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THE ALKALOIDS OF *PICRALIMA KLAINEANA*

BY

T. A. HENRY AND T. M. SHARP

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THE WELLCOME CHEMICAL RESEARCH LABORATORIES
(The Wellcome Foundation Ltd.)
T. A. HENRY, D.Sc., *Director*
6, King Street, Snow Hill
LONDON, E.C. 1

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CCLIV.—*The Alkaloids of* Picralima Klaineana.

By Thomas Anderson Henry and Thomas Marvel Sharp.

In December, 1923, we received from the Imperial Institute a supply of "Akuamma" seeds which had been forwarded by the Department of Agriculture in the Gold Coast Colony, accompanied by herbarium specimens of the plant, which had been identified

by the Director of the Royal Botanic Gardens, Kew, as those of *Picralima Klaineana*, Pierre. A full account of the botany of the plant and of the use of the seeds as a febrifuge in native medicine has been published by Holmes (*Pharm. J.*, 1915, **41**, 758; 1922, **55**, 478). Last year Frère Just Gillet, Curator of the Botanical Gardens at Kisantu, Belgian Congo, sent us a small supply (300 g.) of seeds which also were identified at Kew as derived from a species of Picralima, probably *P. Klaineana*. In spite of its reputation as the source of a useful drug, at least among African natives, *P. Klaineana* does not seem to be widely distributed and Frère Gillet reports that he had to make a tedious journey of some 30 leagues to collect his small sample. We are therefore the more indebted to the authorities of the Imperial Institute and in particular to the officers of the Department of Agriculture in the Gold Coast Colony, for the supplies of seed we have examined.

Beyond the preliminary work referred to in the articles by Holmes (*loc. cit.*), there appeared, when we began this work, to be no record of the chemical examination of any species of *Picralima*, but two papers have been published recently by Clinquart (*Bull. Acad. Roy. Med. Belge*, 1926, 492; *J. Pharm. Belg.*, 1927, **9**, 187) giving results of a preliminary examination of the seeds, bark and leaves also supplied by Frère Gillet. Clinquart obtained a few centigrams of a crystalline alkaloid, m. p. 242—243°, and a large amount of an amorphous alkaloid. The behaviour of these two alkaloids with the usual precipitants and colour-producing reagents is described, but no attempt is made to characterise either alkaloid or its salts in detail. In view of the publication of these two papers it has been considered desirable to place on record the data now presented. The seeds of *P. Klaineana* contain at least four alkaloids, two of which have been crystallised. Only one of these has so far been obtained in sufficient quantity for characterisation and this, which is no doubt identical with Clinquart's crystalline base, it is proposed to call *akuammine* from "akuamma," one of the native names of *P. Klaineana* in the Gold Coast. Akuammine has the formula $C_{22}H_{28}O_4N_2$, yields a series of well-crystallised salts, gives a number of characteristic colour reactions with aromatic aldehydes in presence of hydrochloric acid, and contains one methoxyl, one hydroxyl, and one methylimino-group. The last probably represents a tertiary nitrogen atom, since akuammine furnishes a well-crystallised *monomethiodide*, which decomposes on heating, evolving a fishy odour, doubtless due to dimethylamine. The alkaloid does not react with semicarbazide or other reagents for carbonyl groups and no evidence of a methylenedioxy-group is afforded with Gaebel's reagent. The state of the remaining nitrogen has not been ascer-

tained; on treatment in hydrochloric acid solution with sodium nitrite, a scarlet hydrochloride is formed, which does not give Liebermann's nitrosoamine reaction and appears to be a nitro-derivative of akuammine. Akuammine is converted by alkalis under certain conditions into a *monohydrate*, which crystallises with some difficulty. The latter is also formed on hydrolysis of acetylakuammine by alkali, and an O-*methyl* derivative of the monohydrate appears to be produced when akuammine is treated with methyl iodide in presence of alkali. One or both of the remaining oxygen atoms are no doubt involved in these changes.

