71. *The Alkaloids of* Alstonia *Barks. Part I.* A. constricta, *F. Muell.*

By Thomas M. Sharp.

Since *Cinchona* bark became known to Europeans three centuries ago, its galenical preparations and its chief alkaloid, quinine, have until recently been the only remedies available for the treatment and prophylaxis of malaria. Increased knowledge of the ætiology of the disease has thrown doubt on the status of *Cinchona* as a real anti-malarial specific, and has led to the remarkable activity shown in recent years in the search for additional anti-malarials. This search is being conducted along three main lines: (*a*) synthesis of drugs of new types, (*b*) modifications in the structure of *Cinchona* alkaloids, and (*c*) investigation of natural drugs having local reputations as febrifuges or remedies for malaria. The *Alstonias* belong to group (*c*). Two species, *A. scholaris* and *A. constricta*, were recognised in the British Pharmacopœia of 1914, and *A. scholaris* and other representatives of the genus have been used in West Africa, Malaya, and the Philippine Islands. Previous work in these laboratories has shown that echitamine, $C_{22}H_{28}O_4N_2$ (Goodson and Henry,

J., 1925, **127**, 1640), is the principal alkaloidal constituent of *A. congensis*, *A. scholaris*, *A. Gilletii*, *A. angustiloba*, and *A. spathulata*, but is not present in *A. constricta*, *A. macrophylla*, or *A. villosa* (Goodson, J., 1932, 2626), and it is with the first of the latter group that the present paper is concerned.

Botanically, *A. constricta* differs very much from the typical *Alstonias* (Bentham and von Müller, *Flora Australiensis*, 1869, **4**, 314), and early work of Hesse has shown that its alkaloids are quite different from echitamine (*Annalen*, 1865, Suppl. IV, 40; 1880, **205**, 360; *Ber.*, 1878, **11**, 1546). The bark of this species appears to have been first examined by Palm (*Vierteljahresschr. pr. Pharm.*, 1863, **12**, 161) and later by von Müller and Rummel (J., 1879, **35**, 31) and Oberlin and Schlagdenhauffen (*J. Pharm.*, 1879, **28**, 576). Hesse (*loc. cit.*) isolated three alkaloids, alstonine (chlorogenine), alstonidine, and porphyrine. Alstonine, the most abundant alkaloid, was described as a brown amorphous mass, which could be rubbed to a brownish-yellow powder. Its hydrate (3·5 H_2O) melted under 100°, while the anhydrous base was only liquid at 195°. Its formula was given as $C_{22}H_{20}O_4N_2$. The salts were amorphous and readily precipitated by acids from their aqueous solutions. Solutions of base or salts showed a strong blue fluorescence. Porphyrine was obtained in white flocks melting at 97° and showing a weak blue fluorescence in solution. Alstonidine crystallised in colourless needles, m. p. 181°, and formed colourless crystalline salts, but was obtained in quantity too small for analysis. This alkaloid also showed a blue fluorescence in solution.

Three samples of bark have now been examined; the first was purchased in Australia, the second and third were kindly supplied by Mr. A. R. Penfold, Curator of the Technological Museum, Sydney, and Mr. C. T. White, Government Botanist, Queensland. The last sample was described as *A. mollis*, Benth., which according to Mr. White is now regarded as a variety of *A. constricta*. Mr. T. E. Wallis, B.Sc., who has been good enough to examine the three barks, considers them identical. All three barks yield the same alkaloids, but in different amounts, the bark described as *A. mollis* being the richest.

The isolation of the alkaloids offers some difficulty owing to the instability of the bases and the quantity of tarry alkaloidal material thrown down on the addition of alkali to the acid extracts or on agitation with chloroform; but the method described in the experimental part can be successfully repeated so long as it is carried out rapidly. There are at least four alkaloids present, of which only one has been obtained crystalline. Although it is not easy from Hesse's description to identify these with the alkaloids obtained by him, it is clear that the one obtained crystalline now is Hesse's amorphous alstonine and it is proposed to retain that name for it. The other alkaloids are provisionally named A, B, and C. They have not been obtained pure, but there is sufficient difference in their properties to indicate clearly that they are different. A is a relatively weak base which is liberated from its salts by sodium carbonate, B a strong base only liberated by caustic alkalis, and C is a very soluble base isolated by precipitation with mercuric chloride after removal of the other alkaloids. Definite proof is lacking, but the author inclines to the view that A and the tarry alkaloidal material described above may be decomposition products of alstonine.

