DISSOCIATION CONSTANTS OF SOME COMPOUNDS RELATED TO LYSERGIC ACID

(β-Dimethylaminopropionic Acid, Dihydroarecaidine, Ecgonine and their Derivatives

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IT has been shown[1] that the dissociation constants of the dihydrolysergic acids are in agreement with the view that those dihydrolysergic acids in which the $C_{(8)}$ carboxyl is remote (equatorial) from $N_{(6)}$ are weaker bases than those in which the $C_{(8)}$ carboxyl is axial. Because of the small amount of material available it was necessary to measure these dissociation constants by the method of Craig, Shedlovsky, Gould and Jacobs,[2] in which the *p*H value of a solution half neutralised by the addition of the calculated amount of carbonate-free sodium hydroxide was recorded. Since this method is open to certain objections, the experimental work now described was undertaken to confirm the above conclusions by potentiometric determination of dissociation constants of analagous, but more readily available materials of known structure. Whilst this work was in progress, Stoll, Petrzilka, Rutschmann, Hofmann and Günthard[3] have published dissociation constants for the dihydrolysergic and dihydronorlysergic acids, and other evidence which further substantiates our conclusions.

EXPERIMENTAL

Titrations were carried out in a specially constructed micro-cell thermostatically controlled at 25° C., using a Doran alk-acid glass electrode in conjunction with a Cambridge Direct Reading *p*H meter. An Agla micrometer syringe was used as a burette. Carbon dioxide-free nitrogen was bubbled through the solution, which was stirred by means of a magnetic stirrer.

Solutions of 0·02 millimoles of the hydrochlorides or hydrobromides of the pure substances in carbon dioxide-free double-distilled water were titrated potentiometrically with 0·05N carbonate-free potassium hydroxide, the volume of solvent being calculated to yield a 0·005M solution at half-neutrality. The potassium hydroxide solution was obtained by the method of Steinbach and Freiser,[4] in which 0·05M potassium chloride solution is passed through a column of ion exchange resin IRA 400. The eluant standard alkali was fed directly to the micrometer syringe.

An experimentally determined correction for solvent effect was applied, based on the methods of Tague[5] and Harris[6] in the following way. A blank titration was carried out under the same conditions used in the test, whereby the material under test was replaced by an equivalent quantity of carbon dioxide-free hydrochloric acid. The volumes of 0·05N potassium hydroxide solution required to raise the *p*H 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 *p*H value by interpolation. These corrections were subtracted from the observed titres in the test determinations. The blank determination was repeated for each fresh batch of distilled water.

Dissociation constants were calculated from three points on each of two or more titration curves. Values for all substances quoted were reproducible within $\pm 0{\cdot}05$ units (ecgonine $\pm 0{\cdot}08$ unit).

Preparation of Materials

Ethyl β-dimethylaminopropionate Hydrochloride.—Ethyl β-dimethylaminopropionate, prepared by the method of Adamson,[7] was dissolved in dry ether and treated with excess of dry hydrogen chloride. The product when recrystallised from ethanol-ether gave ethyl β-dimethylaminopropionate hydrochloride, m.pt. 134° C.

β-Dimethylaminopropionic Acid Hydrochloride.—Ethyl β-dimethylaminopropionate (1 g.) was refluxed for 3 hours with concentrated hydrochloric acid. The solution when evaporated to dryness and recrystallised from ethanol gave the required product, m.pt. 191° C. Gresham *et al.*[8] give m.pt. 191–192° C.

Arecoline hydrobromide.—Commercial sample recrystallised to m.pt. 172° C. The British Pharmaceutical Codex 1949 gives m.pt. 168–175° C.

Arecaidine Hydrochloride.—Arecoline was refluxed with concentrated hydrochloric acid for 3 hours. The solution, evaporated to dryness and recrystallised from ethanol-ether, gave arecaidine hydrochloride, m.pt. 263° C. (decomp.). Wohl and Johnson[9] give 262–263° C.

Dihydroarecoline 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 hours. The solution, after filtration, was evaporated and the residue crystallised from a mixture of methanol and ethyl acetate (1 : 1) to give dihydroarecoline hydrobromide, m.pt. 114° C. (after drying *in vacuo* over phosphorus pentoxide). Preobrazhenskiĭ and Fisher[10] give m.pt. 115–116° C. The product was highly deliquescent.

Dihydroarecaidine Hydrochloride.—Arecaidine 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 dihydroarecaidine hydrochloride, m.pt. 173–175° C. from ethanol-ether. Winterstein and Weinhagen[11] give m.pt. 175° C.

