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ECHITAMINE

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Abstract—Results from degradation studies on echitamine, the alkaloid from *Alstonia scholaris*, are discussed.

ALTHOUGH the alkaloid echitamine was isolated[1] nearly a century ago, the first reliable data on this alkaloid were provided by Goodson and Henry[2] who prepared and analysed several crystalline derivatives and furnished evidence for the presence of an indole ring and a carbomethoxy group. There has been a tremendous upsurge of interest recently on the structure of echitamine as evidenced by the spate of publications on this problem during the last two years.[3-6] We have been carrying out studies on echitamine for sometime and parts of our work have been published already in the form of brief communications.[7] Two contrary views emerge from the work carried out in different laboratories, one that echitamine chloride has an N_a—C—N_b system[3,7] and the other that it has a β-type dihydroindole structure.[4,5] The issue has now been settled by determination of structure by the X-ray method (*vide infra*). Under these circumstances we wish to present in this paper full details of our work on echitamine and discuss the results from the point of view of the correct structure.

In agreement with Goodson and Henry[2] the salt obtained by treatment of the alkaloid with hydrochloric acid was found to analyse for the formula $C_{22}H_{29}O_4N_2Cl$. However, potentiometric titration with N/100 alkali indicated that the salt was a quaternary chloride and not a hydrochloride. Echitamine chloride contains one OMe and one NMe. Although the infra-red spectrum in hexachlorobutadiene mull* did not show the expected band at 7·2 μ, Kuhn Roth estimation indicated the presence of one CMe group. The chloride showed ultra-violet absorption maxima at 235 and 295 mμ (log ε 3·93, 3·55) which were unchanged even on addition of strong acid. On the basis of the findings of Hodson and Smith,[8] it can be inferred that the two nitrogen atoms in echitamine chloride are not separated by more than two carbon atoms.

* Kindly determined by Dr. N. Sheppard.

[1] G. Besanez, *Liebigs Ann.* **176**, 88 (1875); O. Hesse, *Ibid.* **176**, 326 (1875); **203**, 144 (1880); E. Harnack, *Ber. Dtsch. Chem. ges.* **13**, 1648 (1880).

[2] J. A. Goodson and T. A. Henry, *J. Chem. Soc.* **127**, 1640 (1925); J. A. Goodson, *Ibid.* 2626 (1932).

[3a] A. J. Birch, H. F. Hodson and G. F. Smith, *Proc. Chem. Soc.* 224 (1959); [b] A. J. Birch, H. F. Hodson, B. Moore, H. Potts, and G. F. Smith, *Tetrahedron Letters* No. 19, 36 (1960).

[4] H. Conroy, R. Bernasconi, P. R. Brook, R. Ikan, R. Kurtz and K. W. Robinson, *Tetrahedron Letters* No. 6, 1 (1960).

[5a] D. Chakravarthi, R. N. Chakravarthi, R. Ghose and Sir Robert Robinson, *Tetrahedron Letters* No. 10, 10 (1960); [b] *Ibid* No. **11**, 25 (1960).

[6a] S. Ghosal and S. Ghosh Majumdar, *Chem. & Ind.* 19 (1960); [b] A. Chatterjee, S. Ghosal and S. Ghosh Majumdar, *Ibid* 265 (1960); [c] A. Chatterjee and S. Ghosal, *Naturwissenschaften* **47**, 234 (1960).

[7a] T. R. Govindachari and S. Rajappa, *Proc. Chem. Soc.* 134 (1959); [b] *Chem. & Ind.* 1154 (1954); [c] *Ibid* 1549 (1959).

[8] H. F. Hodson and G. F. Smith, *J. Chem. Soc.* 1877 (1957).

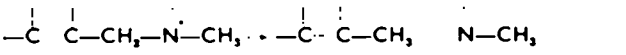
The infra-red absorption spectrum of echitamine chloride showed bands at 3·04 (OH), 3·17 (NH), 5·79 (—COOMe) and 13·2 μ (*o*-di-substituted benzene), but no band near 4·15 μ (⁻⁻NH).

The ultra-violet absorption spectrum of the acetyl derivative of echitamine chloride (λ_{max} 235, 295 mμ log ε 3·94, 3·50) indicated clearly that N_a-acetylation had not taken place. That only O-acetylation had occurred was further supported by the infra-red absorption spectrum which showed bands at 2·92 (NH), 5·70 and 8·14 μ (OAc).

Mild alkaline hydrolysis of echitamine chloride gave demethylechitamine,[2] which was easily shown to be a betaine. In the infra-red absorption spectrum, the ester band at 5·79 μ had disappeared, and instead, the COO^- band made its appearance at 6·25 μ.