The salts of alstonine are yellow to orange in colour and crystallise well, but the base, although it can readily be obtained crystalline, cannot be recrystallised without decomposition and rapidly becomes brown on exposure to air, or even in an evacuated desiccator. The most characteristic salt is the *sulphate*, which is very soluble in methyl alcohol, but practically insoluble in dry ethyl alcohol, whilst the *picrate*, which has a sharp melting point, is most suitable for identification. The salts are soluble in water to yellow or orange solutions, which show a strong blue fluorescence on dilution, and are readily reprecipitated on the addition of acids or alkali metal salts. The freshly precipitated base is soluble in chloroform or benzene, forming yellow solutions which show no fluorescence. On standing, or if the base is not pure, the solutions exhibit a strong green fluorescence, hence Hesse's earlier name "chlorogenine." Analyses of the salts of alstonine indicate the formula $C_{21}H_{20}O_3N_2$. The base, prepared from a pure salt by precipitation with sodium carbonate, forms an orange-coloured varnish, which is converted into a yellow microcrystalline powder on rubbing with water, and forms a *tetrahydrate*. Crystallised from absolute alcohol and

air-dried, it appears to have $1\frac{1}{4}$ mols. of water, but the anhydrous substance could not be obtained owing to decomposition at elevated temperatures.

Alstonine contains one methoxy-group, but no methyl attached to nitrogen, and readily forms a monomethiodide; so one of the nitrogen atoms is tertiary. One only of the nitrogen atoms is basic, but the base is capable of forming acid salts with dibasic acids such as sulphuric and oxalic acids. The function of the second nitrogen has not been determined; the base does not react with acetic anhydride, benzoyl chloride, or nitrous acid. It does not give any of the colour reactions for indole, such as are given by echitamine, and it is unaffected by catalytic hydrogenation. The absence of phenolic hydroxyl groups is indicated by its insolubility in sodium hydroxide solution, and the absence of alcoholic hydroxyl groups by its indifference to acetic anhydride; it also fails to give a reaction for methylenedioxy-groups when tested with Gaebel's reagent.

The pharmacological action of alstonine sulphate has been examined by Dr. A. C. White at the Wellcome Physiological Research Laboratories, whose results are shown in the following table :—

Concentration.	Organ.	Action.
1 : 25,000—1 : 12,000	Isolated rabbit uterus	Causes contraction and increased tone. The adrenaline response is abolished or reduced, and reappears after repeated washing.
1 : 25,000	Isolated guinea pig uterus	Contraction and increased tone.
1 : 25,000	Isolated rabbit intestine	Increased movement.

Alstonine sulphate in doses of 2 mg. per kilogram causes, in the anæsthetised cat, a fall in the blood pressure which is unaffected by atropine. The adrenaline response is considerably reduced, but can be potentiated by the injection of cocaine. Dr. Buttle of the same laboratories reports that alstonine sulphate is inactive in bird malaria. It thus joins echitamine, akuammine, and harmine as drugs whose local reputations as anti-malarials cannot be confirmed by modern methods of investigation (Goodson, Henry, and Macfie, *Biochem. J.*, 1930, **26**, 874).

Experimental.

In the earlier experiments, the finely powdered bark was first exhausted in a Soxhlet extractor with petroleum (b. p. 40—60°) in the hope of isolating Hesse's base porphyrine, but as only traces of alkaloid were removed by the process, this step was omitted in the later batches, and the following method was adopted. The powdered bark (900 g.) was moistened with alcohol, packed into a large copper Tutin extractor (Allen's "Commercial Organic Analysis," 5th Ed., Vol. VII, p. 8) and extracted with alcohol during 4 days. The solvent was evaporated and the black viscous residue extracted with 0·5% sulphuric acid (300 c.c.), filtered under gravity from insoluble fatty material, the filtrate diluted with an equal volume of water and, after an hour, filtered again from precipitated impurities. Soluble impurities were removed from the clear filtrate by many extractions with ether. Chloroform (250 c.c.) was then added to the aqueous solution, and the mixture was treated with 10% sodium carbonate solution (35 c.c.), added in seven portions, the mixture being shaken vigorously after each addition. The almost black chloroform layer was separated, and the aqueous portion extracted several times with fresh chloroform. The mixed chloroform extracts were shaken for about a minute with sodium sulphate, filtered rapidly, and at once extracted with sufficient *N*-sulphuric acid (about 6 c.c.) to make the solution acid to litmus but not to methyl-orange. On evaporation, this solution furnished the sulphate of base A. During the extraction of the alkaline liquid with chloroform a considerable amount of black tarry material was deposited on the sides of the separator and rapid manipulation was necessary in order to reduce this to a minimum. After the removal of base A the solution was treated with a further quantity of 10% sodium carbonate solution (60 c.c.), added in portions of about 10 c.c., several shakings with chloroform being made after each addition. The united chloroform extracts, after drying for a few moments, were treated in the same fashion with *N*-sulphuric acid (usually about 28 c.c.). (The chloroform extract at this stage was of a deep red-brown colour; as the concentration of base in chloroform was lowered by the removal of alkaloid as sulphate to the aqueous layer, the characteristic bright green fluorescence of the impure base in chloroform solution became apparent, and, when all the base had been removed, the fluorescence practically disappeared and the chloroform

