

The Alkaloids of *Holarrhena Antidysenterica*
Part I. Three New Alkaloids from the
Bark of Indian *Holarrhena* and New
Methods of Isolation and Further
Purification of Conessine.

BY SALIMUZZAMAN SIDDIQUI AND P. PARAMESWARAN PILLAY.

A good volume of work has already been done on the different species of *Holarrhena*, resulting invariably in the isolation of conessine ($C_{24}H_{40}N_2$), both from the bark and seeds of the plants. Pyman (*J. Chem. Soc.*, 1919, 115, 163) isolated from the seeds of *Holarrhena congolensis*, Stapf, another alkaloid, holarrhenine ($C_{24}H_{38}ON_2$, m.p. 197-98°), besides conessine (m.p. 125°, Corr., yield 0.25 p.c.). A little earlier Ulrici (*Arch. Pharm.*, 1918, 256, 57), working on the bark and seeds of *Holarrhena africana*, had contended to have isolated from conessine melting at 121.5° two different bases, viz., (i) the "true conessine", $C_{23}H_{38}N_2$, cubical crystals, m.p. 125° and (ii) homo-conessine, $C_{25}H_{42}N_2$, (broad needles which begin to soften and effervesce at 50° and have a persistent solid nucleus which does not clear upto 130°), but Giemsa and Halberkann (*Arch. Pharm.*, 1918, 256, 201), who also worked on the same plant, held them to be identical with conessine as obtained by the previous authors. Ghosh and Ghosh (*J. Indian Chem. Soc.*, 1928, 5, 477) communicated the isolation of two new alkaloids, kurchicine (m.p. 173-75°, yield 0.12 p. c.) and kurchine (m.p. 73-75°) besides conessine (m.p. 120°, yield not mentioned).

Owing, on the one hand, to the immense medicinal interest acquired by *Holarrhena antidysenterica* in later years, and on the other, to the amount of controversy that hangs round the problem of its alkaloidal constituents, we found it of interest to undertake a systematic study of the alkaloids of this plant.

As a result of our investigations we have succeeded in obtaining conessine melting at 126° (Corr.) in a yield of 0.4 p.c. on the weight of dry powdered bark and three new alkaloids, viz.,

(i) Conessimine, $C_{23}H_{38}N_2$, m.p. 100°; distils to a crystalline mass at 230°/1.8 mm; $[\alpha]_D^{25} = -22.5^\circ$; $C_{23}H_{38}N_2$, 2 H_2O , m.p. 91°, yield 0.12 p. c.

(ii) Holarrhimine, $C_{21}H_{36}ON_2$, m.p. 183° (Corr.); $[\alpha]_D^{25} = -14.19^\circ$, (yield 0.12 p.c. in fresh and 0.03 p.c. in older bark).

(iii) Holarrhine, $C_{20}H_{38}O_3N_2$, m.p. 240° ; $[\alpha]_D^{32} = -17.01^\circ$. The formula for holarrhine has been assigned only provisionally, as the nitrogen value was too low and the substance did not suffice for a control combustion.

The method which first led to the isolation of conessimine was based on a repeated fractional precipitation of the sulphuric acid soluble portion of the alkaloids with ammonia, whereby the middle fraction gave conessimine in a very low yield as a hydrate fairly insoluble in moist ethyl acetate. A quantitative isolation of it, however, was only possible on exploiting its two very important properties, viz., the formation of its carbonate by passing carbon dioxide into a solution of the different bases in moist petroleum ether, and the insolubility of its hydroiodide in water and alcohol. Conessimine, which also forms an insoluble crystalline hydroiodide and belongs to the fraction of weaker bases, does not form a carbonate and can be crystallised out from acetone. The separation of holarrhimine and holarrhine was possible only through a fractional crystallisation of the bases, obtained from the water insoluble crystalline sulphates, out of methyl alcohol-ethyl acetate mixture in which holarrhine is much less soluble than holarrhimine.

Conessimine, is diacidic like conessine, contains one active H and 2 $N-CH_3$ groups and forms a crystalline mononitroso derivative. It thus appears to be a secondary-tertiary base. Holarrhimine, is also diacidic, contains no OCH_3 or $N-CH_3$ group, as against $3N-CH_3$ in holarrhenine, and showed the presence of 3 active H, which could be accounted for by assuming, that it contains 1 OH and 2 :NH groups, as this base also gives a non-basic nitroso derivative, which, however, could not be obtained crystalline so far due probably to its being a mixture of mono and dinitroso derivatives.