Ecgonine Hydrochloride.—Ecgonine {$[\alpha]_D^{18}$–44·15° ($c = 4{\cdot}3$ in water); Henry[12] quotes $[\alpha]_D$–45·4°} was dissolved in concentrated hydrochloric acid and the solution allowed to evaporate spontaneously to dryness *in vacuo* over potassium hydroxide. The resulting ecgonine hydrochloride had m.pt. 252° C. (decomp.). Liebermann[13] gives m.pt. 246° C.

Ecgonine Methyl Ester Hydrochloride.—Prepared by the method of Einhorn and Klein[14] m.pt. 216° C. (decomp.). Einhorn and Klein give m.pt. 212° C. (decomp.).

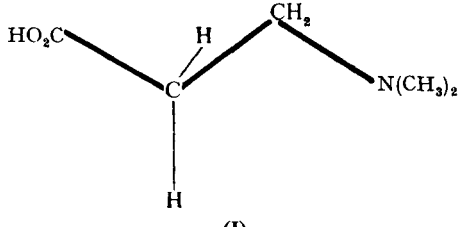
ψ-Ecgonine Hydrochloride.—Prepared by the method of Einhorn and Marquart,[15] m.pt. 236° C. Einhorn and Marquart[16] give 236° C.

ψ-Ecgonine Methyl Ester Hydrochloride.—Crude ψ-ecgonine hydrochloride was dissolved in saturated methanolic hydrochloric acid and the solution refluxed for one hour. The solution was evaporated to dryness, the residue converted to base with aqueous sodium carbonate (10 per cent.) 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 hydrocyloride, m.pt. 197° C. Mixed m.pt. with the hydrochloride from authentic ψ-ecgonine methyl ester, 197° C.

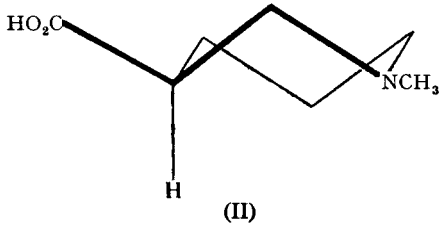
Discussion

Dihydroarecaidine

Neuberger,[17] using ΔpK(pK′a_2 amino-acid–pK′a amino-acid ester) as a function of the distance between the centre of the negative charge of the carboxyl and the dissociating hydrogen of the $-\overset{\diagdown}{\underset{\diagup}{N}}H^+$ group, established that aliphatic amino-acids exist in solution in the zig-zag open chain form (I) in which the two charged groups are remote, a conclusion which was supported by measurements of dipole distances.[18,19] By analogy it would appear therefore that the preferred conformation of dihydroarecaidine in solution should be that in which the two charged groups are remote as in (II) (COOH equatorial) rather than as in III (COOH axial). That such a conformation represents the preferred structure for dihydroarecaidine may also be deduced theoretically by analogy with similar *cyclo*hexane 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 derivatives are not resolvable into optical enantiomorphs). On the other hand, such 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.[20]



(I)



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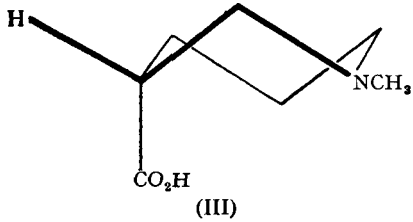


(III)

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.[2] Examination of molecular models reveals that there is identical chain and spacial separation of $-CO_2^-$ and $>NMeH^+$ groups in β-dimethylaminopropionic acid (I) and dihydroarecaidine provided the latter adopts the conformation (II). Consequently we should expect these two substances to have dissociation constants of the same order.

We have measured the second acid dissociation constants of these two amino-acids by potentiometric titration of their hydrochlorides, and the results are recorded in Table I. An experimentally determined blank was

TABLE I

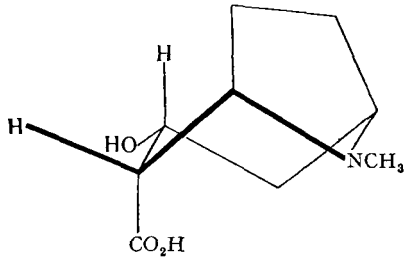
Amino-acid	Acid pK′a at 25° C.	Ester pK′a at 25° C.	Δ pKa
β-Dimethylaminopropionic acid	9·85	8·60 (Et)	1·25
Dihydroarecaidine	9·70	8·45 (Me)	1·25
Arecaidine	9·07	7·70 (Me)	1·37
Ecgonine	10·91 11·15*	9·22 (Me)	1·69
ψ-Ecgonine	9·70	8·21 (Me)	1·49
Benzoylecgonine	11·80*	8·65 (Me)* 8·80 (Me)	