was of a pale yellow colour.) The acid extract was evaporated to dryness, and the residue, on boiling with absolute alcohol, furnished *alstonine sulphate* as an orange-coloured crystalline powder, and a further quantity of amorphous sulphate of A. A third alkaloid, provisionally called B, was obtained by extraction with chloroform after the addition of a strong solution of sodium hydroxide and, after acidification, a fourth alkaloid, C, was precipitated as a pale yellow powder by the addition of mercuric chloride. These have so far not yielded any crystalline derivatives. The tar thrown down on addition of alkali furnished a further quantity of alstonine sulphate and A sulphate after extraction with dilute sulphuric acid and treatment with sodium carbonate and chloroform in the manner described above. The yields of alkaloids from the three barks are shown in the table: B and C were small in quantity and are not included, as the figures are incomplete, several of the earlier batches having been worked through in a number of different ways before this method was adopted. The commercial specimen

			Alstonine sulphate, g.			
Bark.	Weight, kg.	Sulphate of A. Total, g.	From main extract.	From tar.	Alstonine sulphate, %.	Sulphate of A, %.
Commercial sample	15·10	93·2	120·25	19·1	0·92	0·62
Penfold's sample	11·34	91·3	142·50	25·1	1·48	0·81
"Mollis" sample	2·92	44·65	69·00	8·0	2·64	1·53

was about 20 years old, and the amount of crystalline alkaloid recorded is probably lower than the real figure, as the method of isolation was worked out on this bark. The best yield obtained from any individual batch of the bark was 1·33%. The specimen labelled "mollis" gave a much cleaner extract and furnished less tar than the other two barks.

For the purification of the alkaloid, the sulphate was dissolved in methyl alcohol, filtered from a small amount of brick-red substance, and treated with two volumes of absolute alcohol. After some time the sulphate separated in pale orange, stout rods, which frothed at 209° (corr.); $[\alpha]_D$ + 118·6 * (c = 1·042, water) [Found: loss at 120° in a vacuum, 10·2.