About the close of our present investigations appeared second part of "The alkaloids of the bark of *Holarrhena antidysenterica*" by Ghosh and Bose (*Arch. Pharm.*, 1932, 270, 100), in which the authors have characterised in detail the two alkaloids, isolated by Ghosh and Ghosh in 1928 (*loc. cit.*). Also, Haworth (*J. Chem. Soc.*, 1932, 631) has communicated the isolation of a new base from the seeds of *Holarrhena antidysenterica* by distilling the mother liquors of conessine, obtained from Simonsen (*J. Chem. Soc.*, 1926, 2123), which he has called norconessine.

In the face of the analytical data and general characteristics of holarrhimine it is quite apparent that it is different from Pyman's holarrhenine, $C_{24}H_{38}ON_2$, m.p. 197° - 98° , $[\alpha]_D = -7.1^{\circ}$. As to Ghosh's kurchicine, $C_{26}H_{36}ON_2$, m.p. 175° , $[\alpha]_D^{25} = -8.45^{\circ}$, it is either a different base altogether, which we have not yet succeeded in isolating, or a mixture of holarrhimine and holarrhine, as nothing has been done by the authors to separate these two bases, which crystallise together as sulphates.

With regard to conessimine and Ghosh's kurchine, $C_{23}H_{38}N_2$, m. p. 73 - 75° , distilling at $233^{\circ}/1$ mm. to a waxy mass, $[\alpha]_D^{25} = -7.57^{\circ}$, there can be no doubt that the latter, if pure, is quite different from the former. On comparing, however, the methods of isolation of the two bases, it appears more likely that kurchine should prove to be chiefly conessimine in admixture with the non-carbonate forming bases of the conessine group. Also Haworth's norconessine is evidently different from conessimine as the former is a liquid at ordinary temperature, has $[\alpha]_D = +7^{\circ}$ and contains like conessine 3 $N\cdot CH_3$ groups in its molecule as against 2 in conessimine.

As for holarrhine, its melting point and specific rotation are too far apart from those of both kurchicine and holarrhimine to allow of any possibility of its confusion with either.

So far as conessine is concerned we believe to have attained to a higher degree of purity of the base than so far achieved. This was possible through a removal of the last traces of the carbonate-forming, petroleum ether soluble, secondary bases, with nitrous acid, whereby snow-white conessine melting at 126° (Corr.) was obtained. The melting point noted by the different authors for conessine vary from 122° to 125° (Corr.), the latest melting point observed for pure conessine by Späth (*Ber.*, 1930, **63**, 126) being 123° . With regard to the two crystalline forms of conessine, needles and plates, which have formed a source of controversy between Ulrici (*loc. cit.*) and Giemsa and Halberkaun (*loc. cit.*), we have incidentally noted, that the plates are the more stable form for conessine.

EXPERIMENTAL.

The material used was obtained from a vaid dealer in drugs in Lahore and identified by the Sibpur Botanical Institute, Howrah.

The dry powdered bark (about a year old) (17 kg.) was percolated eight times with a mixture of 80 parts of ether with 10 parts of alcohol

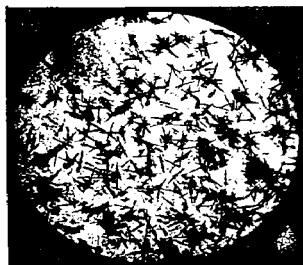
shaken up with 10 parts of liquor ammonia. The percolate was drawn out first after a week and later after every three days, gaseous HCl passed through it till just acid, and the ether decanted off from the precipitated hydrochlorides, made ammoniacal, and used again. After two extractions the ethereal solution decanted from the precipitated hydrochlorides was treated with ammonia and then with acetic acid to faint acidity and the solvent distilled off for further use.

The residue left by the distillation of the extraction medium gave 2 p.c. of neutral matter besides a small quantity of residual alkaloids which was added on to the main alkaloidal hydrochlorides. The total hydrochlorides were dissolved in 2 litres of water and treated with sodium sulphate which gave a cheese-like precipitate of insoluble sulphates, which was filtered and well washed with water. The filtrate from the sulphates was treated with 20 p.c. sodium hydroxide and the alkaloids which were thrown out extracted with ether.