* Kolthoff.[37]

used to correct for solvent effect and applied as described by Tague[5] and Harris.[6] Second acid dissociation constants only have been measured, since these are more clearly indicative of the structural features concerned. Our measurements show that the pK′ of β-dimethylaminopropionic acid (9·85) is distinctly lower than that of the corresponding primary amino-acid, β-alanine (10·36).[21] This is in agreement with the observations of Bredig[22] and Hall and Sprinkle[23] who showed that whereas the introduction of a single methyl group into a primary amine gives a small increase of 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.[3] The concordance of pK′ values for β-dimethylaminopropionic acid (9·85) and dihydroarecaidine (9·70) clearly demonstrates that the steric relationship of basic and acidic groups is the same (at least in solution) in both amino-acids. This not only supports the conformational assignment (II) for dihydroarecaidine, but also the validity of the general principles of conformational analysis to compounds of this type.

Ecgonine and ψ-Ecgonine

Findlay[24,25,26] assigned the structures (IV) and (V) to ecgonine and ψ-ecgonine respectively, and these conformational assignments have since been confirmed by Fodor, Kovács and Weisz.[27,28] We now report second acid dissociation constants for these two substances (Table I). 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



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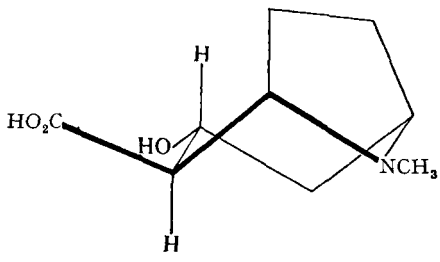
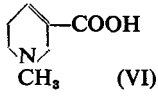


(V)

for ecgonine (IV; pK′ 10·91) than in ψ-ecgonine (V; pK′ 9·70). These results therefore provide a parallel which supports the configurational assignments proposed for the $C_{(8)}$ carboxyl in the dihydrolysergic and lysergic acids.[1] Whilst the agreement between the pK′ for ψ-ecgonine (9·70) and dihydroarecaidine (9·70) would appear to provide further evidence for the assignment of an equatorial carboxyl in ψ-ecgonine (V), 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. We have therefore examined differences in pK′ value between acid-ester pairs in the two series.



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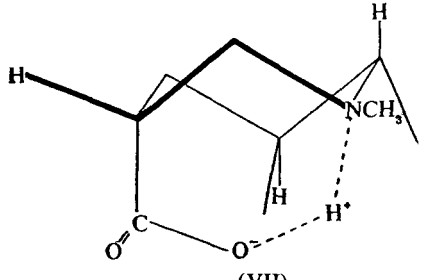

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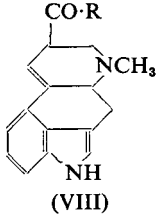

(VIII)

Acid-Ester Differences

It was established by Neuberger[17] that ΔpK (pK′a_2 amino-acid–pK′ a amino-acid ester) provides a measure of the distance between the two charged groups of the amino-acid zwitterion, ΔpK increasing with increasing proximity of the groups. The assumptions made do not permit the use of dissociation constants for accurate measurements of such distances, but this in no way invalidates the use of ΔpK in a qualitative sense. The data in Table I shows that there is reasonable correlation between ΔpK values for dihydroarecaidine (II; ΔpK 1·25), arecaidine (VI; ΔpK 1·37) and ψ-ecgonine (V; ΔpK 1·49) in all of which the carboxyl is equatorial. β-Dimethylaminopropionic acid, in which there is a comparable spacial relationship between the -COOH and $\rangle$NMe groups, also has ΔpK 1·25. On the other hand, ΔpK for ecgonine (IV; ΔpK 1·69), 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 ΔpK 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 ΔpK values are very similar (see Table I). This concordance of ΔpK 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 *cyclo*hexene-like semi-chair conformation.

The effect of the hydroxyl groups in the ecgonine series is obviously more complex. ΔpK for benzoylecgonine (calculated from measurements by Kolthoff[29]) is 3·15 compared with ΔpK 1·69 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).

Solvent Effects on the Dissociation of the Dihydrolysergic Acids

Michaelis and Mizutani[30] and Speakman[31] have shown that a decrease in pK′a value in changing from a mixed organic-aqueous solvent to water is typical of enols and acids. Conversely, an increase in pK′a value for a similar solvent change is consistent with the attachment of a proton to nitrogen in a base-conjugate acid. Comparison of the dissociation constants of the dihydrolysergic acids obtained in 80 per cent. cellosolve by Stoll *et al.*[3] with those obtained in aqueous solution by Craig *et al.*[2] and Stenlake[1] (Table II) reveals that solvent effects are appreciable.