The other three alkaloids, referred to subsequently as A, B, and D, we propose to examine fully as soon as material is available. Names are not suggested for them, as they may prove to be related to akuammine, in which case it may not be necessary to add to the already large trivial nomenclature of alkaloids. Alkaloid D is remarkable in having a specific rotation of $-737°$ in alcohol or chloroform. Neither A nor B has yet been obtained crystalline.

Both the Congo and the Gold Coast seeds yield akuammine and alkaloids A and B, but only one sample of Gold Coast seeds has so far yielded alkaloid D.

EXPERIMENTAL.

The finely ground seeds were extracted in a copper Soxhlet apparatus first with petroleum, b. p. 60—80°, to remove fat and then with 96% alcohol. The alcoholic extract was taken to dryness under reduced pressure, and the residue treated with successive quantities of 2% hydrochloric acid until alkaloid was no longer removed. The mixed acid liquors were shaken out with ether to remove resinous and waxy impurities and then diluted with water until no further precipitation took place. The precipitate at this stage consists of a weakly basic alkaloid or mixture of alkaloids (A), from which no crystalline material has so far been obtained. The filtrate from this was digested with charcoal for 1 hour on a water-bath, filtered, allowed to cool, and then treated with saturated sodium carbonate solution in excess; this precipitated a mixture of alkaloids as a brown powder, which was collected, washed with hot water, and dried in a vacuous desiccator. The dry mixture was then extracted with ether in a Soxhlet apparatus and so separated into a further quantity of material apparently identical with (A), which remained undissolved in the extractor, and a partly crystalline powder, which gradually accumulated in the extraction flask. When the ether ceased to extract alkaloid, the solvent was distilled off and the dry residue macerated with twice its weight of alcohol, which dissolved a second amorphous alkaloid (B), and left a partly

crystalline residue of crude akuammine (alkaloid C). The latter was collected, washed with a little alcohol, allowed to dry in the air, and then treated with a slight excess of *N*-sulphuric acid (2·8 c.c. approx. per gram) and sufficient water to dissolve it on boiling. The hot solution was treated with charcoal and filtered. On cooling, thin, colourless needles (m. p. 214—218°) of crude akuammine sulphate separated. The mother-liquor on concentration in a vacuous desiccator deposited more of the same sulphate, but finally dried up to a brown varnish. From this, by solution in dry alcohol, a further crop of akuammine sulphate can be obtained, but eventually an amorphous residue of the sulphate of alkaloid B is left. If ammonia solution is used in place of sodium carbonate for precipitation, the residue left after the removal of most of the akuammine sulphate sets to a jelly due to the presence of the sulphate of a hydrate of akuammine formed by the action of ammonia on this alkaloid as described later (p. 1958).

The cold alcoholic extract of the ether-soluble portion of the precipitated alkaloids was taken to dryness under reduced pressure, dissolved in sufficient *N*-sulphuric acid to form a faintly acid solution, and to this was added a saturated aqueous solution of sodium iodide in water until no further precipitation occurred. After standing for a week, the clear liquor was poured off and the mixture of tar and crystalline matter that had separated was triturated with a little cold absolute alcohol, which dissolved the tar and left a small quantity of akuammine hydriodide. The alcoholic washings were added to the mother-liquors, and the whole was fractionally precipitated with sodium carbonate solution, the first few drops of precipitant serving to remove a dark brown, sticky base, from which a clear pale yellow mother-liquor was poured off. This on further addition of sodium carbonate solution yielded the crude alkaloid (B) as a pale yellow powder, m. p. 104° (air-dry) or 167° (dried in a vacuum).

The seeds received from the Imperial Institute consisted of several samples collected at different times, and from one of these, on recrystallisation of the crude akuammine sulphate as described above, the first fraction consisted of the well-crystallised sulphate of alkaloid (D) (p. 1958). As only 2 g. of this alkaloid have been obtained, it has been considered advisable to conserve it for the present and only to make such observations upon it as would not entail the loss of material. It is therefore only possible to put its chief characteristics on record.