In this way 38 g. of an insoluble sulphate (30 g. free base) and 326 g. of an ether-soluble reddish yellow treacly alkaloids were obtained, giving a yield of 2.1 p.c. of crude alkaloids.

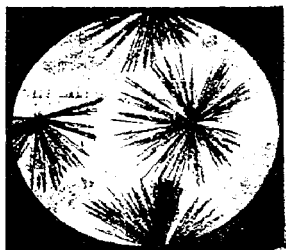
The ether soluble alkaloids were treated with petroleum ether and the soluble portion treated with moist carbon dioxide, which threw down an insoluble carbonate. The petroleum ether insoluble portion was dissolved in ethyl acetate and also separated into carbonate and non-carbonate fractions. After a long process of repeated fractionations, rendered necessary because the separation of the different groups was not very clear cut, the bases were finally separated into three broad fractions: (A) sulphates insoluble in water, (B) carbonates insoluble in petroleum ether, and (C) non-carbonates soluble in petroleum ether.

Fig. 1.



Conessimine.

Fig. 2.



Halarrhimine.

Fig. 8.



Holarrhine.

Isolation of the Bases.

Conessine.—The fraction (C) gave by direct crystallisation from acetone crude conessine (80 g.) which softened at 110° and melted at 110-18°. On recrystallisation out of acetone after removing the last traces of carbonate-forming bases by passing CO₂ into its solution in petroleum ether, suspended over water, conessine which begins to soften at 123° and melts at 124° (yield 68 g.) was obtained. On removing the last traces of secondary bases by treatment with NaNO₂ in acetic acid solution, conessine melting at 126° (Corr.) was obtained, the melting point remaining unaltered on further crystallisation either directly or through its crystalline oxalate.

Conessimine.—The fraction (B) of carbonates was dissolved in hydrochloric acid and fractionally precipitated with ammonia and NaOH. By a repeated fractionation three fractions were finally obtained. The strongest basic fraction gave the residual insoluble sulphates and a fraction of soluble sulphates. The former was added on to the main fraction of sulphates and the base from the latter mixed with the middle fraction. The weakest basic fraction gave some residual conessine and chiefly petroleum ether insoluble carbonates, the base from which was also added on to the middle basic fraction. This was now dissolved in dilute HCl and KI was added to the clear solution in small portions in the cold till the fresh addition of it did not produce any further precipitate. The hydroiodide which came down as a thick oil, soon turned

crystalline (yield 50 g.). After repeated recrystallisations out of a methyl and ethyl alcohol mixture the nearly colourless crystalline hydroiodide, m.p. $308-10^{\circ}$ (decomp.) (pure conessimine hydroiodide melts at $318-19^{\circ}$) was converted into base, which was dissolved in moist petroleum ether and a slow current of CO_2 passed through the solution at ordinary temperature. The base from the precipitated carbonates was dissolved in ethyl acetate, the solution concentrated to a small volume and kept in the cold after adding some water to it, when conessimine hydrate crystallised out in long slender needles. After recrystallisation from the same medium or out of alcohol-water it melted at 91° , turning immediately into a buttery mass, which melts down giving a clear meniscus at 100° , yield 0.12 p.c.

Holarrhine.—The combined insoluble sulphates (38 g.) were treated with 10 p.c. HCl in the cold which dissolved out the major portion, forming a deep red solution. The nearly white, granular residue was then dissolved in hot 10 p.c. methyl alcoholic HCl , the solution neutralised with ammonia and an equal quantity of 1 p.c. H_2SO_4 added to the solution in the hot. On cooling, white silky crystals of the sulphate separated out (yield 10 g.). The base from sulphate, was dissolved in hot methyl alcohol, and ethyl acetate added to the solution till beginning of turbidity. After keeping for a day in the cold a colourless silky crystalline mass separated out, which on recrystallisation out of methyl alcohol and ethyl acetate, yielded holarrhine, m. p. 240° , a subsequent recrystallisation leaving the m.p. unaltered, yield 0.6 g.