Reduction of echitamine chloride in aqueous solution at atmospheric pressure and room temperature in presence of Pd/C gave a tertiary base, m.p. 150–165°, which undoubtedly contained only three oxygen atoms. Since even carbinolamines were unlikely to undergo hydrogenolysis under the extremely mild conditions of this reduction, at an early stage of our work we suggested the possibility[7b] of echitamine chloride being actually $C_{22}H_{27}O_3N_2Cl{\cdot}H_2O$. The correct explanation was first given by Conroy *et al.*[4] who suggested that the proximate product of reduction was so constituted as to lose a molecule of methanol with formation of a lactone. The reduction product was named echitinolide by these authors and correctly formulated as $C_{21}H_{26}O_3N_2$. We came to the same conclusion independently on the basis of experimental evidence to be discussed later. To avoid confusion, we adopt the names echitinolide and isoechitinolide suggested by Conroy *et al.*

The ease of formation of the tertiary base echitinolide from echitamine chloride suggests that the latter is an allyl quaternary ammonium salt. Further, since echitinolide contains two CMe groups as shown by Kuhn–Roth estimation, the principal step in the reduction can be formulated as:



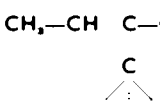
The cleavage of this bond seems to be a necessary condition for the second step, viz., the interaction of the carbomethoxy group with a hydroxyl group with expulsion of methanol and formation of a σ-lactone, since echitamine chloride itself is stable to hot hydrochloric acid.*

The ultra-violet absorption spectrum of echitinolide shows maxima at 247, 310 mμ (log ε 3·90, 3·54), typical of dihydroindole. Both these maxima undergo a hypsochromic shift of about 10 mμ in acid solution. On the basis of the work of Hodson and Smith,[8] it can be inferred that N_a and N_b are separated by a single carbon atom. Echitinolide shows infra-red absorption bands at 2·75 (OH), 2·88 (NH) and 5·80 μ (δ-lactone). Acetylation of echitinolide under vigorous conditions yielded a diacetyl derivative, m.p. 210–214°, in which N_a has been acetylated as indicated by the infra-red (amide band at 6·0 μ) and ultra-violet spectra (λ_{max} 255 mμ; log ε 4·07; λ_{infl} 282 mμ, log ε 3·53) and a positive Otto reaction. Hence echitinolide (and echitamine

* Cf. however, reference 5*a*.

chloride also) should contain a free N_bH group. Mild alkaline hydrolysis of O,N-diacetylechitinolide yielded N-acetylechitinolide, m.p. 160–163° (amide band at 6·02 μ; OH band at 2·71 μ).

Independently, Birch *et al.*[3a] reported results of their work on echitamine in broad agreement with those discussed above.* They further provided conclusive evidence for the presence of an ethylidene group in echitamine chloride and echitinolide, by the isolation in good yield of acetaldehyde on ozonolysis of these compounds. In addition, they obtained α-methylbutyric acid by a modified Kuhn–Roth oxidation of dihydroechitinolide (Base C), proving the presence of the feature



in echitamine chloride. In our experiments it was found that ozonolysis of echitinolide in 2 per cent acetic acid yielded acetaldehyde, but ozonolysis in chloroform solution yielded a small amount of formaldehyde as the only isolable volatile product. Although the reason for this discrepancy is obscure, the presence of an ethylidene group in echitamine chloride can be taken as settled, since there is no indication in the infra-red or N.M.R. spectra of the presence of a vinyl group.

On warming echitinolide with dilute mineral acid, an isomer isoechitinolide, m.p. 180–182°, was formed† whose N-acetyl derivative, m.p. 172–173°, (I.R. band at 6·02 μ; no band in the OH region) was identical with the product obtained by warming N-acetylechitinolide with dilute acid. Isoechitinolide was a tertiary base analysing for the formula $C_{21}H_{26}O_3N_2$ like echitinolide. Isoechitinolide could also be obtained by the following sequence of reactions (Chart I): Demethylechitamine

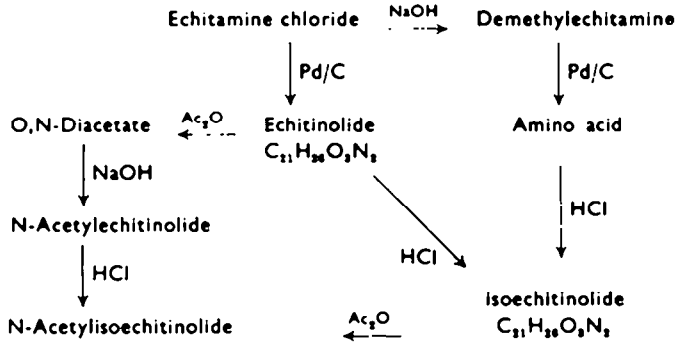


Chart I

was reduced in presence of Pd/C to yield a non-crystalline amino acid (no band in the carbonyl region below 6·3 μ) which on warming with dilute acid yielded isoechitinolide. The latter therefore cannot contain the carbomethoxy group present in echitamine chloride. Two of its oxygen atoms should be present as part of a lactone system. N-Acetylisoechitinolide has no active hydrogen and isoechitinolide can be

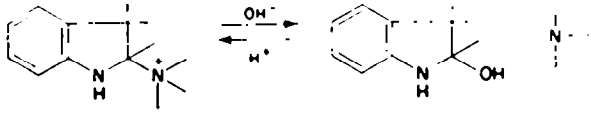
* We have not had access so far to the material in refs. 2 and 3 in the publication of Birch *et al.*[3a]

