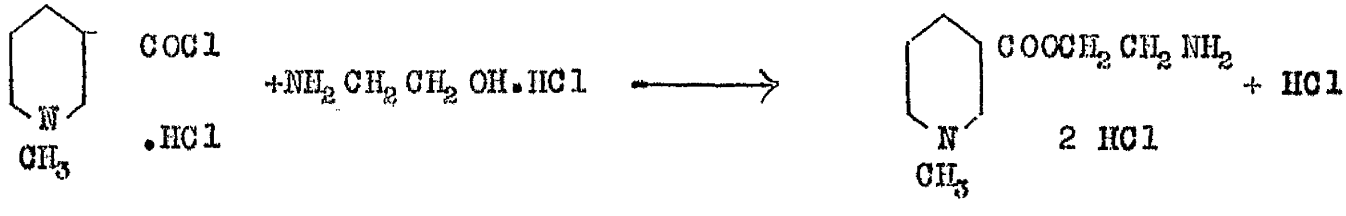
A similar reaction carried out with the homologous (±)-2-aminopropanol hydrochloride gave yields of crystalline (±)-2-aminopropyl 3'-dimethylaminopropionate dihydrochloride varying between 5 and 46%. The percentage yield generally diminished as the scale was increased. The major product, a non-crystallisable hydrochloride, presumably O,N-bis compound, was produced in quantities varying inversely as the yield of amino-ester. This material could not be characterised since it did not appear to form any crystallisable derivatives.

(±)-2-Aminopropyl 3-dimethylaminopropionate was converted to the corresponding propanolamide as before. The product, an oily base, was characterised as its crystalline acid oxalate. (Its succinate was also crystalline but excessively deliquescent). Treatment of the propanolamide with ethanolic hydrogen chloride failed to regenerate aminopropyl ester dihydrochloride as was also the case with the ethanolamide of 3-dimethylaminopropionic acid.

Preparation of the ethanolamide of 1-methylhexahydronicotinic acid through its acid chloride hydrochloride and

aminoethyl ester might appear to be of mainly theoretical interest, since this ethanolamide is more simply produced in good yield by aminolysis of the ethyl ester.[67] Investigation of the process was however of practical interest, since it served as a model for the synthesis of alkanolamides of the related, but much less readily available, arecaidine.

1-Methylhexahydronicotinic acid hydrochloride was readily converted to its acid chloride hydrochloride as already described for 3-dimethylaminopropionic acid hydrochloride. The thermostable, white crystalline product reacted readily with dry ethanol to yield ethyl 1-methylhexahydronicotinate hydrochloride. Reaction of the acid chloride hydrochloride with finely-powdered ethanolamine hydrochloride in dry chloroform, as was used for the dimethylaminopropionic acid derivatives, gave only 5% yield of the required amino ester dihydrochloride (XXXVI).

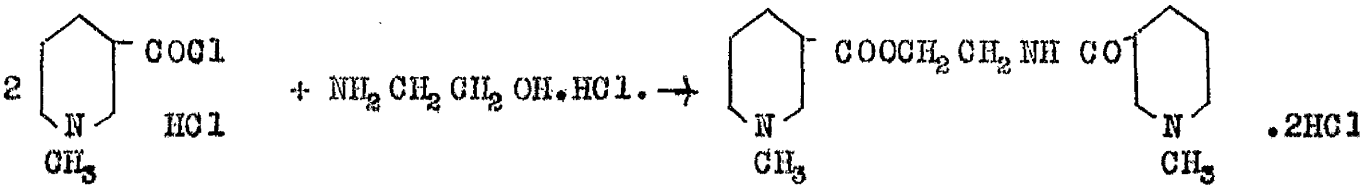


(XXXVI)

(This compound had previously been prepared by Phillips and Baltzly[57] from the ethanolamide of hexahydronicotinic acid by

acyl migration on treatment with ethanolic hydrogen chloride).

The major product of reaction, characterised as its picrate, was the O,N-bis acyl compound (XXXVII)



(XXXVII)

The marked difference in behaviour between the acid chloride hydrochlorides of 1-methylhexahydronicotinic and 3-dimethylamino-propionic acids on reaction with ethanolamine hydrochloride in chloroform (the former producing mainly O,N-bis acyl compound, the latter O-acyl) did not appear to be due to differences in reactivities, since both acids are of very similar strengths and have the same functional groups in the same steric relationship (see the last part of this discussion). The main difference observed between the two acid chloride hydrochlorides was that 3-dimethylaminopropionyl chloride hydrochloride is moderately soluble in the chloroform used as reaction medium, whereas 1-methylhexahydronicotinyl chloride hydrochloride is less soluble, which meant that reaction of the latter was somewhat slower (6 hr. as compared with 2 hr.). Although it was not clear why this should have affected the nature of the reaction, the effect of the solvent appeared to be worthy of further investigation.

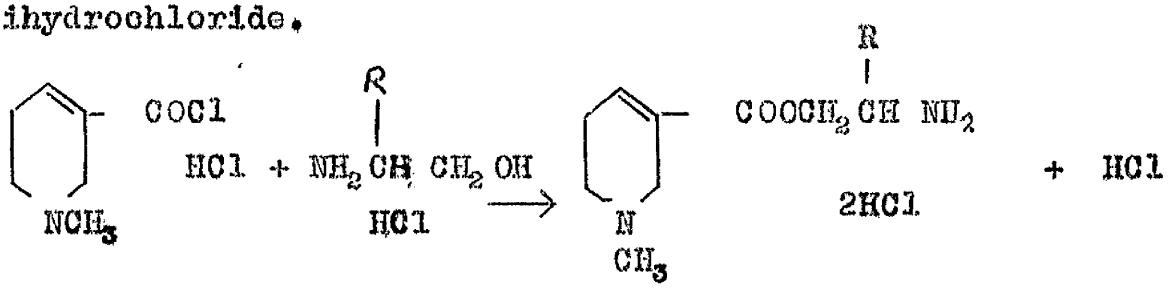
A reaction in dry tetrahydrofuran gave the same result as with chloroform, but this could be explained by the fact that 1-methylhexahydronicotinyl chloride hydrochloride is not very soluble in this solvent either. No suitably inert solvent could be found in which the acid chloride hydrochloride is more soluble than it is in chloroform.

Reaction of 1-methylhexahydronicotinyl chloride hydrochloride with a slight excess of ethanolamine hydrochloride in the absence of solvent was next investigated. Although both compounds are crystalline solids, it is possible to prepare a eutectic mixture of reasonably low melting point by mixing the finely-powdered reactants intimately. (This could be done without exposure to moisture by triturating the powders together under dry light petroleum which was subsequently removed). The mixed powders formed a melt on heating to about 100 to 120°. Evolution of hydrogen chloride occurred at once and continued vigorously for about 20 min., after which further heating produced no more hydrogen chloride and the reaction was assumed to be complete. The yield of pure amino-ester dihydrochloride was 70% of theoretical. It appeared that the higher concentration of reactants and resultant shorter reaction time had depressed the formation of unwanted O,N-bis compound, but the reason for this was not evident.

An attempted preparation of the (±)-2-aminopropyl ester of 1-methylhexahydronicotinic acid by reaction of 1-methylhexahydronicotinyl chloride hydrochloride and (±)-2-aminopropanol hydrochloride in the absence of solvent produced a reaction, evidenced by evolution of hydrogen chloride, but the product was highly deliquescent and could not be crystallised. It seemed probable that the compound was inherently non-crystallisable, since catalytic reduction of pure (±)-2'-aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride (arecaidine (±)-2-aminopropyl ester dihydrochloride) produced a similar non-crystallisable deliquescent hydrochloride. Treatment of the hydrochlorides with aqueous alkali gave amorphous hygroscopic semi-solids, which again did not form any crystalline derivatives.

The aminoethyl ester of arecaidine was now prepared by reaction of the acid chloride hydrochloride (prepared as before) with ethanolamine hydrochloride by heating in the absence of solvent. The reaction went exactly as described for 1-methylhexahydronicotinyl chloride hydrochloride, the required aminoester dihydrochloride (XXXVIII, R = H) being produced in good yield. This was the only method by which this compound could be prepared - attempts to carry out a similar reaction in chloroform gave no crystalline product while unsuccessful attempts at aminolysis and ester-interchange of arecoline

have already been described. The (±)-aminopropyl ester was similarly obtained satisfactorily as its crystalline dihydrochloride.



(XXXVIII)

Treatment of an aqueous solution of the amino-ester dihydrochlorides (XXXVIII; R=H, CH_3)with alkali to produce a pH value of 11-12 resulted in acyl migration to form alkanolamides, which were stable on subsequent neutralisation of the solution. Formation of alkanolamide could be demonstrated by potentiometric titration of aliquot portions of the solution. It is seen (Fig.2) that the equivalent weight of the aminoester dihydrochloride is exactly doubled after treatment with dilute alkali; also the neutralisation curve in the former case is typical of a diacid base, in the latter of a monoacid base. This result could only be attributed to O → N acyl migration with consequent loss of a basic nitrogen atom:

Similar results and conclusions were reported by

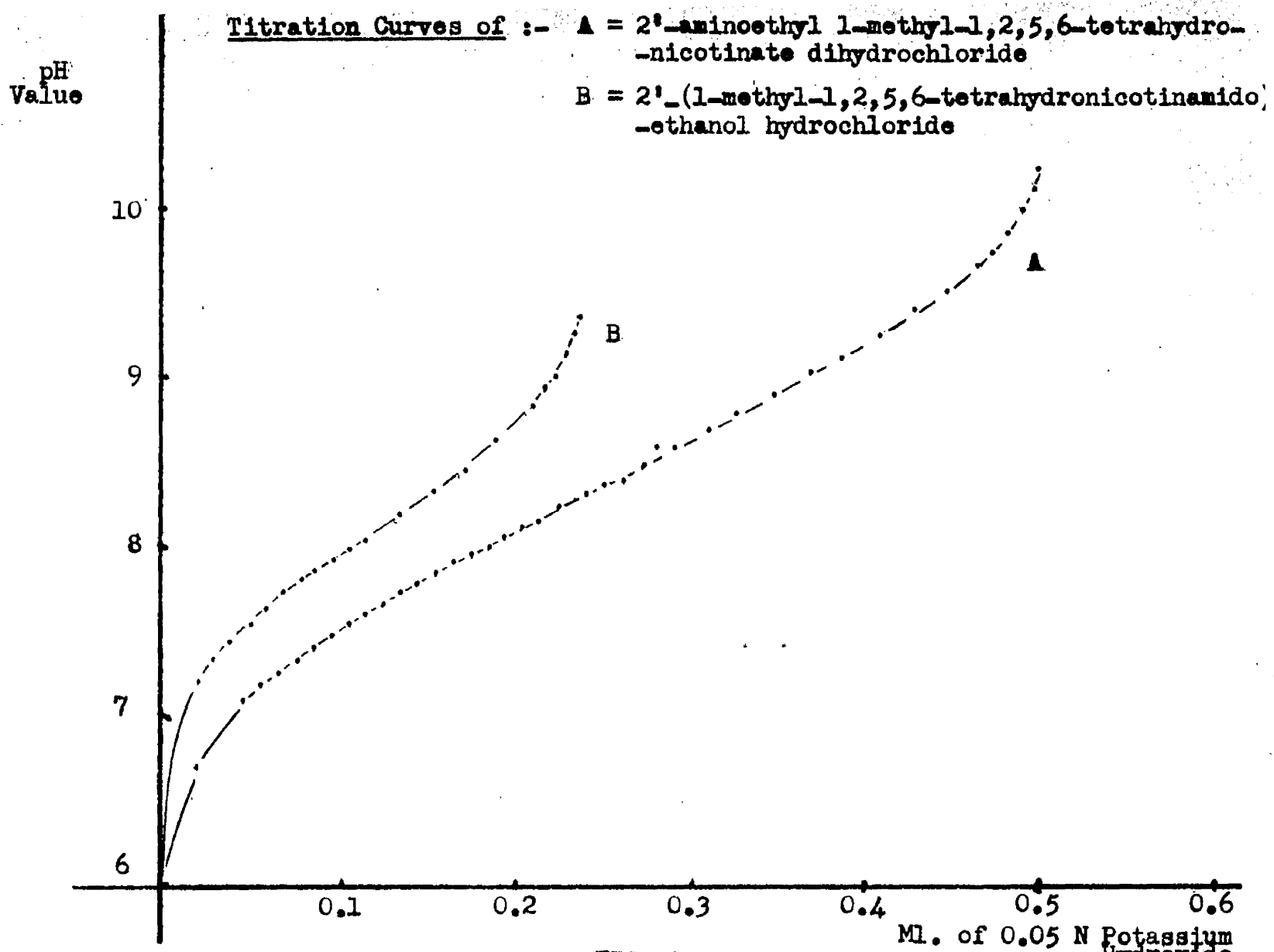

Titration Curves of :- A = 2'-aminoethyl 1-methyl-1,2,5,6-tetrahydro--nicotinate dihydrochloride
B = 2'-(1-methyl-1,2,5,6-tetrahydronicotinamido)-ethanol hydrochloride
pH Value
10
9
8
7
6
A
B
0.1
0.2
0.3
0.4
0.5
0.6
Ml. of 0.05 N Potassium Hydroxide