Holarrhimine.—On completely removing the solvent from the combined mother liquors of holarrhine, the hot ethyl acetate solution of the residue gave star shaped needles on cooling, m.p. 180° . After repeated recrystallisations from the same solvent holarrhimine was finally obtained, m. p. 183° (Corr.), yield 4 g., 0.03 p.c.

Right in the beginning of the present investigations holarrhimine was isolated with comparatively much greater ease in a yield of 0.12 p. c. out of the fresh bark.

Characterisation of the Alkaloids, their Salts and Derivatives.
Conessimine.

Conessimine crystallised out of its concentrated solutions in dry petroleum ether, ethyl acetate or acetone in microscopic white

needles, m.p. 100°. It is fairly soluble in all the common organic solvents. In 2.606 p.c. solution in chloroform it showed a rotation $[\alpha]_D^{25} = -22.25^\circ$. [Found: C, 80.7, 80.6; H, 11.4, 11.1; N, 8.7; M. W. (cryoscopic in benzene), 358. $C_{23}H_{38}N_2$ requires C, 80.7; H, 11.1; N, 8.2 per cent., M. W., 342]. Out of moist ethyl acetate a sparingly soluble dihydrate of the base crystallises out in long slender needles or oftener in aggregates of short spike formed needles, m.p. 91° (cf. Fig. 1). (Found: C, 72.9; H, 11.0; H_2O , 9.8; $C_{23}H_{38}N_2 \cdot 2H_2O$ requires C, 73.0; H, 11.1; H_2O , 9.5 per cent.).

Conessimine distils at 230°/1.8 mm. to a viscous oil which soon starts crystallising and was found to be the unchanged base, although the rotation was slightly reduced— $[\alpha]_D^{25} = -20.0^\circ$ in chloroform in a concentration of 2.476 p.c.

Dissolved in concentrated H_2SO_4 on a watch glass, conessimine forms a colourless solution, which changes to brilliant yellow after standing for sometime or more quickly on heating for a few minutes on the water-bath. If the watch glass is now exposed to the atmosphere, first a green and then a blue ring begins to develop from the outermost edge of the solution slowly masking its preceding colour towards the centre, giving a rainbow effect to the whole. Finally a pinkish violet ring is noticeable from the outer edge of the blue ring. On gradual addition of drops of water to the original bright yellow solution under stirring, it changes the colours in the same sequence, as observed by Warneke and others in the case of conessine. We found Conessimine also to give similar coloured rings as conessimine.

Determination of active H (Zerewitinoff's method) showed the presence of one active H in the molecule. (Found: H, 0.35, $C_{23}H_{38}N_2$ requires H, 0.29 per cent.).

Determination of N-CH₃ (Herzig and Meyer's method) showed the presence of 2 N-CH₃. (Found: CH₃, 8.8. $C_{23}H_{38}N_2$ requires (2N-CH₃) CH₃, 8.8 per cent.).

Conessimine carbonate was obtained on passing CO_2 through a moist petroleum ether solution of the base as a thick voluminous white precipitate, which is soluble in alcohol and insoluble in water and dissolves in dilute acids with effervescence. Dried in the air it begins to soften at 70°, giving off CO_2 , and melts indefinitely upto 105°. It is soluble in cold alcohol and insoluble in petroleum ether saturated with CO_2 , but hot petroleum ether dissolves it owing to decomposition of the unstable salt.

Conessimine hydrochloride, prepared by adding dry ethereal hydrochloric acid to a solution of the base in dry ether, forms a white amorphous powder, is exceedingly soluble in water and alcohol, and showed a rotation in 3.708 p.c. solution in water $[\alpha]_D^{25} = -15.10^\circ$. When dried in *vacuo* at 100° it melts at 342.44° . (Found: Cl, 16.9. $C_{23}H_{38}N_2$, 2 HCl requires Cl, 17.1 per cent.).

Conessimine chloroplatinate, prepared by adding 5 p.c. platinum chloride solution to an aqueous solution of conessimine hydrochloride, formed a cream coloured powder, m.p. 301° (decomp.) and is insoluble in alcohol or water. (Found: Pt, 26.0. $C_{23}H_{38}N_2$, 2 HCl, Pt Cl₄ requires Pt, 25.9 per cent.).