† We thank Prof. Conroy for carrying out an infra-red comparison of our samples of echitinolide and isoechitinolide with theirs. They were found to be identical, although there is a large difference in their reported m.p. for isoechitinolide and ours.

recovered unchanged after refluxing with methanolic sodium borohydride. The third oxygen atom should therefore be present as an ether function. In agreement, the infra-red spectrum of isoechitinolide does not show any absorption in the hydroxyl region, and shows a peak at 5·7 μ characteristic of a δ-lactone. Ozonolysis of isoechitinolide did not yield any volatile product unlike echitinolide, and N-acetylisoechitinolide was recovered unchanged after prolonged treatment with osmium tetroxide–sodium metaperiodate. There could be no doubt that the ethylidene group present in echitinolide had disappeared as a result of addition of some sort. These results could be rationalized only if echitinolide were itself a lactone and isoechitinolide arises from it by addition of a hydroxyl group to the ethylidene group under acid catalysis with formation of a cyclic ether. Modified Kuhn-Roth oxidation of isoechitinolide did not yield any propionic acid (paper chromatography). The N.M.R. spectrum of isoechitinolide, however, showed conclusive evidence for the formation of an ethyl group attached to a carbon bearing no hydrogen atoms, a feature not present in the N.M.R. spectrum of echitinolide. (Triplet centred at τ = 9·38, J = 7 cps; quartet centred at τ = 8·69, J = 8 cps).

Earlier we had inferred on the basis of U.V. absorption data that echitinolide contained an N_a—C—N_b system. A characteristic feature of such systems is the cleavage of the —C—N_b bond on reduction with zinc and hydrochloric acid.[8,9] Echitinolide, however, merely underwent the transformation isoechitinolide (*vide supra*) under these conditions, there being no other change.

Echitinolide yielded a methiodide under forced conditions. Treatment of an aqueous solution of this methiodide with 2 N alkali led to the immediate separation of a tertiary base, echitinolidemethine, $C_{22}H_{30}O_4N_2$, m.p. 193° (decomp). The remarkable facility with which this transformation occurred was suggestive of the presence of an eserine-like system[10] in echitinolide. Echitinolidemethine showed U.V. absorption maxima at 245, 307 mμ in ethanol, unchanged by the addition of alkali. In ethanolic hydrochloric acid the maxima were shifted to 240 and 297 mμ, suggesting the reformation of the —C—N_b bond cleaved by alkali. These transformations could be formulated as:



Echitinolidemethine was also stable to oxidation by potassium ferricyanide, suggesting that the indolinol hydroxyl group was tertiary. Although fully aware that N_a-unsubstituted 2-hydroxyindolines might be too unstable to exist, and readily change over to the corresponding indolenines, it was considered likely that in the present instance some structural feature stood in the way of such dehydration.

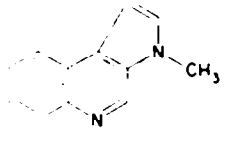
Reduction of echitinolidemethine with zinc and hydrochloric acid yielded desoxyisoechitinolidemethine, $C_{22}H_{30}O_3N_2$, m.p. 200–203°. In the formation of this compound not only had the indolinol hydroxyl group been hydrogenolysed as expected, but the addition of a hydroxyl group to the ethylidene function had also taken place.

[8a] E. Stedman and G. Barger, *J. Chem. Soc.* **127**, 247 (1925); [b] P. L. Julian and J. Pikl, *J. Amer. Chem. Soc.* **57**, 539 (1935).

[10] M. Polonovski and M. Polonovski, *Bull. Soc. Chim.* **23**, 335 (1918); H. F. Hodson, G. F. Smith and J. T. Wrobel, *Chem. & Ind.* 1551 (1958).

The U.V. absorption spectrum of this compound in ethanol solution showed maxima at 247, and 307 mμ (log ε 3·97, 3·59) unchanged by the addition of acid, since the possibility of recyclization with formation of the N_a—C···N_b system no longer existed after the removal of the indolinol hydroxyl group.

In an attempt to obtain a clue to the skeletal structure present in echitamine, echitinolide was submitted to selenium dehydrogenation. The product, echitamyrine, $C_{12}H_{10}N_2$, was identified as 1'-methylpyrrolo (2':3'-3:4)-quinoline (I), by comparison with an authentic sample obtained by silver acetate oxidation of calycanthine.[11]



I

Echitamyrine could conceivably arise either from an α- or a β-type dihydroindole alkaloid with equal facility and did not therefore prove to be of value in the formulation of structure for echitamine.