FIG. 2

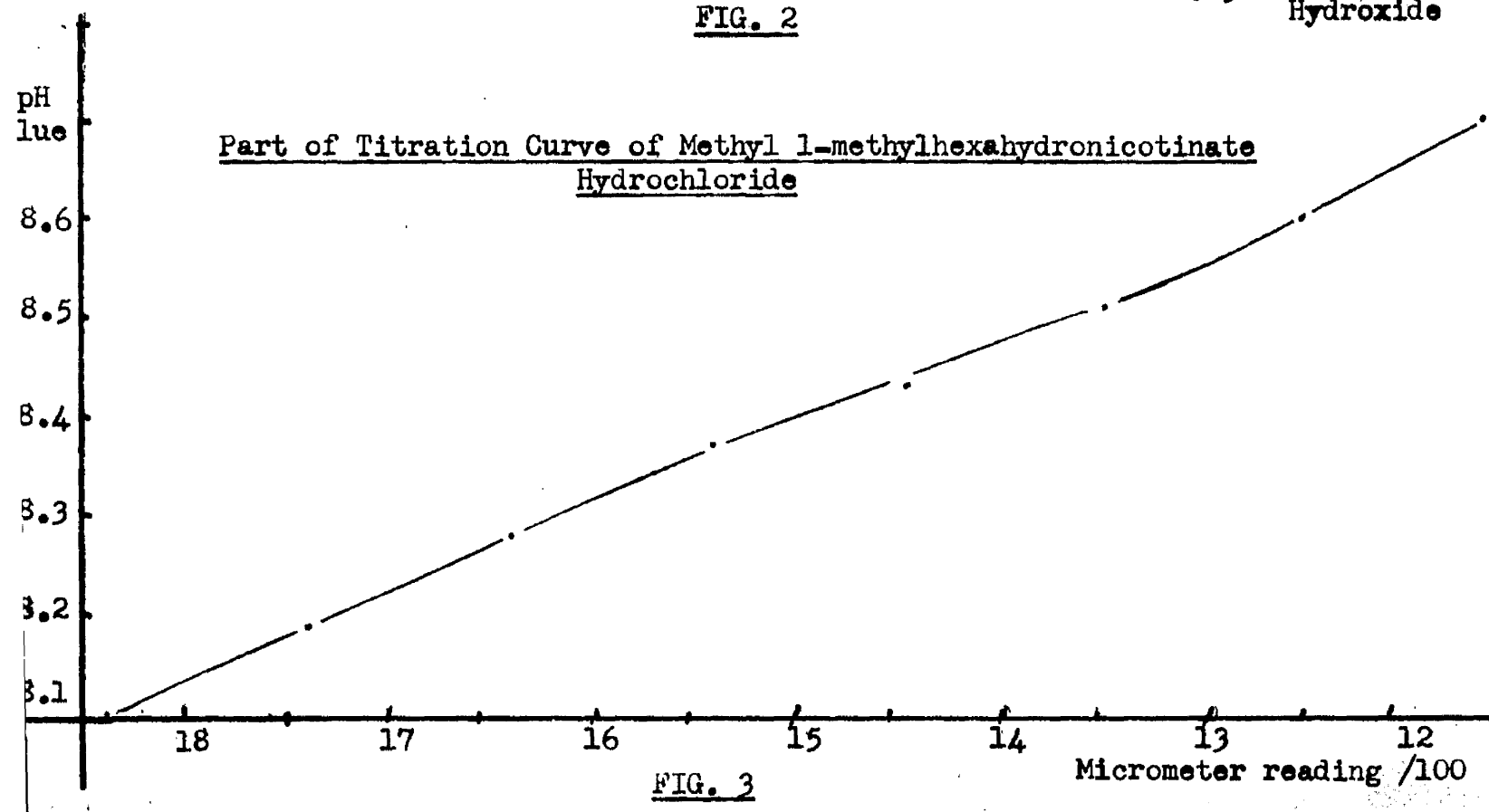

Part of Titration Curve of Methyl 1-methylhexahydronicotinate Hydrochloride
pH lue
8.6
8.5
8.4
8.3
8.2
8.1
18
17
16
15
14
13
12
Micrometer reading /100

FIG. 3

Phillips and Baltzly[67] in studies of the closely-related ethanolamide of 1-methylhexahydronicotinic acid.

Attempts to isolate and characterise the arecaidine alkanolamides were complicated by the high water-solubility of their bases, making extraction by organic solvents difficult and incomplete. The bases were hygroscopic semi-solids which could not be distilled and which did not form any crystalline derivatives. A crystalline oxalate prepared from the ethanolamide base and a slight excess of oxalic acid in ethanol proved to be the aminoethyl-ester oxalate, N→O acyl migration having presumably occurred during preparation of the salt. Amino-ester formation with such a weak acid as oxalic is exceptional, indicating that equilibrium in the N⇌O-acyl system favours the O-acyl form to an unusual extent in this case.

Since the yields of arecaidine alkanolamide bases were so markedly reduced on attempted extraction from the aqueous alkaline solution in which they were formed, and since there was little advantage in such an extraction, the neutralised aqueous solution was used directly for pK'_a determinations and for pharmacological investigation.

The acid chloride hydrochloride of 3-dimethylaminopropionic acid decomposed below the melting point of any eutectic mixture of it with ethanolamine hydrochloride or

2-aminopropanol hydrochloride, thereby preventing acylation in the absence of solvent.

The propanolamide of 3-dimethylaminopropionic acid was now the only one of the required compounds for which no satisfactory synthesis was available. A new route was therefore investigated in which 3-dimethylaminopropionyl chloride was treated with the ethyl ester of a primary 2-amino acid to form a dipeptide ester which was then reduced to the alkanolamide by means of lithium aluminium hydride. This method had the advantage that the material reacting with the acid chloride had only one reactive group, thereby reducing the likelihood of unwanted side-reactions.

Preliminary attempts were carried out using ethyl glycinate, which reacted readily with the acid chloride hydrochloride on heating under reflux in chloroform to give the required dipeptide ester hydrochloride (XXXIX) in good yield,

$$N(CH_3)_2CH_2CH_2COCl.\ HCl + NH_2CH_2COOC_2H_5 \Rightarrow N(CH_3)_2CH_2CH_2CONH\ CH_2COOC_2H_5.\ HCl.$$

(XXXIX)

The product, which was crystalline but deliquescent, was converted to its base by treatment of a suspension in dry ether with an excess of dry ammonia (separation of the base from alkaline aqueous solution being difficult because of its high water solubility).

Reduction of the dipeptide-ester (XXXIX) with lithium aluminium hydride was complicated by the presence in the molecule of two reducible carboxyl groups. It is known however that the ester carboxyl is much more easily reduced than is an amide carboxyl (particularly with an N-substituent)[78] and there are examples in the literature of selective reduction of esters in the presence of amides.[73,74] In the present case it was found possible, by control of time, temperature and proportions of reactants to reduce the dipeptide ester to the required ethanolamide in good yield by means of lithium aluminium hydride in dry ether.

$$N(CH_3)_2CH_2CH_2CONH\ CH_2COOC_2H_5 \xrightarrow{Li\ Al\ H_4} N(CH_3)_2CH_2CH_2CONH\ CH_2CH_2OH$$

Although this method of preparation has no advantage over previously used methods for the ethanolamide of β-dimethylaminopropionic acid, it served as a model for the preparation of the corresponding (and not otherwise readily accessible) propanolamide, which was prepared in good yield by the same method, substituting ethyl (±)-2-aminopropionate for the ethyl glycinate.

Preparation of alkanolamides by reduction of dipeptide esters should have a considerable advantage for the synthesis of amides of optically active amino-alcohols, since optically active amino-acids are available and the mild conditions of reaction

should not tend to cause racemisation. Such a preparation was not attempted however in the present work, since evidence for a relationship between optical activity of amino-alcohols and oxytocic action of lysergic acid amides prepared from them is conflicting,[12,75] and it was felt that preparation of pure stereoisomers in the present series could well await a demonstration of some oxytocic activity by the racemates.

Other mixed anhydrides have been used for the preparation of amides, notably the acid azide which was used by Stoll and co-workers in their partial synthesis of amides of lysergic and isolysergic acids.[12] The main disadvantage of this route was that the intermediate hydrazide was always formed as the racemic hydrazide of isolysergic acid which required epimerisation and resolution before converting to acid azide. This problem did not arise in the present series, since no asymmetric centres are present in the amino-acids which were used.

Attempts to prepare the acid azide of arecaidine were unsuccessful. The hydrazide, prepared from arecoline and hydrazine hydrate by the method of Sapara,[76] and treated with nitrous acid as described by Stoll,[12] showed no sign of having reacted and did not give any ether or chloroform extractive. The aqueous solution on evaporation <u>in vacuo</u> yielded arecaidine hydrazide, indicating that reaction had probably not occurred,

although it is possible that a highly water-soluble acid azide may have been formed and subsequently hydrolysed during the evaporation of the water.

Preparation of the acid azide of 3-dimethylamino-propionic acid was not attempted, since Curtius[77] found that treatment of its hydrazide with nitrous acid failed to produce the required azide.

Recent investigations into the synthesis of polypeptides have stimulated the attention of many workers to the reaction of acid anhydrides and amino-acids, and a number of new acid anhydrides have been prepared and used for this purpose. Garbrecht[69] has reviewed this development and studied the application of various mixed anhydrides of lysergic acid to the preparation of alkanolamides. He found that the most satisfactory acid anhydride was the acid sulphate, which had many advantages over the acid azide previously employed. This route was not employed in the present work, since the problems involved in the use of acid chlorides had largely been solved before the publication of Garbrecht's paper. It is noteworthy that the acid sulphate of lysergic acid, as well as its acid azide, produced mainly N-acylation of amino-alcohols, which raises the question whether the tendency to O-acylation in the present series was due to the chloride or to the amino-acid constituent

of the mixed anhydride. It appears probable that it is the amino-acid moiety which is responsible however, since most other acid chlorides are known to favour N-acylation of amino-alcohols,[68,70,71] with predominant formation of alkanolamides.

The methods which have been described in this section and the results obtained with different amino-acids are summarised in Table I.

TABLE 1

Reactants	Method of Preparation		
	Aminolysis	Acid Chloride in Chloroform	Acid Chloride Hydrochloride in absence of Solvent
Ethanolamine + 3-dimethylaminopropionic acid	Decomposition	Basic conditions:- bis compound Acid conditions:- aminoethyl ester mainly	Decomposition
Nicotinic Acid	Ethanolamide	-	-
N-methylhexahydronicotinic acid	Ethanolamide	Mainly bis compound; some aminoethyl ester under acid conditions	Aminoethyl ester
Arecaidine	Addition compound	Amorphous residue	Aminoethyl ester
2-Aminopropanol + 3-dimethylaminopropionic acid	-	Aminopropyl ester in low and variable yields under acid conditions only.	-
Nicotinic Acid	Propanolamide	-	-
N-Methylhexahydronicotinic acid	Probably propanolamide	-	Probably 2-aminopropyl ester
Arecaidine	-	-	2-Aminopropyl ester
Ethyl glycinate+ 3-dimethylaminopropionic acid	-	Dipeptide ester hydrochloride (reduced with Li Al H_4 to alkanolamide in fair yield).	-
Ethyl 2-aminopropionate + 3-dimethylaminopropionic acid	-	"	-

Pharmacological Action

Pharmacological investigation of the alkanolamides described in this section (for which the author is indebted to Dr. S. Nanjappa of the Department of Experimental Pharmacology, University of Glasgow) showed that none of these compounds had demonstrable oxytocic activity on the isolated oestrous rat uterus in concentrations up to 1 mg/ml.

The propanolamide of arecaidine inhibited acetylcholine-induced contractions of the oestrous rat uterus in concentrations of 0.075 - 0.3 mg./ml., an effect also shown by higher concentrations of the propanolamide of dimethylaminopropionic acid (0.3 - 1.0 mg./ml.) and by the ethanolamides of N-methylhexahydronicotinic acid (1 mg./ml.) and 3-dimethylaminopropionic acid (0.7 - 1 mg./ml.).

No effect on cat blood-pressure was shown by any of the compounds in doses up to 5 mg./Kg., nor did they have any inhibitory effect on the action of adrenaline or noradrenaline on cat blood-pressure.

The lack of oxytocic action shown by these alkanolamides reflects the findings of earlier workers who reported that the alkanolamides of derivatives of ω-amino aliphatic acids,[17,18] piperidine-carboxylic acids[29] and tetrahydroquinoline[35] were either inert as oxytocics or else showed much less activity than

did corresponding esters or simple alkylamides. Amongst purely synthetic compounds, only the propanolamide of N-tetrahydro-naphthyl-N-methyl-3-aminopropionic acid (IV) is reported to have marked oxytocic acticity ('comparable with ergometrine on the isolated uterus of the guinea pig and rabbit').[14,15,16]

It does not seem possible in the light of present evidence to speculate on the nature of the contribution made by the carboxylamido group to the oxytocic activity of the ergot alkaloids and semi-synthetic lysergic acid amides, but it appears that the alkanolamido group is specific in potentiating oxytocic action in only a very small range of compounds.

Ionic Dissociation of Amino-Acids, Amino-Esters and Amino-Alkanolamides.

The apparent dissociation constants (pK'a values) of the alkanolamides described in the preceding section, as well as those of corresponding acids and esters and related compounds were determined potentiometrically in order to provide evidence for an assignment of conformation to these substances. By analogy with the apparent dissociation constants of lysergic and iso-lysergic acids, ergometrine and ergometrinine, additional evidence was obtained for the previously postulated conformation of ring D and configuration at $C_{(8)}$ in these compounds.