The quantity of alkaloids present in *P. Klaineana* seeds is more than 5%, but the exact amount cannot be determined by any of the ordinary methods of estimation, because the alkaloids cannot

be precipitated completely from solution in acids by alkalis, nor can the alkaloid left in such alkaline filtrate be completely removed by any of the ordinary immiscible solvents. The yield of crude akuammine is about 0·6%. The four alkaloids are readily differentiated by their physical properties and colour reactions. Alkaloid A is insoluble in all ordinary solvents except acids and alkalis, and from these it is precipitated on dilution with water. Alkaloid B is readily soluble in all ordinary solvents except water, and solutions of its salts, unlike those of any of the other three, give an intense indigo-blue colour with ferric chloride. Akuammine (alkaloid C) dissolves in concentrated sulphuric acid without coloration, but gives an intense blood-red colour, stable for several days, with nitric acid. The second crystalline alkaloid (D) gives no colour with sulphuric acid but a vivid green, fading to yellow in a few hours, with nitric acid.

In the following descriptions, the " melting points " are corrected; in each case, except for akuammine hydrate, they are points at which the substance froths up the tube. The temperature at which this occurs varies with the rate of heating, *e.g.*, pure akuammine hydrochloride can be made to melt and decompose at any temperature from 215° to 227°. The melting points recorded for the base and its salts were taken alongside the pure hydrochloride, melting at 227°, so that they are comparable among themselves. The optical rotations are for the dried substance in each case.

Akuammine (Alkaloid C).

The base is prepared by adding excess of sodium carbonate solution to a warm, aqueous solution of a purified salt. It is sparingly soluble in cold alcohol, but once dissolved in boiling alcohol it crystallises from the latter only by spontaneous evaporation in minute, colourless needles, m. p. 255°, $[\alpha]_D^{20°}$ −66·7° (in alcohol; $c = 0·504$) or −73·4° (in chloroform; $c = 0·8716$). It is rather more soluble in chloroform and sparingly soluble in acetone or ether (Found: C, 69·0, 69·1; H, 7·2, 7·0; N, 7·5. $C_{22}H_{28}O_4N_2$ requires C, 68·7; H, 7·3; N, 7·3%).

Akuammine hydrobromide, prepared like the sulphate (see above), separates from dilute hot aqueous or alcoholic solutions in faintly grey, long, slender prisms, or from concentrated solutions in the same solvents in small needles, m. p. 228°, $[\alpha]_D^{20°}$ −26·05° (in water; $c = 0·6076$). The air-dry salt loses in a vacuous desiccator 3·61% and no further loss takes place in a vacuum at 100°. (Calc. for $C_{22}H_{28}O_4N_2,HBr,H_2O$: H_2O, 3·7%. Found for dry salt: C, 56·1, 56·3; H, 6·0, 6·1; Br, 16·6. $C_{22}H_{28}O_4N_2,HBr$ requires C, 56·7; H, 6·3; Br, 17·1%). The *hydrochloride* closely resembles the