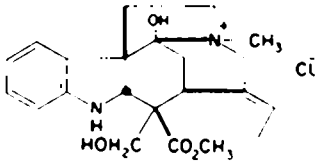
When most of the work outlined above had been concluded, Conroy *et al.* published a paper[4] in which structure II was advanced for echitamine chloride, III for echitinolide and IV for isoechitinolide. Convincing evidence for the presence of a $C\begin{matrix}CH_2OH\\CO_2CH_3\end{matrix}$ unit in echitamine chloride was obtained by heating with potassium t-butoxide and effecting a retro-aldol reaction, the product alloechitamine being formulated as V. The arguments advanced by Conroy *et al.* appeared to be quite in keeping with the experimental evidence we had secured, except in regard to the ultra-violet absorption data, mainly on the basis of which we had suggested the presence of an N_a– C—N_b system. According to the work of Hodson and Smith[8] a compound having the structure II suggested for echitamine chloride would be expected to show indoline absorption in neutral solution and benzenoid absorption in acid solution. In fact compounds of closely related structure such as hemitoxiferine-I[12] and tetrahydrofluorocurarine[13] do exhibit this behaviour. According to Conroy *et al.* the failure to protonate even in strongly acid solution was due to steric hindrance in the neighbourhood of N_a in their structure II. The hypsochromic shift observed in the case of echitinolide and isoechitinolide in acid solution was supposedly due to a change in conformation of the N_a· C_2 bond from axial to equatorial.

The structure proposed by Sir Robert Robinson *et al.*[5] for echitamine chloride differs only in minor detail (about the placement of one of the hydroxyl groups) from that of Conroy *et al.* The structure put forward by Chatterjee *et al.*[6] is clearly untenable since echitamine chloride has undoubtedly a free N_aH group.

In their later publication[3b] Birch *et al.*, while conceding that the structure II advanced by Conroy 'goes a long way towards interpreting the known reactions of the alkaloid' have drawn attention to a number of facts which make this structure less

[11] E. Späth, W. Stroh, E. Lederer and K. Eiter, *Monatsh.* **79**, 11 (1948); K. Eiter, *Ibid.* **79**, 17 (1948); K. Eiter and M. Nagy, *Ibid.* **80**, 607 (1949).
[12] A. R. Battersby and H. F. Hodson, *Proc. Chem. Soc.* 287 (1958).
[13] W. von Philipsborn, K. Bernauer, H. Schmid and P. Karrer, *Helv. Chim. Acta* **42**, 461 (1959).

acceptable. The most pertinent objection in our view is the following: O,O-diacetyl-echitamine chloride was hydrogenated (Emde) to a di-O-acetyl base, in which there is no possibility of any conformational transformation of the type suggested by Conroy *et al.* Still, it shows the hypsochromic shift of 10 mμ of both maxima in acid solution,



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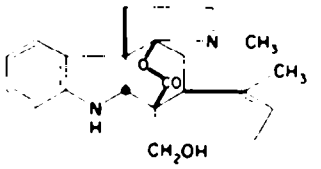

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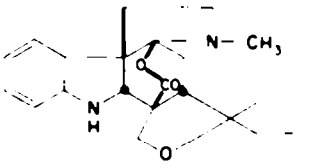

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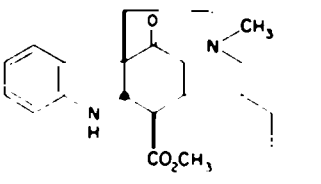


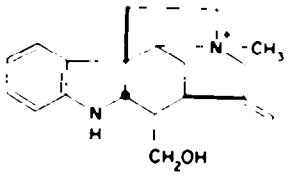
V

associated with the presence of an N_a- C- N_b system. Birch *et al.* have concluded that the most acceptable structure for echitamine chloride would be one in which this system was present.

Subsequent to the publication by Conroy *et al.*, we also carried out certain experiments whose results cannot be satisfactorily explained on the basis of the structures ascribed by these authors to echitinolide and isoechitinolide. Conceding that the presence of an electron-withdrawing carbomethoxy group in the neighbourhood of N_a might prevent protonation of N_a, we reduced echitinolide with lithium aluminium hydride to a non-crystalline alcohol (No I.R. band in the carbonyl region). The reduction product showed indoline absorption in neutral solution, and only the usual hypsochromic shift even in 5N acid solution, but not benzenoid absorption. Similarly isoechitinolide was reduced to a diol which was characterized by the formation of a

crystalline O,O,N-tribenzoate, m.p. 214° (I.R. bands at 5·83, 6·15 μ; no band in the 2·5–3 μ region). On the basis of Conroy's structure for isoechitinolide one would have expected only a O,N-dibenzoate, since the carbinolamine hydroxyl would surely have hydrogenolysed under the conditions of reduction.

In a further attempt to obtain evidence for the correctness of structure II proposed for echitamine chloride, alloechitamine[4] was reduced with lithium aluminium hydride and the non-crystalline alcohol formed was heated with 4 N hydrochloric acid leading to a minute amount of a crystalline quaternary chloride. The last compound should have structure VI on the basis of structure II for echitamine chloride. Two compounds corresponding to this gross structure VI are known, one derived from Wieland–Gumlich aldehyde,[14] the other being tetrahydrofluorocurarin.[13] Comparison of our



VI

compound with the latter showed that there were distinct differences between the two in their infra-red spectra, colour reactions and behaviour in paper chromatography.*