Conessimine aurichloride, obtained as above, forms a yellowish powder, which is insoluble in water and soluble in alcohol. When heated after drying in *vacuo*, it begins to get reddish at 130° , thick red sticky fluid at 140° , and decomposes with evolution of gas at 165° . (Found: Au, 38.3. $C_{23}H_{38}N_2$, 2 HCl, AuCl₃ requires Au, 38.6 per cent.).

Conessimine hydroiodide was obtained by adding KI solution to an aqueous solution of the hydrochloride as a thick oil, which quickly turned crystalline. Recrystallised from water it melts at 318.19° (decomp.). (Found: I, 42.9. $C_{23}H_{38}N_2$, 2 HI requires I, 42.6 per cent.).

Conessimine picrate was obtained by adding a concentrated aqueous solution of picric acid to the aqueous solution of the hydrochloride as a brilliant yellow powder, m.p. 172.74° , which is very sparingly soluble in hot water or alcohol, and comes out of the former solvent in stars of long rectangular planks.

Nitrosoconessimine.—To a cooled solution of the base (0.3 g., 1 mol.) in 8 c. c. of 10 p.c. acetic acid was added a concentrated solution of sodium nitrite (0.18 g., 3 mol.) and the reaction mixture was left overnight, when crystalline amber coloured needles (0.24 g.) separated out, which when filtered and washed first with dilute acetic acid and then with water to remove traces of unchanged base, melted at 240.41° (decomp.) after drying in vacuum. (Found: N, 11.9. $C_{23}H_{37}N_2 \cdot NO$ requires N, 11.8 per cent.).

Holarrhimine.

Holarrhimine was precipitated from aqueous solutions of its salts by concentrated ammonia as a gelatinous mass and in a more filterable form by caustic soda solution. It crystallised out of ethyl

acetate in colourless star-shaped radiating needles (*cf.* Fig. 2), m.p. 183° (*Corr.*). It is very soluble in alcohol and chloroform and almost insoluble in ether and petroleum ether. In 4.798 p. c. chloroform solution it shows a rotation of $[\alpha]_D^{25} = -14.19^\circ$. The rotation was found to diminish on long treatment of the base with hydrochloric acid. After drying in *vacuo* at 100° the base gave the following analysis. (Found: C, 75.6, 75.8; H 11.1, 11.0; N, 8.3. $C_{21}H_{36}ON_2$ requires C, 75.9; H, 10.8; N, 8.4 per cent.).

Holarrhimine is a diacid base, has no OCH_3 or $N-CH_3$ group, but shows the presence of three active H. (Found: H, 0.88. $C_{21}H_{36}ON_2$ requires H, 0.90 per cent.).

Holarrhimine carbonate separated out as a bulky amorphous powder on dissolving the base in moist ethyl acetate and passing CO_2 through the solution. When washed with ethyl acetate saturated with CO_2 and dried in the air it begins to give off CO_2 from 80° upwards without showing any definite sign of melting. It dissolves in acids with effervescence, is fairly soluble in alcohol and insoluble in other organic solvents in the cold.

Holarrhimine hydrochloride was prepared by adding ethereal hydrochloric acid to a chloroformic solution of the base, m. p. 345° (*decomp.*). It is very soluble in alcohol, less so in water, and sparingly soluble in 10 p. c. aqueous or alcoholic HCl. It crystallises from water in star-like aggregates of broad plates. In 2.193 p. c. methyl alcoholic solution it shows a rotation of $[\alpha]_D^{25} = -22.80^\circ$. (Found: Cl, 17.2. $C_{21}H_{36}ON_2 \cdot 2HCl$ requires Cl, 17.5 per cent.).

Holarrhimine chloroplatinate, prepared as in case of conessimine, forms a cream coloured powder, insoluble in alcohol or water, darkens at 270° and chars above 300° without melting. (Found: Pt, 26.2. $C_{21}H_{36}ON_2 \cdot 2HCl, PtCl_4$ requires Pt, 26.3 per cent.).

Holarrhimine hydrobromide was prepared by dissolving the base in dilute HBr and allowing it to cool, when it came out as a crystalline mass. On recrystallisation out of water in which it is sparingly soluble it formed star-shaped aggregates of thin plates and broad needles. m. p. 358-60° (*decomp.*).