hydrobromide in appearance, but is of paler colour and rather more soluble in water or alcohol. It has m. p. 227° and $[\alpha]_D^{22°}$ $-26·6°$ (in water; $c = 0·8769$) or $-32·8°$ (in alcohol; $c = 1·716$) and crystallises with one molecule of water (Found: loss in a vacuum at 120°, 4·0. $C_{22}H_{28}O_4N_2,HCl,H_2O$ requires H_2O, 4·1%. Found for dry salt: C, 62·5, 62·6; H, 6·9, 6·7; N, 7·1; Cl, 8·5, 8·4. $C_{22}H_{28}O_4N_2,HCl$ requires C, 62·7; H, 7·0; N, 6·6; Cl, 8·4%). The *sulphate*, prepared as described above, crystallises from hot water or hot alcohol in rosettes of flattened needles, which rapidly become red on exposure to light. It melts at 221° and has $[\alpha]_D^{19°}$ $-40·3°$ (in water; $c = 2·0868$). The salt is abnormal in composition, crystallises with 10 molecules of water of crystallisation, of which 9 are lost at atmospheric temperature in a vacuous desiccator over calcium chloride. Loss (*a*) from air-dry salt at 100° in a vacuum, 11·5; (*b*) from salt previously dried to constant weight in a vacuous desiccator, 1·8. $(C_{22}H_{28}O_4N_2)_3,2H_2SO_4$, for $10H_2O$, requires H_2O 11·8%, and for $1H_2O$ 1·3% [Found for dry salt: C, 58·8; H, 6·7; S, 4·45. $(C_{22}H_{28}O_4N_2)_3,2H_2SO_4$ requires C, 58·7; H, 6·5; S, 4·7%]. This may be a double compound consisting of one molecule of the acid salt with one molecule of the normal salt (B,H_2SO_4; B_2,H_2SO_4). In this connexion, it may be noted that another dibasic acid, *viz.*, oxalic acid, yields at least three salts, which, however, have not been satisfactorily separated.

The *hydriodide* is precipitated as a crystalline powder on adding potassium iodide to even a dilute solution in water of any soluble salt of akuammine. It cannot be crystallised from hot water without decomposition, but separates from hot alcohol in anhydrous, minute, pale grey needles, m. p. 226° (Found: C, 52·0; H, 6·0. $C_{22}H_{28}O_4N_2,HI$ requires C, 51·5; H, 5·7%).

The *nitrate*, also prepared by precipitation, crystallises from boiling alcohol in pale cream-coloured needles, m. p. 224°. The *thiocyanate*, similarly prepared, crystallises from alcohol in pale greyish needles, m. p. 218°. The *perchlorate*, precipitated by addition of sodium perchlorate to a solution of the hydrochloride in water, crystallises from hot alcohol in colourless, spheroidal granules, m. p. 215°, if the solution is concentrated, and from more dilute solutions in colourless needles also melting at 215°. This salt becomes pink on exposure to air and resinous after several crystallisations from boiling alcohol. The *picrate* crystallises from hot alcohol in rosettes of brilliant yellow needles, m. p. 199°. The *picrolonate*, precipitated in minute, yellow needles, darkens at 160° and froths at 194°. On dissolution in hot alcohol it deposits a deep blue dye.

No chloroaurate has been prepared, as akuammine hydrochloride

reduces gold chloride immediately. It also reduces platinic chloride and silver nitrate in the cold and Fehling's solution on warming.

Methoxyl and methylimino-groups. Determinations of these groups were made on the base and the hydrochloride (Found for base: MeO, 8·87; NMe, 8·2. Calc. for MeO, 8·0 and for NMe, 7·5%. Found for hydrochloride: MeO, 7·73; NMe, 6·6. Calc. for MeO, 7·4 and for NMe, 6·9%).

Akuammine hydrochloride gives (1) a rose-red colour with vanillin or piperonal in presence of hydrochloric acid, (2) a yellowish-brown colour with dimethylaminobenzaldehyde (Ehrlich's reagent), changing on long standing to red with a green fluorescence, (3) with bromine water a pink colour, the solution becoming cloudy on further addition of bromine.