Method of Determination of pK'a values

The potentiometric determination of pK'a values has been fully discussed by Simon and co-workers.[78,79,80] The relationship between values obtained by this method and thermodynamic values (pK a) at a given temperature is known to be affected by concentration, solvent and the properties of the electrodes and meter which are used.

The low concentration (0.005 M.) used in the present work avoided the concentration effect as far as possible. Preliminary attempts at still lower concentration (0.002 M.) yielded similar values, but the end-points were less distinct, thereby reducing the reproducibility of determinations. (Simon[78]

reported satisfactory results with 0.0035 M. solutions of organic acids in various solvents). This low concentration has the additional advantage of economy in materials as well as allowing titration in aqueous solution of sparingly water-soluble compounds. Errors due to traces of undesired electrolyte, especially carbonate, were minimised by passage of water and alkali through ion-exchange resins immediately before use and by bubbling carbon dioxide free nitrogen through the system during titration. The solvent effect, which was appreciable in this high dilution, was corrected for by an experimentally-determined blank as described by Tague[81] Harris[82] and Simon.[79]

Water was used as solvent throughout, except in the titration of lysergic and isolysergic acids the potassium salts of which were precipitated from aqueous solution before half-neutrality had been reached. The addition of a small proportion of ethyl cellosolve was sufficient to keep these acids in solution however, and repeat determinations at different low concentrations of cellosolve showed no appreciable or consistent variation, indicating that the added solvent had little effect (Table 2). Agreement of pK'a values calculated from three points on the titration curves similarly indicated that ionisation in these low cellosolve concentrations was substantially the same as that in water. The hydrophilic

character of the alkanolamido group potentiates water-solubility sufficiently to allow the titration of ergometrine and ergometrinine in aqueous solution. All other compounds investigated were quite soluble in water.

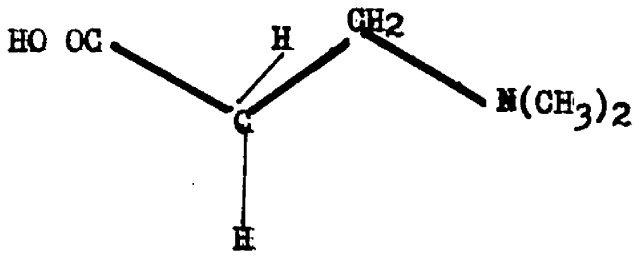
The use of an aqueous system avoids the considerable variation from the thermodynamic value produced by high concentrations of non-aqueous solvents[78,83,84] as well as providing an indication of the behaviour of the compounds under physiological conditions. It also allows confirmation of a pK' value by simple calculation from a number of points on the neutralisation curve.

Errors inherent in the method of pH determination were avoided as far as possible by frequent standardisation of the meter and electrodes against buffers of known pH value and by careful temperature control. The use of a standard technique throughout ensured that the values obtained, if not necessarily identical with the thermodynamic values, should be comparable with one another. The reproducibility of replicate determinations and of values calculated from different points on the same neutralisation curve (Tables 2, 3, 4) is seen to decrease as the pK'a value (and hence the end-point) departs from a value of 7, as might be expected. The differences in pK'a value which form the basis of the following discussion

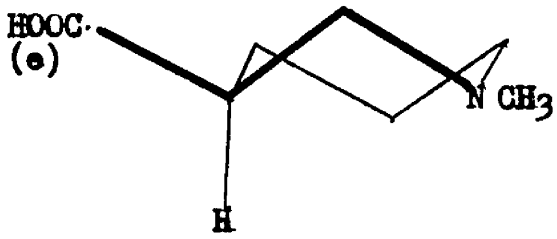
are however much greater than the largest variation observed in any single value, and the validity of the conclusions, which are essentially of a qualitative rather than a quantitative nature, is unaffected.

Discussion of Findings.

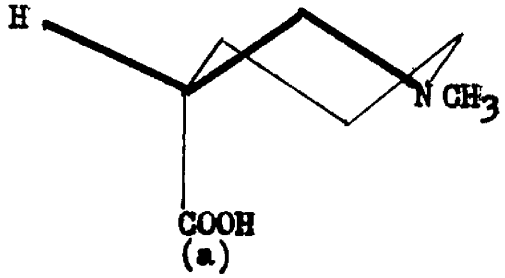
N-Methylhexahydronicotinic acid. Aliphatic amino-acids such as β-dimethylaminopropionic acid are known to exist in solution in the zig-zag open-chain form (XL) in which the two charged groups are remote. (This conclusion originally derived by Neuberger[85] from differences in pK' value between acids and esters has been supported by measurements of dipole distances).[86,87] By analogy it would appear therefore that the preferred conformation of N-methylhexahydronicotinic acid in solution should be that in which the two charged groups are remote as in (XLI) (COOH equatorial) rather than as in (XLII) (COOH axial). That such a conformation represents the preferred structure for N-methylhexahydronicotinic acid may also be deduced theoretically by analogy with similar cyclohexane systems on the grounds that a single substituent will adopt the more stable equatorial configuration (the orientation of the N-methyl group may be ignored, since tertiary nitrogen groups are not resolvable into optical enantiomorphs). On the other hand such



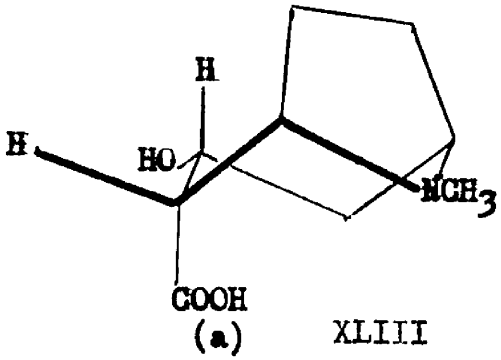
XL



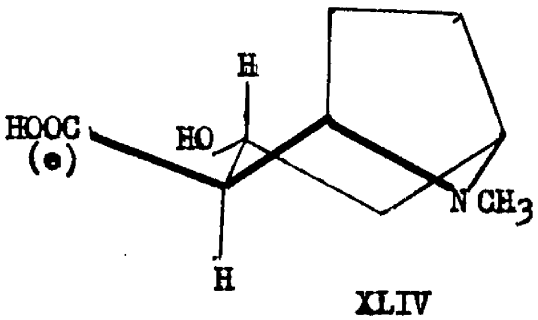
XLI



XLII



XLIII



XLIV

XLV

conformational analogies are of doubtful value when charged groups are involved, since it is known that both steric and electrical repulsion between ring substituents are factors which can affect conformational stability.[88]

Introduction of a carboxyl group into the molecule of an aliphatic amine is base-weakening. The effect is influenced by the position of the carboxyl group in relation to the amino group.[48] Examination of molecular models indicates that there is identical chain and spacial separation of $-CO_2^-$ and $\geq N\ Me\ H^+$ groups in 3-dimethylaminopropionic acid (XL) and N-methylhexahydronicotinic acid provided the latter occupies the conformation (XLI). Consequently we should expect these two substances to have dissociation constants of the same order.

Second acid dissociation constants (which are more clearly indicative of the structural features concerned) for these two amino-acids are given in Table 2. The pK'a value of 3-dimethylaminopropionic acid (9.85) is seen to be distinctly lower than that of the corresponding primary amino-acid, β-alanine (10.36).[89] This is in agreement with the observations of Bredig[90] and Hall and Sprinkle[91] who showed that whereas the introduction of a single methyl group into a primary amine gives a small increase in pK value, introduction of a second methyl group to form a tertiary amine produces a marked fall in pK value.

A similar lowering of pK value in passing from secondary to tertiary base has been observed with corresponding pairs of dihydronorlysergic and dihydrolysergic acids.[49] The concordance of pK' values for 3-dimethylaminopropionic acid (9.85) and N-methylhexahydronicotinic acid (9.70) clearly demonstrates that the steric relationship of basic and acidic groups is the same (at least in solution) in both acids. This not only supports the conformational assignment (XLI) for N-methylhexahydronicotinic acid, but also the validity of the general principles of conformational analysis to compounds of this type.

Lysergic and isolysergic acids. Apparent ionic dissociation constants for these acids obtained in the present work (Table 2) are in general agreement with those obtained less precisely by Craig and co-workers.[92] The interpretation of these figures by Stenlake[48] and by Stoll and co-workers[49] therefore remains unaffected.

Ecgonine and ψ-ecgonine. Findlay[93,94,95] assigned the structures (XLIII) and (XLIV) to ecgonine and ψ-ecgonine respectively, and these conformational assignments have since been confirmed by Fodor, Kovacs and Weisz.[96,97] Second acid dissociation constants for these compounds determined in the

TABLE 2

Second Acid Dissociation Constants of Amino Acids

Name of Compound	pKa from titration to			Mean Value
	half neutrality	1/3 neutrality	2/3 neutrality	
3-Dimethylaminopropionic acid	9.85 9.86	9.87 9.85	9.79 9.89	9.85
1-Methylhexahydronicotinic acid	9.67 9.72	9.67 9.73	9.67 9.72	9.70
1-Methyl-1,2,5,6-tetrahydronicotinic acid (Arecaidine)	9.05 9.08	9.07 9.10	9.05 9.05	9.07
Ecgonine	10.84 10.85	10.91 10.91	11.12 10.78	10.85
ψ-Ecgonine	9.69 9.67	9.59 9.76	9.88 9.62	9.68
Lysergic Acid in 40% cellosolve	7.83	7.86	7.82	
Lysergic Acid in 30% cellosolve	7.81 7.79	7.83 7.77	7.83 7.79	7.82
Lysergic Acid in 15% cellosolve	-	-	7.84	
Isolysergic Acid in 30% cellosolve	8.76	8.63	-	8.67
Isolysergic Acid in 20% cellosolve	8.70	8.61	8.66	

TABLE 3

Dissociation Constants of Esters

Name of Compound	pK'a from titration to			Mean Value	Difference from pK'a of corresponding acid (Δ pK (ester))
	half neutrality	1/3 neutrality	2/3 neutrality		
Ethyl 3-dimethylaminopropionate	8.6	8.61	8.59	8.60	1.25
	8.6	8.58	8.59		
Methyl 1-methylhexahydronicotinate	8.45	8.45	8.44		
	8.46	8.45	8.44	8.44	1.26
	8.41	8.41	8.42		
Methyl 1-methyl-1,2,5,6-tetrahydronicotinate (Arecoline)	7.72	7.71	7.68	7.70	1.37
	7.67	7.68	7.67		
Ecgonine methyl ester	9.22	9.15	9.32	9.22	1.63
	9.16	-	9.26		
ψ-Ecgonine methyl ester	8.23	8.26	8.16		
	8.26	8.20	8.25	8.21	1.47
	8.19	8.17	8.20		

present work are given in Table 2. The expected base-strengthening effect when the carboxyl group is in close spacial proximity to the ring-N is observed in the much higher value for ecgonine (XLIII; pK'a 10.85) than in ψ-ecgonine (XLIV; pK'a 9.68). These results therefore provide a parallel which supports the conformational assignments proposed for the $C_{(8)}$ carboxyl in dihydrolysergic and lysergic acids.[49,51] Whilst the agreement between the pK'a value for ψ-ecgonine (9.68) and N-methylhexahydronicotinic acid (9.70) would appear to provide further evidence for the assignment of an equatorial carboxyl in ψ-ecgonine, (XLIII) this agreement must be regarded as fortuitous, as it takes no account of either the methylene bridge or the $C_{(4)}$ hydroxyl in the latter substance. Differences in pK'a value between acid-ester pairs in the two series have therefore been examined.

Acid-ester differences. It was established by Neuberger[65] that the difference in ionic dissociation constant between an amino-acid and its ester ($\Delta pK_{(ester)}$) provides a measure of the distance between the two charged groups of the amino-acid zwitterion, $\Delta pK_{(ester)}$ increasing with increasing proximity of the groups. The assumptions made do not permit the use of dissociation constants for accurate measurement of such distances, but this in no way invalidates the use of $\Delta pK_{(ester}$

in a qualitative sense. The data in Table 3 shows that there is reasonable correlation between $\Delta pK_{(ester)}$ values for N-methylhexahydronicotinic acid (XLI; $\Delta pK_{(ester)}$ 1.26), arecaidine (XLV; $\Delta pK_{(ester)}$ 1.37) and ψ-ecgonine (XLIV; $\Delta pK_{(ester)}$ 1.47) in all of which the carboxyl is equatorial. 3-Dimethylamino-propionic acid in which there is a comparable spacial relationship between the COOH and $\geq$N Me groups also has $\Delta pK_{(ester)}$ 1.25. On the other hand $\Delta pK_{(ester)}$ for ecgonine (XLIII); $\Delta pK_{(ester)}$ 1.63), in which the carboxyl is axial, is significantly greater.

The influence of structural features such as the ethylenic bond of arecaidine is seen to be largely cancelled out when $\Delta pK_{(ester)}$ values are considered. Both arecaidine and its methyl ester are weaker bases than the corresponding dihydro compounds by about 0.7 pK units, yet their $\Delta pK_{(ester)}$ values are very similar (see Table 3). This concordance of $\Delta pK_{(ester)}$ values demonstrates that this cyclic double bond does not materially alter the space relationship of the carboxyl and ring-nitrogen groups, and supports the view that such unsaturated rings adopt the cyclohexene-like semi-chair conformation (XLV).