Holarrhimine picrate, prepared by adding dry ethereal picric acid to an alcoholic solution of the base, formed a heavy crystalline powder, m. p. 198-200° (*decomp.*). It crystallises from hot water, in which it is fairly soluble, in brilliant yellow plates with a silky lustre, apparently in hydrated form (m. p. 108-10°).

Holarrhimine sulphate was prepared by dissolving the hydrochloride in methyl alcohol and adding dilute sulphuric acid to the solution till beginning of turbidity at water-bath heat, when it soon crystallised out in snow-white silky needles and spangles, m. p. 337°. It is almost insoluble in water and all the organic solvents.

Holarrhine.

Holarrhine crystallised from a mixture of methyl alcohol and ethyl acetate in white needles, m. p. 240°. It is very soluble in methyl and ethyl alcohols, sparingly so in chloroform, and almost insoluble in ethyl acetate, ether and petroleum ether. (Found: C, 67·4; H, 10·3; N, 6·5. $C_{20}H_{18}O_3N_2$ requires C, 67·8; H, 10·7; N, 7·9 per cent.). In 4·992 p. c. methyl alcoholic solution, it shows a rotation of $[\alpha]_D^{25} = -17·01^\circ$. It gives a distinct precipitate on addition of sodium nitrite solution into its acetic acid solution and so appears to be a secondary base.

Holarrhine chloroplatinate was obtained on addition of a cooled 3 p. c. platinum chloride solution to a cooled aqueous solution of the hydrochloride as a yellowish amorphous powder darkening from 270° onwards, and charring above 300°. (Found: Pt, 25·4. $C_{20}H_{18}O_3N_2 \cdot 2HCl \cdot PtCl_4$ requires Pt, 25·5 per cent.).

Holarrhine picrate, prepared as in case of holarrhimine, formed a pale yellow semi-crystalline powder which begins to darken at 275°, but does not melt upto 320°.

Conessine.

Conessine crystallises out of acetone in large prismatic plates, but from more dilute solutions as long flat needles or aggregates of shorter needles, if allowed to crystallise slowly and completely undisturbed. On stirring, however, the needles abruptly changes into plates. If carefully dried, the needles can be collected as such and show the same m. p. as the plates. (Found: C, 80·9; H, 11·3. $C_{24}H_{40}N_2$ requires C, 80·8; H, 11·3 per cent.).

Conessine hydrochloride, prepared by adding ethereal HCl to a solution of the base in dry ether, formed a white powder, which browned up at 335° and melted at 338-40° (decomp.).

Conessine hydroiodide, prepared like conessimine hydroiodide, formed long colourless bars, m. p. 308° (decomp.). (Found: I, 41·2. $C_{24}H_{40}N_2 \cdot 2HI$ requires I, 41·6 per cent.). It is difficultly soluble in

water, fairly soluble in methyl alcohol, less so in ethyl alcohol and in other organic solvents.

Conessine picrate was obtained by adding ethereal picric acid to a dry ethereal solution of the base as a brilliant yellow semi-crystalline powder m. p. 222-24° (decomp.). It crystallises out of hot water in stars of crowded needles.

Conessine oxalate, prepared by dissolving the base in 10 p. c. aqueous oxalic acid, formed a white crystalline powder which on recrystallisation out of water melted at 372° (decomp.).

Colour reactions of the alkaloids of Holarrhena antidysenterica.

Reagent.	Conessimine.	Conessine.	Holarrhimine.	Holarrhine.
Conc. H_2SO_4	Colourless; after standing for $\frac{1}{2}$ hr. or on warming for about 5 min. bright golden yellow to orange; on addition of water, violet.	Same as conessimine.	Yellowish red; then bright red; water lightens colour.	Same as holarrhimine.
HNO_3	Colourless	Do	Colourless	Do
Conc. H_2SO_4 and $K_2Cr_2O_7$	Yellowish green to deep green according to concentration; on adding water colour lightens.	Same as conessimine.	Deep green, water lightens colour.	Same as holarrhimine.
Erdmans reagent	Golden yellow; on adding drops of water turns successively green, blue and then violet.	Do	Deep red	Do
Froehdes reagent	Grass green, then deep green; colour lightens on addition of water.	Do	Yellowish green; on warming brown with a greenish yellow tinge; on adding a little water deep reddish brown. on further dilution deep red.	