Action of methyl iodide on akuammine. The pure base does not dissolve in methyl iodide, but when suspended in the reagent the latter gradually contracts in volume and in about 2 days conversion into a *monomethiodide* appears to be complete. This separates from solutions in hot methyl alcohol in rosettes of colourless prisms, m. p. 274°. On melting, the substance froths vigorously and gives off a vapour with a fishy odour, probably due to dimethylamine. The methiodide in solution gives no precipitate with alkali hydroxides or carbonates and is therefore the iodide of a quaternary base (Found: C, 52·7; H, 5·7; I, 24·4; MeO, 6·8; NMe, 9·7. $C_{22}H_{28}O_4N_2,CH_3I$ requires C, 52·4; H, 5·9; I, 24·1; MeO, 5·9; 2NMe, 11·1%). The results for methoxyl are high and those for methylimino-groups low. In carrying out these estimations, it was noticed that elimination of methyl iodide in the second stage (methylimino-determination) began at the low temperature of 180°, immediately after addition of ammonium iodide, so that it appears likely that part of the methyl iodide collected in the earlier stage (methoxyl determination) is really due to methylimino-groups. The total amount of methyl iodide formed in the estimation, calculated from the amount of silver iodide weighed, was 79·07%, whilst there should be formed, assuming the presence of one methoxyl and two methylimino-groups, 80·9%. Cases in which there is no sharp distinction between the conversion of methoxyl and methylimino-groups into methyl iodide in such estimations have been observed previously, *e.g.*, in ergotoxine (Barger and Ewins, J., 1918, **113**, 235; a summary of literature on this subject is given on page 237).

Action of methyl iodide and alkali on akuammine. Akuammine base (1 g.) was dissolved in *N*-sodium methoxide solution in methyl alcohol (2·7 c.c.), and methyl iodide (4 c.c.) was added. This mixture, after standing for 4 days, was boiled for 4 hours. The liquid was taken to dryness under reduced pressure, and the residue

dissolved in boiling water, from which it separated on cooling as a colourless oil. The solution in water gives a precipitate of a new oily base on addition of sodium carbonate or alkali hydroxide. Neither the base nor any of its salts, except the picrate, have so far been induced to crystallise. The *picrate* is precipitated on adding picric acid in methyl alcohol to a solution of the base or the hydriodide in the same solvent, and can be crystallised either from boiling water or from methyl alcohol, producing in the former case canary-yellow needles or rosettes of needles and in the latter bronze-tinted, dense rosettes of prisms, both forms melting at 205° (Found: MeO, 10·0; NMe, 6·0. $C_{22}H_{29}O_5N_2{\cdot}CH_3,C_6H_3O_7N_3$ requires 2MeO, 9·6; NMe, 4·5%). These results agree with the assumption that in this reaction the akuammine is first hydrated by the alkali (see below) and that the hydrate undergoes *O*-methylation either on the hydroxyl group originally present in akuammine or on a new group formed in the hydration.

Acetylation of akuammine. The pure base (3·8 g.) was boiled for 5 minutes with acetic anhydride (10 c.c.), the solution left to cool, and the excess of reagent removed by distillation under reduced pressure. The residue was dissolved in water, the liquid poured into sodium carbonate solution, and the precipitate collected, sucked dry on the filter, and dissolved in the minimum quantity of boiling alcohol, from which it separated on cooling in rosettes of colourless prisms (yield, 2·4 g.), m. p. 226°. The melting point was not changed by recrystallisation. The concentrated mother-liquors, which had ceased to deposit crystals, were taken to dryness, the residue was dissolved in four times its weight of boiling *N*/2-hydrobromic acid, and the solution filtered hot. On cooling, it deposited a silky mass of hair-like needles, m. p. 236°, of *acetylakuammine hydrobromide* (yield, 1·1 g.), the melting point of which remained unchanged on recrystallisation. This hydrobromide on solution in boiling water and precipitation with sodium carbonate solution furnished the pure *base*, m. p. 226°. From the mother-liquors of the hydrobromide the rest of the acetylated base was precipitated as the *picrate* (0·5 g.), which crystallised with difficulty from concentrated solutions in methyl alcohol, in gelatinous masses of rosettes of minute, yellow needles, m. p. 168° after drying in air. The acetylated base becomes pink on exposure to air and has $[\alpha]_D^{20}$ $-52{\cdot}08°$ ($c = 0{\cdot}64$ in alcohol) (Found for the base: C, 67·5, 67·45; H, 6·9, 6·7; MeO, 9·3; NMe, 6·9; acetic acid formed on alkaline hydrolysis, 13·1. $C_{22}H_{27}O_4N_2{\cdot}CO{\cdot}CH_3$ requires C, 67·6; H, 7·1; MeO, 7·3; NMe, 6·8; acetic acid, 14·1%. Found for hydrobromide: C, 57·3, 57·1; H, 5·9, 6·0; Br, 15·95. $C_{22}H_{27}O_4N_2{\cdot}CO{\cdot}CH_3,HBr$ requires C, 56·8; H, 6·2; Br, 15·75%).