The effect of the hydroxyl groups in the ecgonine series is obviously more complex. Δ pK for benzoylecgonine

TABLE 4

Name of Compound	pK'a from titration to			Mean Value	Difference from pK'a of corresponding acid. (Δ $pK_{(amide)}$)
	Half neutrality	1/3 neutrality	2/3 neutrality		
2-(3'-Dimethylaminopropionamido)--ethanol. (3-Dimethylaminopropionic acid ethanolamide).	8.65 8.65	8.66 8.67	8.65 8.64	8.65	1.20
(±)-2-(3'-Dimethylaminopropionamido)-propanol. [(±)-3-Dimethylaminopropionic acid propanolamide].	8.84 8.82 8.82	8.77 8.84 8.81	8.80 8.82 8.84	8.82	1.03
2'-(1-Methylhexahydronicotinamido)-ethanol.(1-Methylhexahydronicotinic acid ethanolamide).	8.66 8.66 8.65	8.67 8.68 8.64	8.65 8.66 8.63	8.65	1.05
2'-(1-Methyl-1,2,5,6-tetrahydro-nicotinamido)-ethanol. (Arecaidine ethanolamide)	8.02 8.02	8.02 8.04	8.01 8.02	8.02	1.05
(±)-2'-(1-Methyl-1,2,5,6-tetrahydro-nicotinamido)-propanol. [(±)-Arecaidine propanolamide]	8.07 8.08	8.08 8.06	8.06 8.06	8.07	1.00
Ergometrine	6.78 6.80	6.78 6.80	6.82 6.78	6.79	1.03
Ergometrinine	7.38 7.38	7.35 7.36	7.37 7.40	7.37	1.30

(calculated from measurements by Kolthoff[98]) is 3.15, compared with Δ pK 1.63 for ecgonine. This large difference could be ascribed to hydrogen-bond formation between the adjacent hydroxyl and carboxyl groups in ecgonine and its methyl ester. Such a hydrogen bond would have opposite effects in acid and ester, and it is observed that whereas the pK' value of ecgonine (10.91) is less than that of benzoylecgonine (11.8), the pK' value of ecgonine methyl ester (9.22) is greater than that of cocaine (8.80).

Acid-propanolamide differences. Comparison of $\Delta pK_{(ester)}$ values for lysergic acid and isolysergic acid (I) in aqueous solution was limited by the low water-solubility of most lysergic acid derivatives. Since the acids themselves were only titratable in very low concentration and in the presence of some added organic solvent, it did not seem likely that their esters could be titrated in any predominantly aqueous system. This difficulty was overcome by determining pK' values of the more water-soluble propanolamides (ergometrine and ergometrinine; I, R = $NHCH(CH_3)CH_2OH$) and comparing these acid - propanolamide differences ($\Delta pK_{(amide)}$) with acid-propanolamide differences for 3-dimethylaminopropionic acid and arecaidine and with acid-ethanolamide differences for 3-dimethylaminopropionic acid, N-methylhexahydronicotinic acid and arecaidine

A further advantage of this procedure was that it provided direct evidence for the detailed stereochemistry of the pharmacologically-active ergometrine in aqueous solution.

Table 4 shows that there is excellent agreement between the $\Delta\ pK_{(amide)}$ values for lysergic acid -ergometrine ($\Delta\ pK_{(amide)}$ 1.03), 3-dimethylaminopropionic acid -propanolamide ($\Delta\ pK_{(amide)}$ 1.03) and arecaidine -propanolamide ($\Delta\ pK_{(amide)}$ 1.00). Values for the ethanolamides are comparable, a slight variation being shown only by the ethanolamide of 3-dimethylaminopropionic acid ($\Delta\ pK_{(amide)}$ 1.20). By analogy with the $\Delta\ pK_{(ester)}$ values, this conformity of $\Delta\ pK_{(amide)}$ values offers evidence that the carboxyl-ring nitrogen distance is similar in all these compounds. This would be consistent with the adoption of a chair conformation by ring D of lysergic acid and ergometrine, combined with an equatorial carboxyl substituent at $C_{(8)}$ (structure XIX) corresponding to structures (XLI), (XLIV) and (XLV) for the other amino-acids.

The higher $\Delta\ pK_{(amide)}$ value for isolysergic acid - ergometrinine (1.30) provides confirmation for the validity of the above interpretation and for the existence of an axial carboxyl substituent at $C_{(8)}$ in the isolysergic acid series, since the closer proximity of the ionised carboxylic acid and

ring nitrogen groups produced by such a configuration (XX) would have a base-strengthening effect in the free acid,[48,49] whereas the effect of a proximate carboxypropanolamido group would only be the relatively weak one due to hydrogen bonding with the amide and hydroxyl hydrogen atoms. The effect of such bonding would be the same as that already discussed for ecgonine methyl ester, the pK'a value of the isolysergic acid propanolamide being increased and thereby reducing the $\Delta pK_{(amide)}$ value as compared with the $\Delta pK_{(ester)}$ value where hydrogen bonding could not occur. This is shown clearly in values published by Stoll's school for the dihydroisolysergic acid series (for dihydroisolysergic acid I and its monoethyl amide, $\Delta pK_{(ester)} = 2.85$, $\Delta pK_{(amide)} = 1.97$). The effect is much less marked in the dihydrolysergic acid series where the groups concerned are more widely separated, (for dihydrolysergic acid I $\Delta pK_{(ester)} = 1.65$, $\Delta pK_{(amide)} = 1.17$). (The differences in actual value between Stoll's figures and those in the present work are doubtless due to the use of 80% cellosolve as solvent by Stoll). It follows that the $\Delta pK_{(amide)}$ value does not vary so much with differences in distance between the carboxyl and ring-nitrogen groups as does the $\Delta pK_{(ester)}$ value. The relatively small (though significant) differences between the

$\Delta\ pK_{(amide)}$ values for lysergic and isolysergic acids would therefore probably have corresponded to a much greater difference in $\Delta\ pK_{(ester)}$ values for the same compounds, if these had been determinable in an aqueous system. The two methods should accordingly be equally valid as a means of comparing differences in distance between charged groups in related molecules, although the sensitivity of the $\Delta\ pK_{(ester)}$ method will be higher.

Conclusions

(a) 1-Methylhexahydronicotinic acid and 3-dimethylamino-propionic acid appear to have the same spacial relationship of carboxyl and amino groups, as indicated by their similar pK'a values. From the known steric structure of 3-dimethylamino-propionic acid (XL) it may be deduced that 1-methylhexahydro-nicotinic acid has structure (XLI).

(b) The spacial separation of carboxyl and amino groups in 3-dimethylaminopropionic acid, 1-methylhexahydronicotinic acid, arecaidine and ψ-ecgonine and their esters is considered to be similar, since these compounds all have similar $\triangle pK_{(ester)}$ values. This would indicate that the last three compounds have structures (XLI), (XLV) and (XLIV) respectively. The higher $\triangle pK_{(ester)}$ value for ecgonine is in accordance with the known axial configuration of the carboxyl substituent in this compound (XLIII).

(c) Concordance of $\triangle pK_{(amide)}$ values for 3-dimethylamino-propionic acid, 1-methylhexahydronicotinic acid, arecaidine and lysergic acid and their alkanolamides offers evidence for the existence of a similar spacial relationship of carboxyl and amino groups in all of these compounds, consistent with the adoption of structure (XIX) by lysergic acid and a corresponding

configuration for ergometrine. The higher Δ $pK_{(amide)}$ value for isolysergic acid supports the previously postulated steric structure for this compound (XX) and for ergometrinine in aqueous solution.[48,49]

E X P E R I M E N T A L

P A R T III

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Melting points are uncorrected.

Preparation of 3-Dimethylamino-2-styrylpropionic Acid

(i) Attempted Mannich condensation of ethyl styrylacetate, formaldehyde and dimethylamine.

(a) Ethyl styrylacetate, prepared by the method of Linstead and Williams,[99] (0.95 g., 0.005 mole) in ethanol (3.5 ml.) was heated under reflux for 4 hr. with dimethylamine hydrochloride (0.45 g., 0.005 mole) and 40% aqueous solution of formaldehyde (0.95 ml.). The ethanol was removed by evaporation *in vacuo*, the residue dissolved in water (2 ml.), acidified with hydrochloric acid and extracted with ether. Evaporation of the ether *in vacuo* yielded 0.90 g. of unchanged ethyl styrylacetate.

The aqueous fraction remaining after the ether extraction was basified with solution of sodium hydroxide and again extracted with ether. The ethereal extract yielded on evaporation *in vacuo* 24 mg. of an oily residue which failed to form a crystalline hydrochloride or picrate.

(b) Substitution of methanol for the ethanol and increase in the time of refluxing to 72 hr. gave the same result.

(c) The reaction was repeated using butanol as solvent and replacing the solution of formaldehyde by paraformaldehyde (0.4 g.) Heating under reflux for 72 hr. yielded 26 mg. of non-volatile basic material which did not form a crystalline hydrochloride or picrate.

(d) The previous reaction was repeated using amyl alcohol as solvent. The same result was obtained.

(e) A mixture of ethyl styrylacetate (0.95 g., 0.005 mole), dimethylamine hydrochloride (0.45 g., 0.005 mole) and para-formaldehyde (0.3 g.) was heated in a sealed tube at 206° for 6 hr. A yield of 28 mg. of non-volatile base similar to that described in the previous reactions was obtained.

(f) Dimethylamine (0.23 g., 0.005 mole) was dissolved in a mixture of acetic acid (4 ml.) and acetic anhydride (0.4 ml.). Ethyl styrylacetate (0.95 g., 0.005 mole) and paraformaldehyde (0.3 g.) were added and the mixture heated under reflux for 6 hr. Most of the solvent was removed by evaporation in vacuo, the residue mixed with water (15 ml.) and extracted with ether. The ether extract yielded on evaporation 0.9 g. of unchanged ethyl styrylacetate. The residual aqueous fraction was basified with sodium hydroxide and again extracted with ether to yield a small trace of basic material on evaporation.

(g) Styrylacetic acid (0.8 g., 0.005 mole) was suspended in water, dimethylamine (0.23 g., 0.005 mole) added and the mixture stirred until solution was effected. Solution of formaldehyde 40% (1 ml.) was added and the mixture heated under reflux for 6 hr. The solution was cooled and acidified with hydrochloric acid to give a precipitate of unchanged styrylacetic acid.

Extraction of the supernatant liquid with ether yielded more unchanged acid, the total recovery being 0.78 g.

(ii) Mannich condensation of styrylmalonic acid, formaldehyde and dimethylamine.

Styrylmalonic acid (1.03 g., 0.05 mole), prepared by the method of Ivanov and Pschenichnii,[52] was dissolved in 60% aqueous solution of dimethylamine (0.38 ml.) and treated with 40% solution of formaldehyde (0.4 ml.) at 0°. There was an immediate transient effervescence. The mixture was left overnight at 0°, acidified with dilute hydrochloric acid, and the amorphous solid which separated was extracted with ether. Evaporation of the ethereal extract in vacuo yielded 0.49 g. of a solid which could not be crystallised butwhich gave a crystalline derivative with p-bromophenacyl bromide, m.p. 142° (from ethanol), probably p-bromophenacyl 2-styrylacrylate. (Found: C,60.5; H,4.01; Br,20.8. $C_{19}H_{15}O_3Br$ requires C,61.41; H,4.01; Br,21.3).

The aqueous fraction remaining after extraction of the acid was treated with a slight excess of sodium bicarbonate and extracted with ether to remove any unchanged dimethylamine, then a slight excess of solution of sodium picrate was added. On the addition of a few drops of dilute hydrochloric acid, crystals (23 mg., 0.9%) m.p. 146° (from ethanol) of the picrate of 3-dimethylamino-2-styrylpropionic acid were obtained. (Found:

C,51.5; H,4.5; N,12.3. $C_{19}H_{20}N_4O_9$ requires C,50.9; H,4.5; N,12.5).

(iii) Attempted preparation of 3-methylamino-2-styrylpropionic acid.

The preceding reaction was repeated, replacing the dimethylamine by an equivalent amount of methylamine. The acidic fraction obtained was identical with that from the previous reaction. A picrate m.p. 114° was obtained as described above, but in quantity too small for recrystallisation and characterisation.

Preparation of 4-Dimethylamino-2-styrylbutyric Acid.

(a) Sodium (0.575 g., 0.025 mole) was dissolved in dry ethanol (20 ml.) and ethyl styrylacetate (4.75 g., 0.025 mole) added with stirring and cooling. After 60 hr. at room temperature the mixture was treated with a solution of dimethylaminoethyl chloride (obtained from dimethylaminoethyl chloride hydrochloride, 3.58 g., 0.025 mole) in xylene (7 ml.) added dropwise with continuous stirring. On heating under reflux for 2 hr. a white precipitate was obtained. The mixture was cooled, acidified with dilute hydrochloric acid, extracted with ether and the ethereal extract reserved. The aqueous fraction was basified with solution of ammonia and again extracted with ether. The ether extract was dried over anhydrous sodium sulphate, evaporated *in vacuo* and the oily residue fractionally distilled *in vacuo* to yield 0.92 g., 14% of a colourless viscous oil, b.p. 100-110° 0.4 mm., n_D^{18}, 1.515, probably ethyl 4-dimethylamino-2-styrylbutyrate.

(Equivalent weight by potentiometric titration, 258. $C_{16}H_{23}O_2N$ requires equivalent weight 261).

The product resinified and darkened rapidly on storage.

The picrate of this base was initially oily but slowly formed crystals m.p. 106° (from aqueous ethanol)of the *picrate* of *4-dimethylamino-2-styrylbutyric acid*. (Found: C,51.4; H,4.7;

N,12.2. $C_{20}H_{22}O_9N_4$ requires C,51.95; H,4.8;N,12.1).