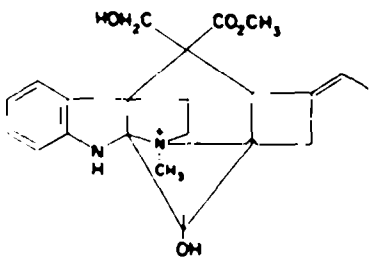
The deadlock about the structure of echitamine has been resolved by the brilliant elucidation of structure of echitamine bromide by the X-ray crystallographic method effected by Prof. Monteath Robertson *et al.*,[15] according to which echitamine bromide should be formulated as VII, containing the N_a—C—N_b system suggested by us and by Birch *et al.*, on the basis of ultra-violet absorption characteristics. Independent confirmation of this structure has been obtained by Dr. S. Ramaseshan[16] working on anhydrous crystals of echitamine iodide and chloride, also by the X-ray method. On the basis of this structure, echitinolide, isoichitinolide and alloechitamine can be formulated as VIII, IX and X respectively. The transannular interaction between a carbonyl group and N_b observed by Conroy *et al.*, is equally feasible with the new correct formulation of alloechitamine. It is indeed a matter for surprise that two structures like II and VII for echitamine chloride which are so utterly dissimilar should be able to account for most of the experimental results with equal facility.

The structure VII now established for echitamine chloride could conceivably arise from an intermediate of the type XI derivable from a normal yohimbine type precursor by Woodward fission of ring E and cleavage of the C_3– N_b bond. Oxidative coupling of C_{16} with C_7 of this intermediate in the indolenine form and formation of a N_b—C_2 bond would lead to structure VII. The hydroxyl group at C_3 is at a position compatible with this scheme.

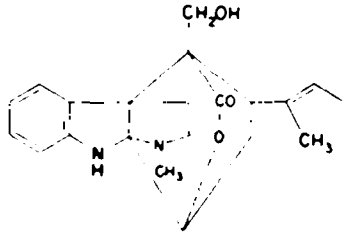
On the basis of structure VII for echitamine chloride, desoxyisoechitinolide methine should be formulated as XII. This compound exhibits indoline absorption

* We are extremely grateful to Prof. P. Karrer who carried out this comparison.

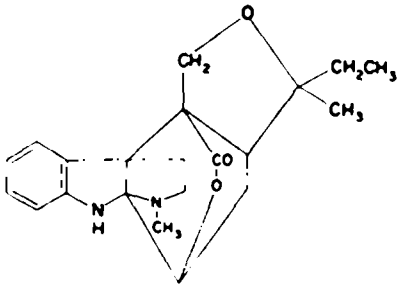
[14] K. Bernauer, F. Berlage, W. v. Philipsborn, H. Schmid and P. Karrer, *Helv. Chim. Acta* **41**,2 293 (1958).
[15] J. A. Hamilton, T. A. Hamor, J. Monteath Robertson and G. A. Sim, *Proc. Chem. Soc.* 63 (1961).
[16] H. Manohar and S. Ramaseshan, *Curr. Sci., India* **30**, 5 (1961). We are deeply indebted to Prof. Robertson and Dr. Ramaseshan for communicating their results to us in advance of publication.



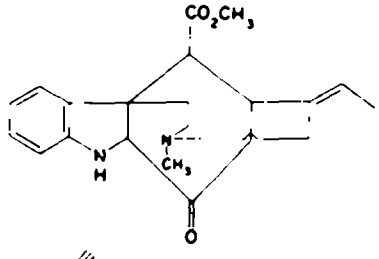
VII



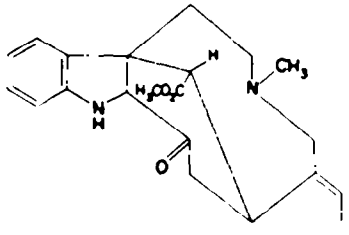
VIII



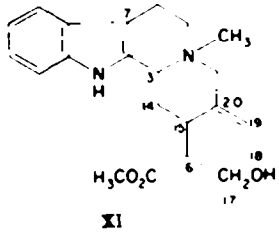
IX



X



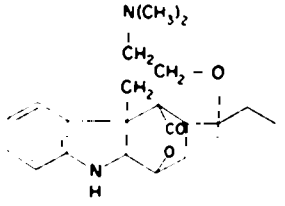
even in 0·1 N acid solution, instead of benzenoid absorption as expected. Alloechitamine, now formulated as X exhibits indoline absorption in 0·1 N hydrochloric acid, but benzenoid absorptions in 5 N acid. In both these compounds N_b is separated by more than three carbon atoms from N_a and the spectral behaviour should be ascribed to the proximity of the carbonyl function in these compounds to N_a. The situation is analogous to that in compound XIII which shows indoline absorption in 0·2 N hydrochloric acid, and benzenoid absorption only in 2·5 N acid.[17]



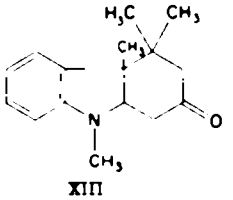
XI

[17] B. Robinson and G. F. Smith, *J. Chem. Soc.* 4574 (1960).

Echitamine base was obtained as a crystalline benzene solvate Conroy *et al.*[4] On the basis of the new formula for echitamine chloride, the base can be ascribed structure XIV in keeping with the properties ascribed to it by these authors.