The base recovered from the acetic acid estimation appeared to be akuammine hydrate, m. p. above 300° (see below).

Action of alkalis on akuammine. Akuammine dissolves slowly in solutions of ammonia or alkali hydroxides, forming in the course of a few days dark brown liquids, which on neutralisation by acids deposit precipitates of a mixture of bases. For the examination of these products it has been found most convenient to treat akuammine with a known quantity of a normal solution of sodium ethoxide in alcohol, in which it dissolves immediately, keep the solution for several days, remove the alcohol, and treat the residue with water; the sodium derivatives presumably first formed and stable in alcohol are then almost completely dissociated, leaving a dark brown precipitate. The latter on digestion first with alcohol and then with chloroform is obtained as a nearly colourless powder, which does not sinter up to 300° and after heating to this temperature can usually be shaken out of the melting point tube as an orange-brown powder. The product is insoluble in all ordinary solvents, and is finally purified by dissolving it in a known quantity of sodium ethoxide in alcohol, adding sufficient alcohol to dilute the solution to a concentration of about 1 part in 5 parts, and then neutralising the filtered liquid with the calculated quantity of $N/2$-acetic acid; after a few minutes, practically the whole of the product crystallises in small, spheroidal granules of minute needles, which do not sinter or melt up to 310°. It dissolves immediately in solutions of ammonia or alkali hydroxides, but such solutions gelatinise when carbon dioxide is passed into them: the jelly can be filtered out and dries up to a brown powder having the characters of the original substance. The product appears to be an *akuammine hydrate* formed by addition of a molecule of water to akuammine (Found: C, 65·0, 65·1; H, 6·9, 6·8. $C_{22}H_{28}O_4N_2,H_2O$ requires C, 65·6; H, 7·5%). The yield in the above process has varied from 15 to 41% of the akuammine used. The by-products soluble in alcohol and chloroform are brown, amorphous substances soluble in alkalis, but they also form salts, *e.g.*, hydrobromides, which are sparingly soluble in cold water or saline aqueous solutions but very readily soluble in alcohol, acetone, or chloroform.

Base D.

The akuamma seeds used for this work consisted of a number of samples and from one of these the crude alkaloidal sulphates, prepared as described on p. 1953, on recrystallisation from water gave a fraction crystallising in colourless, transparent, flattened cubes. This *sulphate*, m. p. 161°, $[\alpha]_D^{14\cdot5°}$ $-594\cdot1°$ ($c = 0\cdot4226$ in water) or $-539\cdot8°$ ($c = 0\cdot2860$ in water), contains 8·9% of water

of crystallisation, of which 2·4% is lost in a vacuous desiccator at atmospheric temperature. The *base*, obtained by adding sodium carbonate solution to an aqueous solution of the sulphate, crystallises from hot alcohol in brilliant, colourless leaflets, m. p. 177·5°, $[\alpha]_D^{19°}$ $-737·5°$ ($c = 0·4932$ in alcohol), $-737·7°$ ($c = 0·3504$ in chloroform). The *nitrate* forms colourless needles, m. p. 180—181°. The *picrate* can be crystallised by dissolving it in boiling chloroform and adding an equal volume of boiling alcohol. From this mixture on cooling, it separates in dull yellow needles, m. p. 169°.

Base D gives a grass-green coloration when a mere trace is added to a drop of concentrated nitric acid. Like akuammine, it gives colours with aromatic aldehydes in presence of hydrochloric acid, *viz.*, indigo-blue with vanillin and magenta with piperonal, both changing to bright ultramarine-blue after a few days and remaining stable for at least 14 days. The alkaloid gives no coloration with Gaebel's test for methylenedioxy-groups.

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