(b) The reaction was repeated, heating the sodium ethoxide and ethyl styrylacetate under reflux for 2 hr. initially. The non-basic ether-soluble fraction was again reserved. The yield of base, identical with that from the previous reaction was 0.88 g., 13%.

(c) The initial period of heating under reflux was increased to 18 hr. No basic material was recovered.

The non-basic ethereal fractions from the previous reactions were combined and fractionally distilled in vacuo to yield, in addition to unchanged ethyl styrylacetate, 14.9 g. of a very viscous yellow liquid b.p. 206-208° 0.8 mm. The latter was hydrolysed by heating under reflux for 30 min. with ethanolic potassium hydroxide. Removal of the alcohol by evaporation in vacuo, acidification with hydrochloric acid and extraction with ether produced an acid, m.p. 153° (from benzene and light petroleum), probably a dimer of styrylacetic acid (Found: C,74.2; H,6.6; equivalent weight by potentiometric titration, 164; approximate molecular weight from freezing-point depression of camphor, 294. $C_{20}H_{20}O_4$ requires C,74.1; H,6.2; equivalent weight, 162; molecular weight, 324). Styrylacetic acid has m.p. 54°.

(d) Ethyl styrylacetate (4.75 g., 0.025 mole) was added to

'molecular' sodium (0.575 g., 0.025 mole) suspended in xylene (30 ml.). No reaction was evident in the cold; on warming a red resin was formed without evolution of hydrogen.

(e) The first reaction in the series was repeated, adding the dimethylaminoethyl chloride at a temperature of -30°, leaving 48 hr. at 0° then for four days at room temperature. The non-basic ether-soluble fraction consisted entirely of ethyl styrylacetate and none of the required basic product was obtained.

(f) The sodio derivative of ethyl styrylacetate was prepared as in the first reaction of the series, the alcohol removed by evaporation in vacuo and the residue dried in a vacuum desiccator over phosphorus pentoxide for 48 hr. The residue was suspended in xylene (40 ml.) and a solution of dimethylaminoethyl chloride (from 3.58 g. of hydrochloride, 0.025 mole) in xylene (20 ml.) was added. The mixture was heated under reflux with stirring for 20 min. The basic product, isolated as before, (3.8 g.) yielded on fractional distillation 0.5 g. of the required base. The remainder could not be distilled at a pressure of 0.1 mm. and did not form a crystalline picrate or hydrochloride.

(g) The previous reaction was repeated twice, heating under reflux for 7 min. and for 2 hr. Recoveries of the desired base were 0.7 g., 11% and 0.65 g., 10% respectively.

Alkanolamides of 3-Dimethylaminopropionic Acid.

(i) Reaction of ethyl 3-dimethylaminopropionate and ethanolamine.

(a) Equal volumes of ethyl 3-dimethylaminopropionate, prepared by the method of Adamson,[100] (14.5 g., 0.1 mole) and ethanolamine were heated together in a still fitted with a short fractionating column so that the fraction distilling below 90° was continually removed. After 6.12 g. of distillate had been obtained no more product of this boiling-range could be separated and heating was discontinued. The presence of an alcohol in the distillate was indicated by a positive iodoform reaction (theoretical yield of ethanol, 4.6 g.). Addition of alcoholic solution of picric acid to the distillate gave crystals of dimethylamine picrate, m.p. 150° undepressed on mixture with the picrate of authentic dimethylamine.

The residue in the still was fractionally distilled at 1 mm. pressure. After a fore-run of ethanolamine there was obtained a few drops of oily liquid distilling over a range of 140-190°. The residue was brown and resinous and could not be distilled. Neither it nor the distillate formed crystalline hydrochlorides, picrates or oxalates.

(b) A repeat reaction in which the ester and ethanolamine were heated together at 100° for 4 hr., yielded only unchanged starting material.

(ii) Reaction of 3-dimethylaminopropionyl chloride hydrochloride and ethanolamine.

(a) A suspension of 3-dimethylaminopropionic acid hydrochloride (6.12 g., 0.04 mole) in thionyl chloride (20 ml.) was heated in a water bath at 65° until effervescence ceased and the mixture became clear. Heating above this temperature caused resinification. Excess thionyl chloride was removed by evaporation *in vacuo* and the white crystalline residue washed with light petroleum and again dried *in vacuo*. The acid chloride hydrochloride so obtained was suspended in dry chloroform (20 ml.) and treated with a solution of ethanolamine (2.44 g., 0.04 mole) in dry chloroform (10 ml.) added in one quantity at room temperature with stirring. After standing overnight at room temperature, the chloroform was removed by evaporation *in vacuo* and the syrupy residue dried *in vacuo* over potassium hydroxide. Ethanol (30 ml.) was added and set aside overnight at 0°. The crystalline residue was filtered off and recrystallised from aqueous ethanol to give 2-aminoethyl 3'-dimethylaminopropionate dihydrochloride (1.1 g., 12%) m.p. 181°. (Found: C,35.9; H,7.7; N,12.0; Cl,30.6. $C_7H_{18}O_2N_2Cl_2$ requires C,36.0; H,7.7; N,12.0, Cl,30.5).

The alcoholic mother-liquors left after removal of the amino ester dihydrochloride were concentrated *in vacuo* and treated with dry ether to yield 4.1 g. 50% of a deliquescent hydrochloride,

m.p. 154° which formed on the addition of sodium picrate solution the dipicrate of O, N,-bis-(3-dimethylaminopropionyl)-ethanolamine. (m.p. 146°) (Found: C,41.05; H,4.5; N,17.3. $C_{24}H_{31}O_{17}N_9$ requires C,40.2; H,4.3; N,17.6).

(iii) Reaction of 3-dimethylaminopropionyl chloride hydrochloride and ethanolamine in the presence of triethylamine.

(a) A suspension in chloroform (20 ml.) of 3-dimethylamino-propionyl chloride hydrochloride (0.02 mole) prepared as described in the previous reaction was treated with triethylamine (2.02 g., 0.02 mole) added dropwise with stirring and cooling, then with a solution of ethanolamine (1.22 g., 0.02 mole) in chloroform (10 ml.) added in one quantity. There was an immediate exothermic reaction and a dark-brown colour developed. The mixture, after standing at room temperature for 4 hr., became homogeneous. It was cooled to 0°, shaken with a slight excess of solution of sodium hydroxide and the chloroform layer separated. The aqueous phase was extracted with two further portions of chloroform (20 ml.) and the combined chloroform extracts dried over sodium sulphate and evaporated in vacuo. The oily liquid obtained (5.3 g., 80%) yielded a hydrochloride m.p. 154° and picrate m.p. 147° undepressed on mixture with the corresponding derivatives of O, N,-bis-(dimethylaminopropionyl)-ethanolamine from the previous reaction.

(b) The previous reaction was repeated, keeping the reactants at a temperature below 0° and adding the ethanolamine solution slowly with continual stirring. The mixture was kept at 0° overnight, then the resulting suspension was filtered to yield ethanolamine hydrochloride (1.96 g., 100%). The filtrate was shaken with a slight excess of solution of sodium hydroxide, the chloroform layer separated and the aqueous phase extracted with a further two portions of chloroform (20 ml.). The combined chloroform fractions were dried over anhydrous sodium sulphate and evaporated to yield a brownish oily liquid (130 mg.) which formed on treatment with alcoholic picric acid, the picrate of 3-dimethylaminopropionic acid dimethylamide m.p. 131° (from ethanol) undepressed on mixture with the picrate ofthe amide produced by reaction of 3-dimethylaminopropionyl chloride hydrochloride and an excess of dimethylamine. (Found: C,42.9; H,5.4; O,33.8; N,18.2. $C_{13}H_{19}O_8N_5$ requires C,41.8; H,5.1; O,34.4; N,18.7).

(c) A suspension of 3-dimethylaminopropionyl chloride hydrochloride (0.05 mole prepared from 3-dimethylaminopropionic acid hydrochloride, 7.65 g.) in dry chloroform (50 ml.) was added slowly with stirring to a solution of ethanolamine (6.10 g., 0.10 mole) in dry chloroform (20 ml.), the temperature being kept below 0° throughout. The suspension was left overnight at room temperature then filtered. The

residue (13.3 g.) was recrystallised from ethanol-ether to give 5.5 g. of ethanolamine hydrochloride. The concentrated mother-liquors on treatment with sodium picrate solution gave a picrate, m.p. 147°, undepressed on mixture with the picrate of the bis compound from previous reactions.

The original chloroform filtrate was treated with a slight excess of solution of sodium hydroxide and the basic fraction obtained as described in the previous preparation. The base (1.31 g.) formed a picrate which was identical with that of the bis compound obtained previously.

(iv) Reaction of 3-dimethylaminopropionyl chloride hydrochloride with ethanolamine hydrochloride to yield 2-aminoethyl 3'-dimethylaminopropionate dihydrochloride.

A suspension of 3-dimethylaminopropionyl chloride hydrochloride (0.05 mole) in dry chloroform (30 ml.) was treated with ethanolamine hydrochloride (4.85 g., 0.05 mole) which had been finely powdered by levigation under dry light-petroleum. No immediate reaction was evident, but, on heating under reflux, hydrogen chloride was evolved and the amount of insoluble matter increased. After 2 hr., evolution of hydrogen chloride ceased, the mixture was allowed to cool and the chloroform removed by evaporation in vacuo. The residue was dissolved in a minimum quantity of boiling ethanol,

filtered and allowed to cool, yielding 2-aminoethyl 3'-dimethylaminopropionate dihydrochloride (7.5 g.,) m.p. 181°, identical with that obtained previously. Evaporation of the mother liquors yielded a further 1.0 g. of product and a total yield of 8.5 g. (73%).

(v) Preparation of 2-(3'-dimethylaminopropionamido)ethanol from 2-aminoethyl 3'-dimethylaminopropionate dihydrochloride.

A solution of 2-aminoethyl 3'-dimethylaminopropionate dihydrochloride (7.5 g., 0.033 mole) in a minimum volume of water was shaken with chloroform (30 ml.) and treated under cooling with a slight excess of potassium hydroxide dissolved in a minimum of water. The mixture was well shaken, the chloroform separated and the residue extracted with a further two portions of chloroform (30 ml.). The combined chloroform extracts were dried over exsiccated magnesium sulphate and the chloroform removed by evaporation in vacuo to leave 3.0 g., 57% of an oil, n_D^{18}, 1.477 probably slightly impure 2-(3'-dimethylaminopropionamido)-ethanol. (Found equivalent weight, potentiometric, 164, 165. $C_7H_{16}O_2N_2$ requires 160).

Short-path distillation of the oil at a pressure of 0.8 mm. yielded a colourless oily distillate of b.p. 155-160°. Some loss of vacuum occurred during heating, and there was a small residue of volatile base in the solid carbon dioxide - acetone cooled vapour trap. This base gave a picrate m.p. 150°

undepressed on mixture with the picrate of authentic dimethylamine.

The distillate fraction of b.p. 155-160° had n_D^{18}, 1.489 and an equivalent weight of 193. A portion of it was mixed with an excess of dimethylamine and left at room temperature for 48 hr. Excess dimethylamine was removed by heating to 100° in vacuo and the equivalent weight of the residue found to be 166.

Equimolecular proportions of the base (E.Wt. 166) and oxalic acid were separately dissolved in minimum amounts of ethanol and mixed. The ethanol was removed by distillation in vacuo and the resultant syrup treated with boiling acetone. This gave, on cooling, crystals m.p. 85° (from acetone) of 2-(3'-dimethylaminopropionamido)-ethanol acid oxalate. (Found: C,43.1; H,7.4; N,11.0. $C_9H_{18}O_6N_2$ requires C,43.2; H,7.2; N,11.2).

The succinate of 2-(3'-dimethylaminopropionamido)-ethanol was prepared as described for the oxalate, using 2 moles of base to each mole of succinic acid. It consisted of hygroscopic crystals, m.p. 108° (from acetone). (Found: C,49.5; H,8.8; N,12.8. $C_{18}H_{38}O_8N_4$ requires C,49.32; H,8.68; N,12.78).

Attempts to prepare crystalline picrates, picrolonates, hydrochlorides and naphthylisocyanates of the base were unsuccessful.

(vi) Ethyl 2-(3'-dimethylaminopropionamido)-acetate.

A suspension of 3-dimethylaminopropionyl chloride hydrochloride (0.05 mole) in dry chloroform (30 ml.) was treated with a solution of ethyl aminoacetate (5.15 g., 0.05 mole) in chloroform (20 ml.) added in one quantity. The mixture was heated under reflux until no further hydrogen chloride was evolved (about 2 hr.) and the chloroform removed by evaporation *in vacuo* to leave 9.8 g. (82%) of a gummy solid which crystallised on kneading under dry ether. Recrystallisation from ethanol-ether gave crystals m.p. 124° of a highly deliquescent substance, probably slightly impure ethyl 2-(3'-dimethylaminopropionamido)-acetate hydrochloride (Found: Cl, 16.1. $C_9H_{19}O_3N_2Cl$ requires Cl,14.9). Repeated recrystallisation did not improve the analysis.