XII



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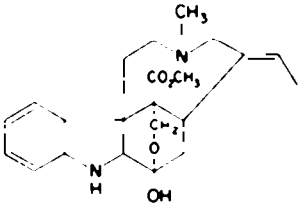

XIV

EXPERIMENTAL

Melting points are uncorrected. Ultra-violet absorption spectra were measured with a Beckmann Model DU Spectrophotometer. Unless otherwise stated, infra-red spectra were measured by Mr. S. Selvavinayakam using a Perkin-Elmer Infracord Spectrophotometer.

Isolation of echitamine

The alkaloid was isolated as its chloride according to the method described by Goodson and Henry.[3] λ_{max} in ethanol 235, 295 mμ (log ϵ 3·93, 3·55) (Found: C, 62·7; H, 6·9; OMe, 7·55; CMe 2·13; NMe, 4·49. $C_{22}H_{29}O_4N_2Cl$ requires: C, 62·8; H, 6·9; one OMe, 7·4; one CMe, 3·6; one NMe, 6·9%).

Echitinolide

Echitamine chloride (2 g) in water (50 ml) was shaken with hydrogen in the presence of Pd/C (5%; 2 g) at 15 lbs/in^2 for 6 hr. The solution was then filtered, basified with ammonia and extracted with ether. The ether extract was dried (Na_2SO_4) and the solvent distilled off leaving the tertiary base as a froth (1·3 g). Crystallization from benzene–light petroleum gave *echitinolide*, m.p. 150–165°; λ_{max} in ethanol, 247, 310 mμ (log ϵ 3·90, 3·54); λ_{max} in ethanolic hydrochloric acid, 237, 295 mμ (log ϵ 3·85, 3·47). (Found: C, 71·1; H, 7·6; OMe, nil; act. H, 0·57. $C_{21}H_{28}O_3N_2$ requires: C, 71·2; H, 7·3; 2 act. H, 0·56%).

Ozonolysis of echitinolide

Echitinolide (0·5 g) in 2% acetic acid (30 ml) was ozonized at 0° (1 hr). The excess ozone was then driven off in a current of nitrogen (1·2 hr), while the solution was still cooled in ice. The acetic acid solution was then taken out and steam-distilled, the distillate being collected in a flask cooled in ice. The distillate (50 ml) was treated with alcohol (25 ml) and 2,4-dinitrophenylhydrazine (100 mg), refluxed for 3 hr, and left overnight at 30°. The crystalline derivative that had separated was collected, washed with alcohol, dried and chromatographed in benzene solution over alumina. The eluate was evaporated and the residue crystallized from alcohol to give acetaldehyde 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 166–167°.

In another experiment, the ozonolysis was performed in chloroform solution, and the volatile aldehyde after decomposition of the ozonide with zinc dust, collected in a solution of dimedone. Formaldehyde dimethone (10 mg from 0·45 g echitinolide), m.p. and mixed m.p. 191°, was obtained.

O,N-*Diacetylechitinolide*

Echitinolide (0·5 g) was refluxed with pyridine (a few drops) and acetic anhydride (15 ml) for 12 hr. It was then cooled and poured on crushed ice. The solution was basified with ammonia and ether extracted. The dried (Na_2SO_4) ethereal extract was evaporated and left in vacuum to remove pyridine. Crystallization of the residue from methanol gave colourless crystals (300 mg) of the O,N-*diacetyl derivative*, m.p. 210–214°. λ_{max} 255 mμ (log ε 4·07); λ_{infl} 282 mμ (log ε 3·53). (Found: C, 68·7; H, 7·0. $C_{33}H_{38}O_8N_2$ requires: C, 68·5; H, 6·8%).

N-*Acetylechitinolide*

The above diacetate (0·5 g) in methanol (12 ml) was refluxed with potassium hydroxide (1 g) in water (5 ml) for 2½ hr. The methanol was then removed *in vacuo*, water was added and the material which had separated was extracted in ether. The ether extract was dried and the solvent distilled off. Crystallization of the residue from dry ether gave N-*acetylechitinolide*, m.p. 160–163°. (Found: C, 69·4; H, 7·1. $C_{31}H_{34}O_6N_2$ requires: C, 69·7; H, 7·1%).

Selenium dehydrogenation of echitinolide

Echitinolide (1·2 g) was intimately ground with black selenium (2 g). The dehydrogenation was carried out in two batches as follows: The mixture was introduced into a metal-bath at 250°, and the temp raised slowly to 340° (5 min), at which stage hydrogen selenide started evolving. The temp of the bath was maintained at 330–340° for 10 min. It was then cooled, the solid mass, along with the container, crushed in a mortar and mixed with alumina. Two such batches were combined and soxhleted with methanol for 10 hr. The methanol extract was filtered and the solvent distilled off. The residue, in ether, was repeatedly extracted with N HCl till the aqueous layer was no more coloured. The aqueous layer was once extracted with ether. The combined ether extracts were washed thoroughly with water and worked up for non-basic material.