$(C_{21}H_{20}O_3N_2)_2,H_2SO_4,5H_2O$

requires loss of $5H_2O$, 10·2%. Found, on dry salt: C, 63·3, 63·1; H, 5·35, 5·4; N, 6·8, 6·9; S, 4·2, 4·1; OMe, 7·8, 7·8; NMe, nil. $(C_{21}H_{20}O_3N_2)_2,H_2SO_4$ requires C, 63·45; H, 5·3; N, 7·05; S, 4·0; OMe, 7·8%]. The *acid sulphate*, obtained by addition of the calculated quantity of dilute sulphuric acid to the normal sulphate, separates from dry alcohol in yellow prismatic needles, m. p. 246—248° (decomp., corr.); $[\alpha]_D$ = 113·1° (c = 1·034, water) (Found, on salt dried at 110° in a vacuum: C, 57·1, 57·2; H, 5·25, 5·3; N, 6·05, 6·2; S, 7·1; OMe, 7·15, 7·1; NMe, nil. $C_{21}H_{20}O_3N_2,H_2SO_4$ requires C, 56·5; H, 5·0; N, 6·3; S, 7·2; OMe, 7·0%). The *hydrochloride*, obtained by treatment of the sulphate with barium chloride, forms aggregates of yellow, stout, pentagonal plates from absolute alcohol, m. p. 286° (decomp., corr.); it shows a brilliant purple fluorescence in alcoholic solution; $[\alpha]_D$ + 131·9° (c = 1·064, water) (Found: C, 66·0, 66·15; H, 5·3, 5·6; N, 7·3, 7·2; Cl, 9·55; OMe, 8·3, 8·2; NMe, nil. $C_{21}H_{20}O_3N_2,HCl$ requires C, 65·5; H, 5·5; N, 7·3; Cl, 9·2; OMe, 8·1%). Some of the analyses for this salt and for the nitrate and acid sulphate agree better with the formula $C_{22}H_{22}O_3N_2$, but the analyses as a whole are more in agreement with the formula given. The *acid oxalate* separates from alcohol in rosettes of soft yellow needles, m. p. 239° (decomp., corr.) (Found: C, 63·05, 63·05; H, 5·3, 5·4; N, 6·4, 6·5; OMe, 7·2, 7·2. $C_{21}H_{20}O_3N_2,C_2H_2O_4$ requires C, 63·0; H, 5·1; N, 6·4; OMe, 7·1%). The *nitrate* forms rosettes of stout orange-coloured needles from alcohol, m. p. 262—263° (decomp., corr.) after blackening at 230° (Found: C, 61·85, 61·8; H, 5·2, 5·3; N, 9·9, 10·0; OMe, 7·5, 7·6. $C_{21}H_{20}O_3N_2,HNO_3$ requires C, 61·3; H, 5·15; N, 10·2; OMe, 7·55%). The *picrate* forms rosettes of stout reddish-orange needles from alcohol, m. p. 194—195° (corr.) (Found: C, 56·4, 56·4; H, 4·3, 4·25; N, 12·3, 12·2. $C_{21}H_{20}O_3N_2,C_6H_3O_7N_3$ requires C, 56·1; H, 4·0; N, 12·1%). The *hydriodide* separates from methyl alcohol in pale yellow, triangular leaflets, m. p. 291° (decomp., corr.) (Found: I, 26·6, 26·5; OMe, 6·6, 6·7. $C_{21}H_{20}O_3N_2,HI$ requires I, 26·65; OMe, 6·5%). Alstonine base is thrown down as an orange-coloured resin, which adheres to the sides of the vessel, on the addition of excess of sodium carbonate to an aqueous solution of a salt. After being washed with water, the resin on long stirring with water is converted into a yellow microcrystalline powder, which appears to be a *tetrahydrate* (Found, on air-dried material: C, 60·0, 59·8; H, 6·8, 6·6; N, 6·7, 6·5; OMe, 7·9, 7·8. $C_{21}H_{20}O_3N_2,4H_2O$ requires C, 60·0; H, 6·7; N, 6·7; OMe, 7·4%. Found, on base dried at 55° in a vacuum: C, 70·65, 70·6; H, 5·9, 5·8; N, 7·55, 7·8; OMe, 9·0. $C_{21}H_{20}O_3N_2,\frac{1}{2}H_2O$

* All the rotations were done on solutions of the dry salts.

requires C, 70·55; H, 5·9; N, 7·85; OMe, 8·7%). The base darkens considerably at a higher temperature and the anhydrous base could not be obtained. The tetrahydrate sinters at 77° and forms a transparent red froth at 130°. It is soluble in cold absolute alcohol and the solution deposits fine canary-yellow crystals almost at once. These, after air-drying, appear to contain $1\frac{1}{4}H_2O$ (Found: C, 67·7, 67·8; H, 6·1, 6·15; N, 7·5, 7·6; OMe, 8·3, 8·3. $C_{21}H_{20}O_3N_2,1\frac{1}{4}H_2O$ requires C, 68·0; H, 6·1; N, 7·6; OMe, 8·4%). This hydrate became brown on drying at 55° in a vacuum and satisfactory analytical numbers could not be obtained on the dried substance. The base could not be recrystallised without considerable loss. It sinters at 87°, resolidifies on further heating, and decomposes sharply at 254° (corr.). A *monomethiodide*, obtained by warming the base gently with excess of methyl iodide, crystallised from methyl alcohol in rosettes of soft yellow needles, which decomposed at 246° (corr.) after shrinking at 242° (Found: C, 54·1; H, 4·9; N, 5·8; OMe, 6·35; NMe, 5·6. $C_{21}H_{20}O_3N_2,CH_3I$ requires C, 53·9; H, 4·7; N, 5·7; OMe, 6·3; NMe, 5·9%). The quaternary base was obtained as a reddish-brown resin by treatment with thallium sulphate and barium hydroxide and evaporation on a water-bath in an open dish. It is soluble in water to an orange-coloured liquid, which shows a strong blue fluorescence. Attempts to degrade the base by Hofmann's method were unsuccessful. With acetyl chloride alstonine was converted into the hydrochloride, but no acetyl derivative was formed.

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