The hydrochloride was suspended in dry ether (30 ml.) and dry ammonia passed through it for 10 min. with continual stirring. The product was filtered, the solid resuspended in ether and the process repeated. After a third repetition the ethereal fractions were combined and the ether removed by evaporation *in vacuo*. The oily residue was left overnight in a desiccator at 0.5 mm. pressure in the presence of potassium hydroxide and paraffin wax to yield ethyl 2-(3'-dimethylamino-propionamido)-acetate, n_D^{18}, 1.4621. (Found: C,53.6; H,8.6;

N,13.6. $C_9H_{18}O_3N_2$ requires C,53.5; H,8.9; N,13.9).

(vii) Preparation of 2-(3'-aminopropionamido)-ethanol by reduction of ethyl 2-(3'-aminopropionamido)-acetate.

Ethyl 2-(3'-dimethylaminopropionamido)-acetate (3 g., 0.0015 mole) was dissolved in dry ether (30 ml.) and treated with lithium aluminium hydride (0.68 g., 0.002 mole) added in small portions with continuous stirring and ice-cooling. The mixture was allowed to attain room-temperature with continuing constant stirring, the excess of lithium aluminium hydride was decomposed by the addition of moist ether followed by pieces of ice, and the product was filtered. The solid residue, on extraction with chloroform and evaporation of the extract *in vacuo* yielded an oily base (1.1 g., 46%) which formed an oxalate identical with the acid oxalate of 2-(3'-dimethylamino-propionamido)-ethanol obtained previously.

(viii) Preparation of (+)-2-aminopropyl 3'-dimethylaminopropionate dihydrochloride.

Finely powdered (±)-2-aminopropanol hydrochloride (0.76 g., 0.02 mole) prepared by the method of Vogl and Pöhm,[101] was added in one quantity to a suspension of 3-dimethylaminopropionyl chloride hydrochloride (0.005 mole) in dry chloroform and heated under reflux until no more hydrogen chloride was evolved (about 45 min.). The chloroform was removed by evaporation

in vacuo and the solid residue recrystallised from aqueous ethanol to yield (±)-2-aminopropyl 3'-dimethylaminopropionate dihydrochloride (0.47 g.) m.p. 189°. (Found: C,38.6; H,7.8; N,11.4. $C_8H_{20}O_2N_2Cl_2$ requires C,38.9; H,8.1; N,11.3). Concentration of mother liquors yielded a further 0.11 g. of product; total 0.58 g. (46%).

The residual fraction in the mother liquors (0.49 g.) could not be crystallised and did not give a crystallisable picrate on treatment with sodium picrate solution.

Numerous repetitions of the reaction gave yields varying from 5% to 46%. Increase in scale generally diminished the percentage yield.

(ix) Preparation of (±)-2-(3'-dimethylaminopropionamido)-propanol from (±)-2-aminopropyl 3'-dimethylaminopropionate dihydrochloride.

The method was identical with that described for the corresponding ethanol derivative (Preparation No.(v) in this section). An oily base was obtained which formed on treatment with oxalic acid the acid oxalate of (±)-2-3'-dimethylaminopropionamido)-propanol, m.p. 114° (from acetone-ethanol). (Found: C,45.8; H,7.9; N,10.5. $C_{10}H_{20}O_6N_2$ requires C,45.5; H,7.6; N,10.6)

The succinate was crystalline but excessively deliquescent.

(x) (±)-Ethyl 2-(3'-dimethylaminopropionamido)-propionate.

As described for the corresponding acetate (Preparation No.(vi) in this section) 3-dimethylaminopropionyl chloride

hydrochloride (0.05 mole) was treated with (±)-ethyl 2-amino-propionate to yield an extremely deliquescent hydrochloride, m.p. 96° (from ethanol-ether). (Found: Cl,14.3. $C_{10}H_{21}O_3N_2Cl$ requires Cl,14.1).

Treatment of a suspension of this hydrochloride in dry ether with excess dry ammonia as described previously gave (±)-ethyl 2-(3'-dimethylaminopropionamido)-propionate, a colourless oil, n_D^{18}, 1.4558, (8.78 g., 81%) (Found: C,55.7; H,9.3; N,12.9. $C_{10}H_{20}O_3N_2$ requires C,55.6; H,9.3; N,13.0).

(xi) Preparation of (±)-2-(3'-dimethylaminopropionamido)-propanol by reduction of (±)-ethyl 2-(3'-dimethylaminopropionamido)-propionate.

The total product from the previous reaction was dissolved in dry ether (50 ml.) and excess of lithium aluminium hydride (2 g.) added in portions with stirring and ice-cooling. Stirring was continued for 2 hr., during which time the mixture was allowed to come to room-temperature. Excess lithium aluminium hydride was decomposed by the cautious addition of ice and the product filtered. The solid residue was extracted twice with 50 ml. portions of boiling ethanol, which were combined and evaporated in vacuo to a syrupy residue. This was extracted with 25 ml. of boiling ethanol, and the extract

was treated with ether to form an opalescent solution. After filtration, this was again evaporated in vacuo to yield 4.95 g. (70%) of a viscous oil. Oxalic acid (4 g.) was added as an ethanolic solution and the resulting precipitate, consisting mostly of inorganic salts of oxalic acid, was removed by filtration. The filtrate on evaporation to dryness and extraction with boiling acetone gave crystals, m.p. 114° identical with those of the acid oxalate of (±)-2-(3'-dimethylaminopropionamido)-propanol obtained previously.

Alkanolamides of Nicotinic Acid and Related Substances

(i) 2-(1'-Methylhexahydronicotinamido)-ethanol acid oxalate. - The method of Phillips and Baltzly[57] was modified as follows:- A solution of 1-methylhexahydronicotinic acid hydrochloride (3 g., 0.0166 mole) in dry ethanol (30 ml.) was saturated with hydrogen chloride then heated under reflux for 1 hr. The mixture was evaporated *in vacuo* to a syrup which was treated with a slight excess of 20% aqueous sodium hydroxide and at once extracted with chloroform. The chloroform extract was dried over anhydrous sodium carbonate and the chloroform removed by evaporation *in vacuo* to yield 2.2 g. of ethyl 1-methylhexahydronicotinate. The base was mixed with excess ethanolamine (2 ml.) and heated to 180° in a distillation flask fitted with a short fractionating column. Ethanol, b.p. 78° was evolved for about 20 min., after which no distillate boiling below 160° could be obtained and heating was stopped. Excess ethanolamine was removed by evaporation *in vacuo* to yield 2.1 g. of viscous oil. The oil was dissolved in a little ethanol and mixed with a slight excess of an ethanolic solution of oxalic acid, then evaporated to a syrup *in vacuo*. On extraction with boiling acetone and cooling, 2-(1'-methylhexahydronicotinamido)-ethanol acid oxalate (1.8 g., 46%), m.p. 124° (from acetone) was obtained. (Found: C,48.3; H,7.4; N,10.0. $C_{11}H_{20}O_6N_2$ requires C,47.8; H,7.25; N,10.15).

Treatment of the product of this reaction with a large excess of ethanolic hydrogen chloride gave crystals of 2-aminoethyl 1'-methylhexahydronicotinate dihydrochloride, m.p. 216° (from ethanol). Phillips and Baltzly[67] give m.p. 214°

(ii) Preparation of 2-aminoethyl 1'-methylhexahydronicotinate dihydrochloride from 1-methylhexahydronicotinyl chloride and ethanolamine hydrochloride. -

(a) A suspension of 1-methylhexahydronicotinic acid hydrochloride, prepared by the method of Preobrazhenskiĭ and Fishe[102] (4.49 g., 0.025 mole) in thionyl chloride (25 ml.) was heated under reflux for 15-20 min. until evolution of hydrogen chloride ceased and the mixture became homogeneous. Excess thionyl chloride was removed by evaporation in vacuo and the crystalline residue washed with dry light petroleum until the washings were colourless. Light petroleum was removed by decantation followed by drying in vacuo and the product suspended in dry chloroform (60 ml.). Finely powdered ethanolamine hydrochloride (2.85 g., 0.03 mole) was added, and the mixture heated under reflux until hydrogen chloride ceased to be evolved (5-6 hr.). The chloroform was evaporated in vacuo and the residue dissolved in a minimum volume of boiling ethanol. On cooling, 2-aminoethyl 1'-methylhexahydronicotinate dihydrochloride (315 mg., 5%), m.p. 216°

(from ethanol) was obtained.

Evaporation of the alcoholic mother liquors gave 4.1 g. of an uncrystallisable residue which was almost completely soluble in chloroform. The small portion of chloroform-insoluble material was identical with the aminoester dihydrochloride m.p. 216° obtained initially. The chloroform-soluble portion could still not be crystallised, but yielded on treatment with sodium picrate, O,N-bis-(1-methylhexahydronicotinyl)-2-aminoethanol dipicrate, m.p. 227° (dec.)(from aqueous ethanol) (Found: C,43.4; H,4.4; N,16.5. $C_{28}H_{36}O_{17}N_8$ requires C,43.7; H,4.6; N,16.4).

(b) The previous preparation was repeated, replacing the dry chloroform by dry tetrahydrofuran. The yield of aminoester dihydrochloride was as before.

(c) The acid chloride hydrochloride was prepared as before from 1-methylhexahydronicotinic acid hydrochloride (0.18 g., 0.001 mole). The washed and dried product was then mixed intimately with dry and finely powdered ethanolamine hydrochloride (0.12 g., 0.0012 mole) and heated slowly on an oil-bath. The mixture melted at a bath temperature of about 120° and evolved hydrogen chloride, whereupon heating was continued at 120°-140° until no more gas was evolved (about 20 min.). It was then allowed to cool and dissolved in a minimum quantity of boiling

ethanol, which on cooling yielded 177 mg. (60%) of crystals m.p. 216° (from ethanol) identical with the 2-aminoethyl 1'-methylhexahydronicotinate dihydrochloride obtained previously.

(iii) Attempted preparation of (±)-2-(1'-methylhexahydronicotinamido)-propanol.

Ethyl 1-methylhexahydronicotinate (1.71 g., 0.01 mole) was heated with (±)-2-aminopropanol (2 ml.) as described in reaction (i) of this series. Reaction occurred with evolution of ethanol which was complete after 30 min. The product, after removal *in vacuo* of excess aminopropanol, was semi-solid, amorphous, hygroscopic and did not form a crystallisable acid oxalate. Short path distillation of the product (bath temperature 120°, pressure 0.05 mm.) gave a distillate similar in appearance, of equivalent weight (potentiometric) 218. (The required product $C_{10}H_{20}O_2N_2$ has equivalent weight 200).

A portion of the distillate was dissolved in ethanol and saturated with hydrogen chloride. After standing overnight at room temperature, the ethanol was removed by evaporation *in vacuo* to leave a very hygroscopic amorphous residue which could not be crystallised.

(iv) Attempted preparation of (+)-2'-aminopropyl 1-methylhexahydronicotinate hydrochloride.

(a) The acid chloride hydrochloride, prepared from 1-methyl-

hexahydronicotinic acid hydrochloride (0.18 g., 0.001 mole), was treated with (±)-2-aminopropanol hydrochloride (0.14 g., 0.0012 mole) as described in reaction ii(c) of this series. Reaction occurred with evolution of hydrogen chloride, but the product was very hygroscopic and could not be crystallised.

(b) (±)-2'-Aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride (0.27 g., 0.001 mole), prepared as described in reaction (xii) of this series, was dissolved in water (5 ml.) and hydrogenated at atmospheric pressure in the presence of a platinum oxide catalyst. Reduction was complete in 4 hr. The product, on evaporation to dryness *in vacuo* was again amorphous, hygroscopic and could not be crystallised.

The non-crystalline residue was treated with aqueous alkali, extracted with chloroform, the chloroform extract evaporated to dryness *in vacuo* and the residue treated with a slight excess of ethanolic solution of oxalic acid. The resultant oxalate could not be crystallised.

(v) Preparation of 2-nicotinamido-ethanol.

Ethyl nicotinate (3.02 g., 0.02 mole)and ethanolamine (5 ml.) were treated as described for the corresponding reaction with 1-methylhexahydronicotinic acid (i) . The product left after evaporation *in vacuo* of the excess ethanolamine crystallised spontaneously and yielded, after recrystallisation from acetone - ether, 2-nicotinamido-ethanol (2.9 g., 87%), m.p. 90°. Phillips and Baltzly[57] give 89-90°. 2-Nicotinamido-ethanol acid oxalate, m.p. 116° (from acetone) was prepared as before. (Found: C,47.0; H,4.0; N,10.9. $C_{10}H_{12}O_6N_2$ requires C,46.9; H,4.7; N,10.9%).

(vi) Preparation of (+)-2-nicotinamido-propanol.

Treated as in the previous reaction, ethyl nicotinate (3.02 g., 0.02 mole) and (+)-2-aminopropanol (5 ml.) yielded 2.8 g. (78%) of a base which did not crystallise, but which yielded, on treatment with excess of oxalic acid, (+)-2-nicotinamido-propanol acid oxalate, m.p. 128° (from acetone). (Found: C,49.3; H,5.5; N,10.2. $C_{11}H_{14}N_2O_6$ requires C,48.9; H,5.2; N,10.4%).

(vii) Reaction of methyl 1-methyl-1,2,5,6-tetrahydronicotinate and ethanolamine.