The basic fraction: The aqueous acidic layer was well-cooled and basified with conc NaOH solution. The solution was ether extracted, dried (K_2CO_3) and distilled. The residue was sublimed at 180°/0·04 mm, and the sublimate resublimed. The sublimate was then taken in alcohol (3 ml) and picric acid (50 mg) added. The solution was boiled and decanted. The residual picrate was repeatedly digested with small quantities of hot alcohol and decanted to remove the green colour. At this stage, the yield of greenish-yellow picrate was 19 mg. The traces of green colour could only be removed as follows: The picrate was decomposed with sodium hydroxide solution and the base extracted in ether. The ether layer was washed twice with water, dried (Na_2SO_4), distilled and the residue sublimed at 180°/0·04 mm. The sublimate was reconverted to the picrate. Recrystallization from alcohol gave *echitamyrine picrate*, m.p. 252° (decomp) with blackening and shrinking from 242°. The m.p. was unchanged on admixture with authentic 1′-methylpyrrolo(2′,3′-3,4)quinoline picrate obtained from calycanthine. (Found: C, 53·4; H, 3·8. $C_{18}H_{13}O_7N_5$ requires: C, 52·6; H, 3·2%.)

Echitamyrine base itself was obtained by liberation from the picrate, followed by sublimation. λ_{max} in ethanol 245, 307 mμ (log ε 4·45, 3·97); λ_{max} in ethanolic HCl 245, 327, 355 mμ (log ε 4·29, 3·90, 3·95). The I.R. spectrum of the base (in $CHCl_3$) was superposable on that of 1′-methylpyrrolo-(2′,3′-3,4)quinoline.

The non-basic fraction: Careful fractional sublimation of the non-basic fraction gave only indolic material. No trace of carbazole could be detected (U.V. and colour reactions).

Attempted Zn/HCl reduction of echitinolide

Echitinolide (250 mg) in 5 N HCl (30 ml) was treated with zinc granules (10 g) in portions at 100° over 2 hr. The aqueous solution was decanted, cooled, diluted with water, and basified with ammonia. The product was extracted in ether, dried, and after removal of the solvent, crystallized from benzene light petroleum to give *isoechitinolide* (see below) (200 mg), m.p. and mixed m.p. 182°.

Isoechitinolide

(a) Echitinolide (100 mg) in methanol (10 ml) was saturated with dry HCl at 0° and left at 30° for 18 hr. The solution was then evaporated to dryness *in vacuo*, the residue dissolved in water, basified with ammonia, and repeatedly extracted with ether. The ether extract was dried, and distilled. The residual solid (50 mg) was crystallized twice from benzene–pet ether to give *isochitinolide*, m.p. 180–182° (Conroy *et al.*, report m.p. 149–154°; Birch *et al.*, report m.p. 182–184°). λ_{max} in ethanol 247, 307 mμ (log ε 3·94, 3·57); λ_{max} in ethanolic HCl 237, 295 mμ (log ε 3·89, 3·48). (Found: C, 71·1; H, 7·0; OMe, nil. $C_{21}H_{24}O_3N_2$ requires: C, 71·2; H, 7·3%).

In the qualitative chromic acid oxidation method of Karrer, thic compound gave only acetic acid. No trace of propionic acid could be found.

(b) Echitinolide (1 g) was heated at 100° with 5 N HCl (20 ml) for 1½ hr. Dilution with water, basification with ammonia, ether extraction and crystallization from benzene–pet ether gave *isoechitinolide* (0·8 g) m.p. 180–182°.

Isoechitinolide from demethylechitamine

Demethylechitamine (0·6 g) in 50% aqueous alcohol (40 ml) was shaken with hydrogen at 15 lbs/in² in presence of Pd/C (5%; 0·6 g) for 6 hr. The solution was then filtered and evaporated to dryness *in vacuo*. The residual amino acid showed no carbonyl absorption band in the 5–6 μ region. The COO band occurred at 6·3 μ. This solid was dissolved in 5 N HCl (20 ml) and heated on a steam-bath for 1½ hr, diluted, and basified with ammonia. The product was extracted in ether, dried (K_2CO_3) and the solvent removed. The residue (0·4 g) was passed in benzene solution through a short column of acid-washed alumina. The product crystallized from benzene–pet ether as white clusters of needles, m.p. 179–182°, undepressed by admixture with isoechitinolide. The I.R. spectra of the two samples were identical.

N-Acetylisoechitinolide

(a) Isoechitinolide (0·3 g) was refluxed with pyridine (a few drops) and acetic anhydride (12 ml) for 8 hr. The cooled mixture was poured on crushed ice, basified with ammonia and extracted with ether. After removal of the solvent, the residue was crystallized from ether–petroleum ether to give *N-acetylisoechitinolide* (0·25 g), m.p. 172–173°. (Found: C, 69·7; H, 7·1. $C_{23}H_{28}O_4N_2$ requires: C, 69·7; H, 7·1%).

(b) N-Acetylechitinolide (75 mg) in 5 N HCl (10 ml) was heated on a steam-bath for 1 hr. The solution was cooled, diluted to 25 ml, basified with ammonia and ether extracted. The ether solution was dried (K_2CO_3), evaporated, and the residue crystallized from dry ether to give N-acetylisoechitinolide (50 mg), m.p. and mixed m.p. with the above sample, 172°.