TABLE II

Amino-acid	80 per cent.[3] cellosolve	Water[2] 24° C.	Water[1] 20° C.	Shift in pK′
Dihydrolysergic acid I. $pK'a_1$	4·85	3·57	—	−1·28
$pK'a_2$	7·85	8·45	8·50	+0·65
Dihydro*iso*lysergic acid II. $pK'a_1$	4·97	3·60	—	−1·37
$pK'a_2$	8·38	8·57	8·52	+0·14
Dihydro*iso*lysergic acid I. $pK'a_2$	9·25	—	8·91	−0·34

Dihydrolysergic acid-I and dihydro*iso*lysergic acid-II (in which the COOH is remote from $N_{(6)}$) behave as most amino-acids in that the pK′ value shows a negative shift on changing solvent from 80 per cent. cellosolve to water for the first dissociation constant (proton attached to carboxyl) while there is a positive shift for the second dissociation constant (proton attached to nitrogen). On the other hand the behaviour of dihydro-*iso*lysergic acid-I is anomalous in that its second dissociation constant shows a negative shift on changing solvent from 80 per cent. cellosolve to water. Such an effect is consistent with the axial orientation of the carboxyl group in dihydro*iso*lysergic acid-I which favours chelation of the

proton between the carboxylate ion and $N_{(6)}$ as in (VII), thus permitting behaviour typical of carboxylic acid ionisation.

Conclusion

The evidence cited above and elsewhere indicates that lysergic acid (VIII, R=OH) has the $C_{(8)}$ carboxyl remote from $N_{(6)}$ (equatorial carboxyl). It would appear that the physiologically active series of ergot alkaloids, which are related to lysergic acid, have a similar configuration. We are now investigating synthetic model substances based on this essential structural feature.

SUMMARY

1. Dissociation constants have been recorded for a number of substances structurally related to lysergic acid.

2. The dissociation constant of dihydroarecaidine is consistent with a chair piperidine conformation, with the carboxyl group equatorial.

3. ΔpK values between acid and ester for dihydroarecaidine, arecaidine, ψ-ecgonine, and β-dimethylaminopropionic acid are comparable and significantly smaller than for ecgonine, indicating that in stereoisomeric pairs of amino-acids of this type, the isomer in which the basic and acidic groups are remote is a weaker base than that in which the groups are in propinquity. These results also indicate that the double bond in arecaidine does not materially alter the conformation of the ring as compared with its dihydro derivative.

We wish to thank Messrs. T. and H. Smith, Ltd., for a gift of ecgonine.

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DISCUSSION

The paper was presented by DR. J. B. STENLAKE.

DR. F. HARTLEY (London) said that until there was more detailed knowledge of stereochemical structures no more than partial progress would be made in any attempt to correlate structure with action. In applying the principles which Barton had so successfully applied in steroids and triterpenes the authors were doing a very worthwhile job. Inevitably in order to sort out whether a group was axial or equatorial, Dr. Stenlake had made studies of the dissociation constants of closely related substances.

DR. L. SAUNDERS (London) said that the author had stirred his solution with a magnetic stirrer which he thought unwise when using a high resistance potentiometer such as the *p*H meter. He suggested that a stream of nitrogen was a better method. He wondered whether it would be more satisfactory if, instead of taking *p*H at half neutralisation and determining dissociation constants direct from titration curves, one used more precise techniques.

DR. A. H. BECKETT (London) said that his own results using nitrogen stirring agreed with the authors for the dissociation constants of ecgonine and pseudoecgonine. He felt that there were a number of complexities, for instance, in the case of tropine and pseudotropine one would expect that when the OH group was near the nitrogen the cation would be stabilised by hydrogen bonding and give a stronger base. However, that was not the case. He had reduced ecgonine and pseudoecgonine to primary alcohols, and where the OH group of the primary alcohol was near the nitrogen there was a completely predictable effect in terms of hydrogen bonding.

MR. H. D. C. RAPSON (Dorking) said he had experienced no trouble as a result of using a magnetic stirrer with a *p*H meter.

DR. J. B. STENLAKE, in reply, said that he was aware the magnetic stirrer affected the *p*H meter, but before making the measurements care was taken to switch it off. The danger of stirring with nitrogen was that the nitrogen could be contaminated with carbon dioxide. Owing to the small amount of material sometimes available it was necessary to effect a compromise by using a reasonably accurate method giving useful results. The question of salt effect on concentrations had been investigated. On the question of interference from other groupings, there were two opinions as to the correct interpretation of the dissociation constant of tropine and pseudotropine. He drew attention to the measurements by Cavalieri on adenylic and cytidylic acids which paralleled his own results with lysergic acid.