Methyl 1-methyl-1,2,5,6-tetrahydronicotinate hydrobromide (Arecoline Hydrobromide B.P.C., 1.18 g., 0.005 mole) was dissolved in water (0.8 ml.) and treated with a solution of potassium hydroxide (0.4 g.) in water (0.5 ml.). The mixture was extracted

with three successive portions of chloroform (20 ml.), which were combined, dried over anhydrous sodium carbonate and evaporated to a syrup *in vacuo*. An equal volume of ethanolamine was added and the mixture heated as described for the previous two reactions to yield 0.774 g. of a viscous oil on evaporation of excess ethanolamine. Treatment with an excess of ethanolic solution of oxalic acid gave a gummy residue which crystallised from aqueous methanol, m.p. 205-206° (dec.) (Found: C,49.0; H,7.6; N,11.0, 11.3%). This is not in agreement with the required values for 2'-(1-methyl-1,2,5,6-tetrahydronicotinamido)-ethanol acid oxalate ($C_{11}H_{18}O_6N_2$ requires C,48.2; H,6.6; N,10.2%).

The product may have been N,N-*bis*-4-[1-methyl-3-(2'-hydroxyethyl)-carbonamidopiperidyl]-ethanolamine dioxalate ($C_{24}H_{43}N_5O_{13}$ requires C,47.3; H,7.1; N,11.5%) since a sample of hydrochloride (prepared by treatment of an aqueous solution of the oxalate with an equivalent amount of calcium chloride solution and filtering) showed no absorption maximum between 200 and 250 mμ, compared with arecoline hydrobromide which had $E^{1\%}_{1\ cm.} = 563$ at 205 mμ (Fig. 1).

(viii) *Attempted preparation of 1-methyl-1,2,5,6-tetrahydronicotinic acid azide.*

1-Methyl-1,2,5,6-tetrahydronicotinic acid hydrazide

(310 mg., 0.002 mole) prepared by the method of Sapara,[76] was dissolved in 0.1 N hydrochloric acid (20 ml.) and cooled to 0°. Normal solution of sodium nitrite (2 ml.) was added in one portion with vigorous stirring, which was continued during the further gradual addition of 0.1 N hydrochloric acid until the solution was distinctly acid to congo-red paper. There was no evidence of any reaction.

The mixture was stirred for 5 min. at 0°, then neutralised by the addition of N solution of sodium bicarbonate and extracted with ether. The ethereal extract was dried over sodium sulphate in the dark at 0° for 15 min., then a portion was evaporated in vacuo. A negligible amount of residue was obtained.

A chloroform extract of the aqueous reaction mixture similarly gave only a trace of residue on evaporation in vacuo.

The aqueous phase was finally evaporated to dryness in vacuo and the residue extracted with boiling benzene, which yielded on cooling a small amount of crystalline material, m.p. 156° (from benzene) undepressed on mixture with the starting hydrazide.

(ix) Reaction of arecoline hydrobromide and ethanolamine hydrobromide.

Heating equal weights of arecoline hydrobromide and ethanolamine hydrobromide on an oil bath up to 220° had no visible effect. Above 220° decomposition occurred to yield a product which could not be crystallised.

(x) Preparation of 2'-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride.

An acid chloride hydrochloride was prepared from 1-methyl-1,2,5,6-tetrahydronicotinic acid hydrochloride (0.89 g., 0.005 mole) as described for the corresponding hexahydronicotinic acid derivativeii(a) and mixed intimately with finely powdered ethanolamine hydrochloride (1 g.) by trituration under light petroleum. The solvent was removed by decantation, the solid residue dried *in vacuo* and heated on an oil bath. Frothing and evolution of hydrogen chloride occurred at about 140°, whereupon the temperature was raised slowly to 180° and maintained at that temperature until no further reaction was visible (about 20 min.). After cooling, the product was dissolved in a minimum volume of boiling ethanol which yielded on cooling 0.84 g., (65%) of brownish platelets. Recrystallisation from ethanol (charcoal) gave 2'-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride, m.p. 258° (dec.) (Found: C,41.8; H,6.9; N,10.7; Cl,27.1. $C_9H_{18}O_2N_2Cl_2$ requires C,42.0; H,7.0; N,10.9; Cl,27.6%).

(xi) Preparation of 2'-(1-methyl-1,2,5,6-tetrahydronicotinamido)-ethanol

A saturated aqueous solution of 2'-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride (1.28 g., 0.005 mole) was treated with a slight excess of 40% aqueous potassium hydroxide and extracted with three portions of chloroform (30 ml.). The combined chloroform extracts were dried over anhydrous sodium

sulphate and evaporated *in vacuo* to a very viscous syrup. Treatment with an ethanolic solution of a very slight excess of oxalic acid gave a product (1.02 g.) m.p. 92° (from aqueous ethanol). Recrystallisation from methanol and acetone gave a product m.p. 158° (dec.), but in poor yield. This material on further recrystallisation from aqueous acetone had m.p. 92°, undepressed on mixture with the original product. (Found: for oxalate, m.p. 92°; C,40.6; H,7.7; N,8.8. $C_{11}H_{18}O_6N_2 3H_2O$ requires C,40.2; H,7.3; N,8.5%).

Titration of a solution of the hydrochloride prepared from this oxalate as described in the final section of this work gave a neutralisation curve characteristic of a diacid base. The product must therefore have been the oxalate of 2'-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate.

A further portion of 2'-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate hydrochloride (25.7 mg., 0.1 millimole) was dissolved in electrolyte-free water (10 ml.) and an aliquot portion (2 ml., 0.02 millimole) titrated with carbonate-free potassium hydroxide solution as described in the following section. The volume required for complete neutralisation was 1.10 ml. and the titration curve (Fig.2A) characteristic of a diacid base. The remainder of the solution was adjusted to a pH value of 13 by the addition of solution of potassium

hydroxide and allowed to stand overnight. It was then brought to pH3 by the addition of hydrochloric acid and made up to 10 ml. with electrolyte-free water. A further aliquot portion (2.5 ml., 0.02 millimole) was again titrated under identical conditions. The volume required for complete neutralisation was now 0.512 ml. and the neutralisation curve was typical of that for a monoacid base as shown by comparison of the pH values at ½, 2/3 and 1/3 neutrality (Fig.2B). This indicated that O⟶N acyl-migration had occurred and that the solution contained the required 2'-(1-methyl-1,2,5,6-tetrahydronicotinamido)-ethanol.

Further attempts to prepare a crystalline oxalate of this base were unsuccessful, as were attempts to prepare crystalline succinates tartrates and phthalates.

(xii) Preparation of (±)-2'-aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride.

Using the method described for the corresponding aminoethyl ester (No. x in this section) 1-methyl-1,2,5,6-tetrahydronicotinic acid hydrochloride (890 mg., 0.005 mole) and (±)-2-aminopropanol hydrochloride (1 g.) gave 825 mg. (61%) of a crystalline product which was recrystallised from ethanol (charcoal) to yield (±)-2'-aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride, m.p. 215° (dec.). (Found: C,43.9; H,7.4; N,10.0; Cl,25.4. $C_9H_{18}O_2N_2Cl_2$ requires C,44.3;

H,7.4; N,10.3; Cl,26.2%).

(xiii) Preparation of (+)-2'-(1-methyl-1,2,5,6-tetrahydronicotinamido)-propanol.

(+)-2-Aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride was treated with alkali as described under preparation No. xi in this section. The product was a hygroscopic, amorphous semi-solid which failed to form a crystallisable oxalate, succinate, picrate, tartrate or phthalate.

A solution of the dihydrochloride (27 mg., 0.1 millimole) in water (10 ml.) was then treated as described in preparation No. xi of this section. A portion equivalent to 0.02 millimole had an initial titre of 0.009 ml. of potassium hydroxide solution and a titration curve typical of that for a diacid base. After treatment with alkali and neutralisation the titre was 0.404 ml. and the curve characteristic of a mono-acid base, indicating that the solution contained (±)-2'-(1-methyl-1,2,5,6-tetrahydronicotinamido)-propanol.

Determination of Ionic Dissociation Constants

(i) Preparation of materials under test.

(a) Acids.

3-Dimethylaminopropionic Acid Hydrochloride. - Ethyl 3-dimethylaminopropionate prepared by the method of Adamson,[100] was heated under reflux for 3 hr. with concentrated hydrochloric acid. The solution, when evaporated to dryness and recrystallised from ethanol gave the required product, m.p. 191°. Gresham et al.[103] give m.p. 191-192°.

1-Methyl-1,2,5,6-tetrahydronicotinic Acid Hydrochloride. - Arecoline, prepared from commercial arecoline hydrobromide, was heated under reflux with concentrated hydrochloric acid for 3 hr. The solution, evaporated to dryness and recrystallised from ethanol-ether gave arecaidine hydrochloride, m.p. 263° (decomp.) Wohl and Johnson[104] give 262-263°.

1-Methylhexahydronicotinic Acid. - 1-Methyl-1,2,5,6-tetrahydronicotinic acid hydrochloride (0.3 g.) in ethanol (10 ml.) was hydrogenated at atmospheric pressure in the presence of a platinum catalyst. The solution, after filtration and concentration, yielded the desired product, m.p. 173-175° (from ethanol-ether). Winterstein and Weinhagen[105] give m.p. 175°.

Ecgonine Hydrochloride. - Ecgonine ($[\alpha]_D^{18}$ -44.15° (c,4.3 in water); Henry[106] (quotes $[\alpha]_D$ - 45.4°) was dissolved in a slight excess of concentrated hydrochloric acid and the solution allowed spontaneously to evaporate to dryness over potassium hydroxide in vacuo. The resulting ecgonine hydrochloride had m.p. 252° (dec.) Liebermann[107] gives m.p. 246°.

ψEcgonine Hydrochloride. - Prepared by the method of Einhorn and Marquart,[108] m.p. 236°. Einhorn and Marquart give 236°.[109]

Lysergic Acid. - Prepared from ergotoxine by the method of Stoll and Hoffmann, m.p. 239° (dec.) from water. Stoll and Hoffmann[110] give 240-250° (dec.).

Isolysergic Acid. - Prepared from lysergic acid by the method of Smith and Timmis,[47] m.p. 238° (dec.), from water. Stoll and Hoffmann[110] give 240-245°.

(b) Esters.

Ethyl 3-Dimethylaminopropionate Hydrochloride. - Ethyl 3-dimethylaminopropionate prepared by the method of Adamson,[100] was dissolved in dry ether and treated with excess of dry hydrogen chloride. The product, when recrystallised from ethanol-ether gave ethyl 3-dimethylaminopropionate hydrochloride, m.p.134°.

Methyl 1-Methyl-1,2,5,6-tetrahydronicotinate Hydrobromide. - A commercial sample of arecoline hydrobromide was recrystallised from ethanol to a constant m.p. of 172°. The British Pharmaceutical Codex 1949 gives m.p. 168-175°.

Methyl 1-Methylhexahydronicotinate Hydrobromide. - Arecoline hydrobromide (1 g.) in methanol (20 ml.) was hydrogenated at atmospheric pressure in the presence of a platinum catalyst. Hydrogenation was complete in 4 hr. The solution, after filtration, was evaporated and the residue recrystallised from a mixture of methanol and ethyl acetate (1:1) to give methyl 1-methylhexahydronicotinate hydrobromide, m.p. 114° (after drying *in vacuo* over phosphorus pentoxide). Preobrazhenskiĭ and Fisher[102] give m.p. 115-116°. The product was highly deliquescent

Ecgonine Methyl Ester Hydrochloride. - Prepared by the method of Einhorn and Klein,[111] m.p. 216° (dec.). Einhorn and Klein give m.p. 212° (dec.).

ψ -Ecgonine Methyl Ester Hydrochloride. - Crude ψ-ecgonine hydrochloride was dissolved in saturated methanolic hydrogen chloride and the solution refluxed for 1 hr. The solution was evaporated to dryness, the residue converted to base with aqueous sodium carbonate (10%) and the base extracted with ether. The base in the dry ether was treated with dry hydrogen chloride and the precipitated hydrochloride recrystallised from ethanol to give ψ-ecgonine methyl ester hydrochloride,

m.p. 197°. Mixed melting point with the hydrochloride from authentic ψ-ecgonine methyl ester, 197°.

(c) Amides.

Hydrochlorides of (+)-2-(3'-Dimethylaminopropionamido)propanol, 2-(3'-Dimethylaminopropionamido)-ethanol and 2'-(1-Methylhexahydronicotinamido)ethanol.

These were obtained, as described in the preceding sections, as acid oxalates and were converted in aqueous solution to their hydrochlorides as follows:- 0.1 millimole of the acid oxalate was dissolved in electrolyte-free water (0.5 ml.) and treated with a very slight excess of solution of calcium chloride. Precipitated calcium oxalate was removed by centrifugation, washed, and the supernatant liquid plus washings made up to 10 ml. with electrolyte-free water. Aliquot portions of 2 ml. (equivalent to 0.02 millimole) were used for each determination.

Hydrochlorides of 2'-(1-Methyl-1,2,5,6-tetrahydronicotinamido)-ethanol and (+)-2'-(1-Methyl-1,2,5,6-tetrahydronicotinamido)-propanol. - Prepared in solution from the corresponding amino-ester dihydroochlorides as described in Parts xi and xiii of this section

Ergometrine, Ergometrinine and 2-Nicotinamido-ethanol Hydrochlorides. - The bases (0.02 millimole) were dissolved

in a known slight excess of 0.5 N. hydrochloric acid immediately before titration.

(ii) Reagents and solvents

Carbonate-free potassium hydroxide solution was prepared by the method of Steinbach and Freiser,[112] in which 0.05 N. solution of potassium chloride in electrolyte-free water is passed through a column of strongly basic ion-exchange resin (I.R.A. 400). Alkali was prepared in small quantities as required and stored in a specially designed vessel to prevent access of atmospheric carbon dioxide (see Fig.4).