Ozonolysis of isoechitinolide

Isoechitinolide (0·5 g) in chloroform (15 ml) was subjected to ozonolysis (1 hr). The solvent was then evaporated *in vacuo*, the residue heated in water with zinc dust at 100° (5 hr), and then steam-distilled. The distillate gave no precipitate with aqueous dimedone solution.

Action of osmium tetroxide–sodium metaperiodate on N-acetylisoechitinolide

N-Acetylisoechitinolide (0·25 g) in ether (30 ml) and water (25 ml) was treated with osmium tetroxide (50 mg). Sodium periodate (0·25 g) was then added to the mixture in portions with vigorous shaking during the course of 1 hr. The mixture was left overnight. The ether layer was separated, dried (Na_2SO_4) and the solvent removed by distillation. The residue on two crystallizations from ether–petroleum ether gave the original material (75 mg), m.p. and mixed m.p. 172°.

Echitinolidemethine

Echitinolide (0·6 g) was refluxed with methyl iodide (3 ml) in chloroform (5 ml) for 10 hr. The chloroform was then removed and the residue dissolved in water (5 ml). The aqueous solution was added with stirring to 2 N NaOH (12 ml). An oil separated almost immediately, and after digestion for 15 min, was extracted with ether. The ether solution was dried (Na_2SO_4) and the solvent distilled off. The residue, on crystallization from benzene–pet ether, gave *echitinolidemethine* (0·3 g), m.p. 193° (decomp). (Birch *et al.*,[30] report m.p. 169–173°) λ_{max} in ethanol 245, 307 mμ (log ε 3·92, 3·52)

unaffected by addition of alkali; λ_{max} in ethanolic hydrochloric acid 240, 297 mμ (log ε 3·88, 3·47). (Found: C, 68·9; H, 7·9. $C_{22}H_{30}O_4N_2$ requires: C, 68·4; H, 7·8%).

Desoxyisoechitinolidemethine

The above methine (100 mg) in 5 N HCl (15 ml) was treated with zinc granules (5 g) in portions at 100° over 2 hr, such that a steady rate of evolution of hydrogen was maintained. The solution was then decanted from the zinc, diluted, basified with ammonia and ether extracted. After removal of the ether, the residue was crystallized twice from benzene pet ether to give *desoxyisoechitinolidemethine* (50 mg), m.p. 200-203°. λ_{max} in ethanol 247, 307 mμ (log ε 3·97, 3·59) unchanged on addition of acid. (Found: C, 71·6; H, 8·2. $C_{22}H_{30}O_3N_2$ requires: C, 71·4; H, 8·1%).

Attempted ferricyanide oxidation of echitinolidemethine

The methine (0·9 g) in alcohol (2 ml) was added to potassium ferricyanide (2·4 g) in water (33 ml) containing sodium hydroxide (0·8 g). The solution was refluxed for 5 min, cooled and extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and after removal of the solvent, the residue passed through a column of acid-washed alumina in benzene solution. Evaporation of the eluate, and crystallization of the residue (0·5 g) from benzene-pet ether gave back the methine, m.p. and mixed m.p. 193° (decomp).

Lithium aluminium hydride reduction of echitinolide

Echitinolide (0·5 g) in dry tetrahydrofuran (5 ml) was added to a suspension of lithium aluminium hydride (1 g) in dry ether (40 ml). The mixture was stirred for 5 hr, left overnight and worked up as usual. The product (0·5 g) was amorphous and could not be crystallized. λ_{max} in ethanol 250, 307 mμ (log ε 3·84, 3·41); λ_{max} in 0·1 N or 5 N HCl, 240, 295 mμ (log ε 3·82, 3·39). It had no band in the carbonyl region of the infra-red spectrum. Benzoylation or acetylation of the compound led to no crystalline material.

Lithium aluminium hydride reduction of isoechitinolide

Isoechitinolide (0·2 g) in tetrahydrofuran (10 ml) was added to lithium aluminium hydride (0·5 g) in dry ether (40 ml). The mixture was stirred for 3 hr, left overnight, and worked up as usual. The product was a froth, having no carbonyl band in the infra-red. The product (0·2 g) in pyridine (5 ml) was treated with benzoyl chloride (2 ml) and left for 3 days at 30°. It was then poured into water (50 ml), extracted in benzene, and the benzene layer washed with 2N Na_2CO_3 solution. After removal of benzene and pyridine *in vacuo*, the residue was chromatographed in benzene over alumina. Crystallization from methanol gave the *benzoate*, m.p. 214°. (Found: C, 75·2, 75·3; H, 6·6, 6·4. $C_{43}H_{42}O_6N_2$ requires: C, 75·2; H, 6·3%).

Lithium aluminium hydride reduction of alloechitamine

Alloechitamine (0·12 g) in dry ether (20 ml) was added to lithium aluminium hydride (0·25 g) in dry ether. The product was non-crystalline. The product was heated on a water-bath with 4 N HCl (10 ml) for 3 hr, evaporated to dryness *in vacuo*, and the residue crystallized from methanol to give a product (ca. 1 mg), λ_{max} in ethanol 245, 295 mμ (log ε 3·83, 3·43).

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