Nitrogen was freed from carbon dioxide by passage through soda-lime then bubbled through water at 25° to warm, humidify and remove any entrained particles of soda-lime.

Electrolyte-free water was prepared by passing tap water first through a 'Deminrolit' two-bed ion exchanger followed by a mixed bed of strongly acidic and basic ion-exchange resins immediately before use.

Ethyl cellosolve was prepared from a commercial sample which was fractionally distilled from solid potassium hydroxide, the fraction of boiling-range 132-134° being taken.

(iii) Apparatus (Fig.4)

Titrations were carried out in a cylindrical glass cell of about 8 ml. capacity which had been silicone-treated to facilitate draining and washing. The cell rested in a copper

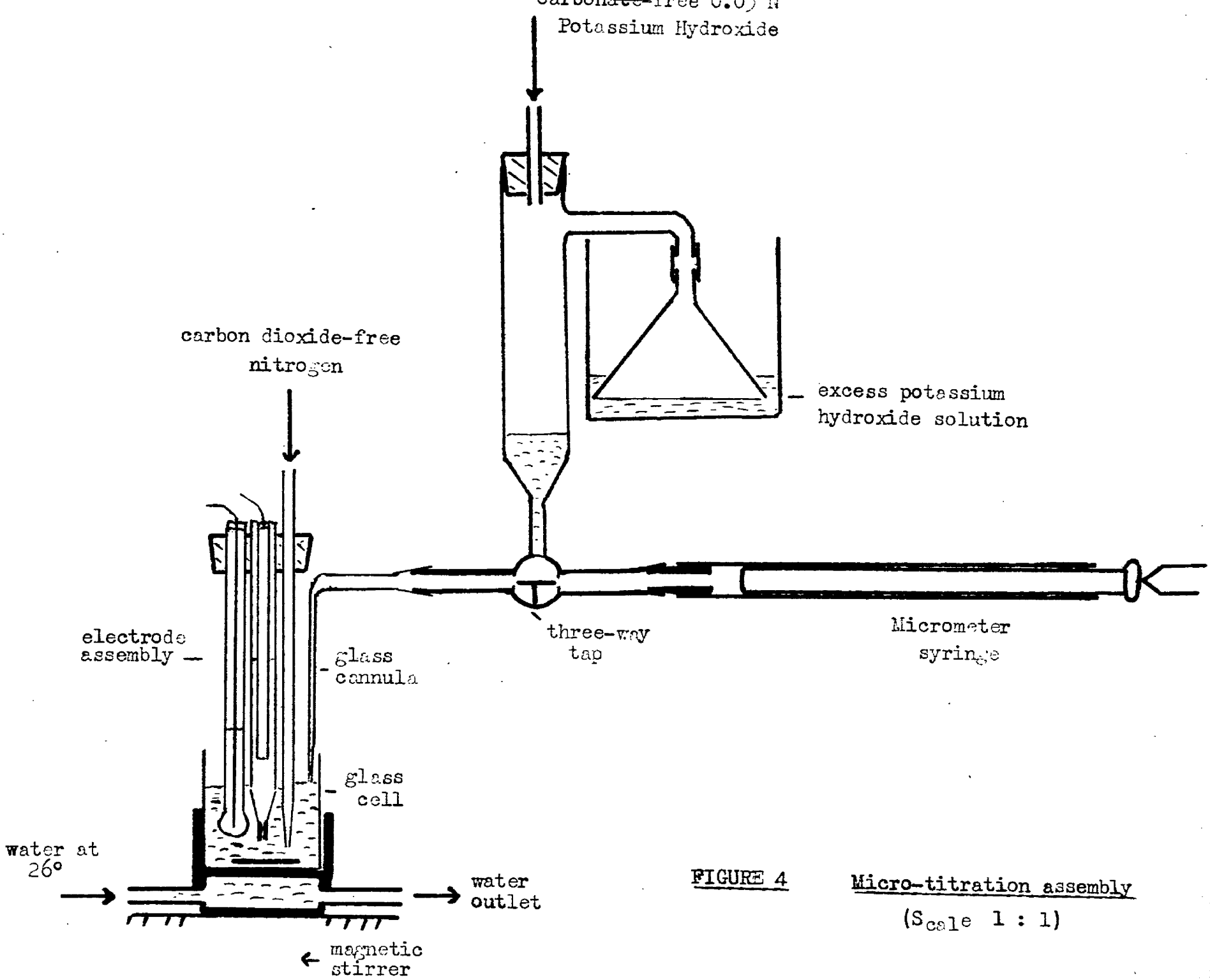


FIGURE 4 Micro-titration assembly

(S_{cale} 1 : 1)

cup which could be heated by means of circulating water from a thermostatically-controlled reservoir to maintain the contents of the cup at 25° ± 0.1°. A magnetic rotor below the cell and cup activated a stainless-steel stirrer. A separate glass cell fitted with a drain tube was found to be convenient for washing and standardising the electrodes.

A 'Doran' micro-electrode assembly, fitted with a glass capillary through which carbon dioxide-free nitrogen could be bubbled, was arranged so that it could easily be lowered into the cell. The pH meter used was a direct-reading type (Electroni Industries Ltd.) with a Δ pH scale allowing accurate observation to 0.01 units.

Alkali from the reservoir was passed through a glass tube to a two-way adaptor attached to an 'Agla' micrometer syringe and to a glass cannula, thereby allowing the syringe to be filled and emptied without exposure of alkali to the atmosphere. The tip of the cannula was just in contact with the surface of the liquid during titration.

(iv) Method

The material under test (0.02 millimole) in the form of its hydrochloride was dissolved in such a volume of electrolyte-free water that its calculated volume on titration to half-neutrality would be 4.0 ml. This solution was transferred

to the thermostatically-controlled cell and titrated potentiometrically with carbonate-free potassium hydroxide solution from the micrometer syringe under a stream of carbon dioxide-free nitrogen and stirring by means of the magnetic stirrer.

An experimentally determined correction for solvent effect was applied, based on the methods of Tague[81] and Harris[82] in the following way. A blank titration was carried out under the same conditions as used in the test, whereby the material under test was replaced by an equivalent quantity of carbon dioxide-free hydrochloric acid. The volumes of 0.05 N potassium hydroxide solution required to raise the pH value in the blank determination from 7.0 to various pre-selected values were determined accurately from a mean of eight titrations. From this it was possible to determine solvent corrections for any desired pH value by interpolation. These corrections were subtracted from the observed titres in the test determinations.

The procedure for converting oxalates to hydrochlorides was shown to be satisfactory by carrying out a blank titration in which the oxalate under test was replaced by an equivalent amount of sodium oxalate. The titration curve did not differ from that for an equivalent amount of sodium chloride.

For lysergic and isolysergic acids the method was as follows:- the acid (4 mg., 0.013 millimole) was suspended in a

mixture of water and ethyl cellosolve calculated to give a volume of 4 ml. and a known cellosolve concentration on titration to half-neutrality. The acid was dissolved by the addition of an accurately measured volume of 0.05 N carbonate-free potassium hydroxide (0.4 ml.) and immediately titrated with 0.05N hydrochloric acid. Titration was discontinued if the acid was precipitated, the end-point then being calculated from the known equivalence of the acid and alkali used. A solvent-correction was calculated as before from a blank titration in which the lysergic and isolysergic acids were omitted. A separate correction was calculated for each concentration of cellosolve.

(iv) Results.

Dissociation constants were calculated from the pH values at $\frac{1}{2}$, $2/3$ and $1/3$ neutrality, a mean being taken of at least two titrations. The second acid dissociation constant of amino acids was taken. The results are tabulated in Tables 2, 3 and 4.

Details of a typical titration are as follows:

Determination of pK'a value for methyl 1-methylhexahydronicotinat

Weight of sample (as hydrobromide) 5.5 mg.

Volume of electrolyte-free water 3.8 ml.

pH value	Volume of 0.05N potassium hydroxide (1 unit = 0.0002 ml.)	Volume corrected (v)	$\frac{d\ pH}{d\ v}$
4.80	2500		
7.60	2200		
7.99	1900		
8.10	1800	1830	
8.19	1700	1732	
8.28	1600	1635	
8.37	1500	1537	
8.43	1400	1440	
8.51	1300	1345	
8.60	1200	1250	
8.70	1100	1160	
8.98	800		
9.10	700	790	
9.17	650	750	$\frac{0.07}{400} = 0.0017$
9.22	600	710	$\frac{0.05}{400} = 0.0013$
9.38	500	620	$\frac{0.16}{900} = 0.0018$
9.52	400	540	$\frac{0.14}{800} = 0.0018$
9.62	350	500	$\frac{0.10}{400} = 0.0025$
9.72	300	470	$\frac{0.10}{300} = 0.0033$
9.82	250	440	$\frac{0.10}{300} = 0.0033$

Volume of alkali at end-point = 2500 - 470 = 2030 units.

Volume of alkali at half-neutrality = 1015 units.

Reading at half-neutrality = 1015 + 470 = 1485 (Or 2500 - 1015 = 1485)

Reading at 1/3 neutrality = $\frac{2030 \times 2}{3}$ + 470 = 1824

Reading at 2/3 neutrality = $\frac{2030}{3}$ + 470 = 1147

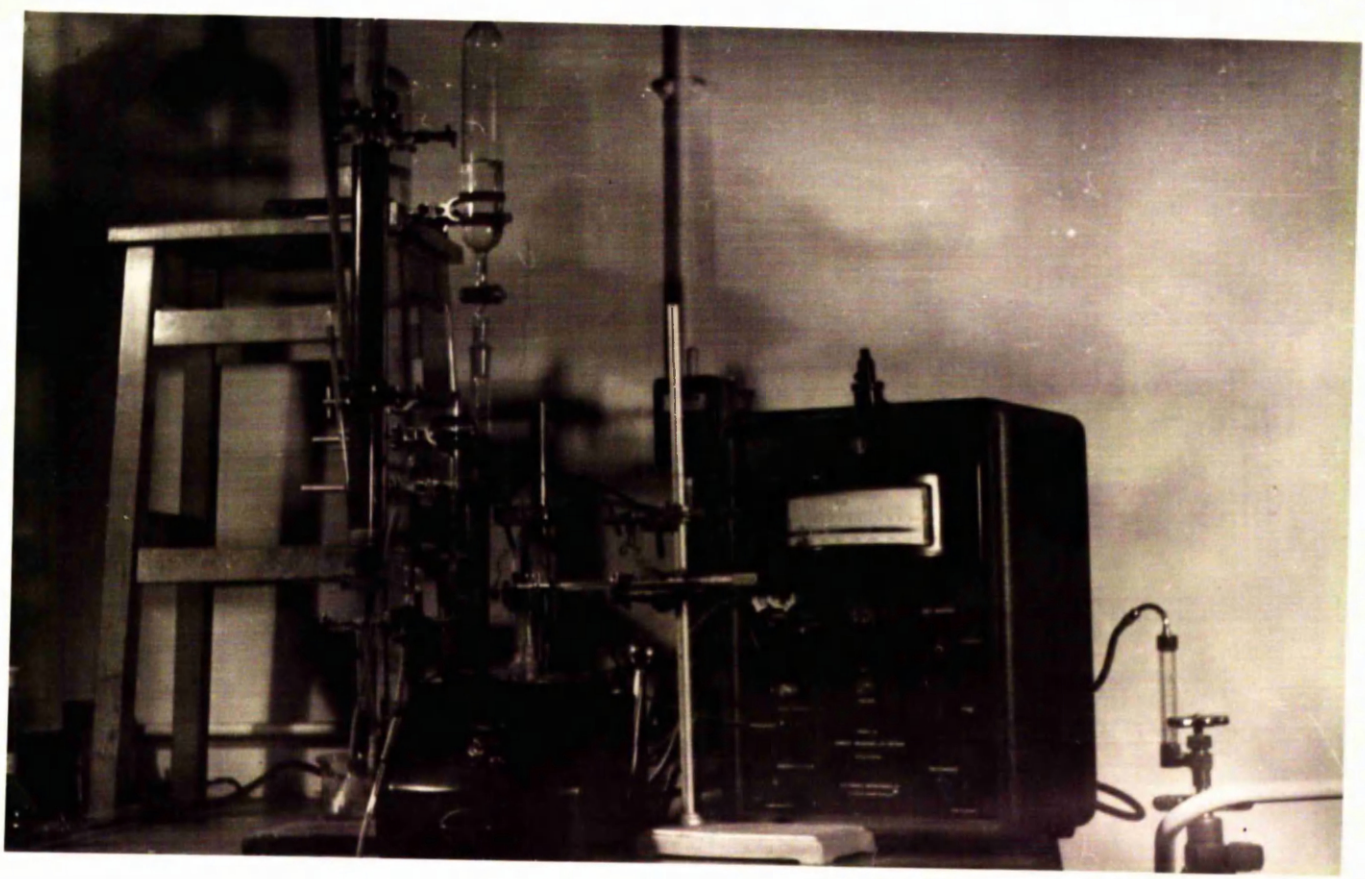
Values of pH at these corrected titres were obtained by interpolation in a graph (Fig.3) of pH value : corrected titre and were as follows:-

At half neutrality 8.41 (= pK'a)

At 1/3 neutrality 8.11 (pK'a value = 8.11 + Log. 2 = 8.41)

At 2/3 neutrality 8.72 (pK'a value = 8.72 - Log. 2 = 8.42)

∴ Mean value for pK'a = 8.41.



Micro-titration apparatus. As Fig. 4.

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