Chemical Investigations of *Alangium lamarckii* I. Isolation of a New Alkaloid, Ankorine, from the Leaves

By B. DASGUPTA

A new crystalline phenolic alkaloid, Ankorine, m.p. 174–176°, has been isolated from the leaves of *Alangium lamarckii* Thwaites. Several salts of the alkaloid have been prepared; the alkaloid has been assigned the molecular formula, $C_{19}H_{29}O_4N$, with two OCH_3 groups, one phenolic OH group and possibly one furan ring.

ALANGIUM LAMARCKII Thw. (N.O. *Alangiaceae*) is a small tree distributed throughout India, Ceylon, S. China, Malaya, and the Philippines. It has various synonyms in India, *viz.*, Bengali—akarkanta, angkula, angkura, ankoda; Hindi—akhaul, akola, ankora, dhera; Sanskrit—ankola, ankota, etc. The plant has been used in the Indian indigenous systems of medicine from ancient times (1). The root, root-bark, and bark have been claimed to be useful as anthelmintic, emetic, diaphoretic, antipyretic, and purgative agents, particularly in the treatment of leprosy and skin diseases; the leaves are used as a poultice to relieve rheumatic pains. Different workers have claimed, from time to time, the isolation of amorphous and crystalline alkaloids from the root-bark and the seeds of this plant; some of the alkaloids have been found to be hypotensive and have interesting cardiovascular properties (2–13).

No chemical and pharmacological work has been reported on the leaves of this plant, which appear to contain a considerable amount of a mixture of alkaloids. However, preliminary pharmacological studies with the total alkaloidal extract indicate mild adrenolytic, nonspecific antispasmodic, hypotensive, and anticholinesterase activity of the alkaloids (14). The present report describes the isolation of a crystalline phenolic alkaloid, m.p. 174–176°, $[\alpha]_D^{20°} - 53.11°$ ($CHCl_3$) from the leaves of *A. lamarckii* Thw. (0.033% yield) by column chromatography of the chloroform soluble tertiary bases. The alkaloid is given the molecular formula $C_{19}H_{29}O_4N$ from the analysis of the base and its hydrochloride. Mass spectrum[1] of the alkaloid shows a molecular weight of 335, a confirmation of the above formula. The NMR spectrum of the alkaloid determined in deuterochloroform as solvent and tetramethylsilane as an internal standard indicates that the alkaloid contains (*a*) 29 protons (may be also 28), (*b*) no N—CH_3 group, (*c*) possibly no >NH group, (*d*) one exchangeable hydrogen [may be OH (τ 9.12, cycles 53)], (*e*) two O-Me groups to aromatic rings [may be based on furan ring (τ 6.17, cycles 230)], (*f*) one hydrogen in β-position to furan ring (τ 3.70, cycles 378), (*g*) a few aliphatic hydrogens (τ/cycles, 6.36/218, 6.95/183, 7.15/171, 7.33/160, 7.82/131, 8.18/109, 8.70/78), (*h*) one aliphatic CH_3 group, which may be angular between two six-membered fused rings (τ 9.05, cycles 57). The analysis of the alkaloid indicates the possible presence of one OCH_3 group and one NCH_3 group in the molecule; but the NMR spectrum rules out the possibility of one NCH_3 group. Instead, it confirms the presence of two OCH_3 groups. The ultraviolet absorption spectrum of the alkaloid, determined in ethanol solution (concentration, 0.20 mg./ml.; absorbance, 0.548) in a Cary spectrophotometer, shows $\lambda_{min.}$ at 253 mμ and $\lambda_{max.}$ at 272 mμ, with a shoulder at 273 mμ. This may be due to aromatic absorption of the furan ring. The infrared spectrum of the alkaloid, determined in chloroform solution in a Perkin-Elmer Infracord, shows absorption at 2.8, 3.4, 3.6 (s), 6.1, 6.25, 6.6, 6.8, 7 (s), 7.3, 7.5, 8.9, 9.2, and 9.9 μ. There is no carbonyl absorption in the I.R. spectrum. The alkaloid is freely soluble in 2% caustic potash solution and does not seem to have a methylenedioxy group. It does not have unsaturation in the molecule since it failed to absorb hydrogen in ethanol and glacial acetic acid solution over platinum oxide catalyst. Initial attempts to prepare the acetate and benzoate derivatives of the alkaloid in crystalline forms were unsuccessful. Several salts of the alkaloid —*viz.*, hydrochloride, hydrobromide, oxalate and sulfate—were prepared. No picrate could be prepared. The crystalline alkaloid (colorless shiny flakes) appears to be different from any of the alkaloids reported to have been isolated previously from the bark and seeds, and as such, is given the name ankorine. It is a one spot material on paper chromatogram. The alkaloid is sparingly soluble in benzene, moderately soluble in acetone, and freely soluble in chloroform and alcohol. Further work on the elucidation of the chemical structure of the alkaloid and its pharmacological studies are in progress.

EXPERIMENTAL

Extraction of Alkaloids from the Leaves of A. lamarckii.—Coarsely ground *A. lamarckii* (air-dried leaves, 2.5 Kg.) was extracted with rectified spirit by percolation at room temperature. The plant material was covered with fresh charges of solvent six times over a period of 10–12 days. Evaporation of the whole extract on a boiling waterbath left a semisolid residue. The residue was triturated with 2% hydrochloric acid with mechanical stirring. The acid solution was filtered, and the insoluble solid was extracted again with 2% hydrochloric acid as above. The combined acid extract (about 3.5 L.) was filtered, then made alkaline with ammonium hydroxide when a flocculent precipitate (A) was obtained. It was filtered under suction.

The filtrate (B) was extracted with chloroform three times. The chloroform extract yielded a brown pasty mass (C) on removal of solvent. The

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aqueous solution (D) gave a positive test with Mayer's reagent.

The precipitate (A) and the residue from the chloroform extract (C) were dissolved in 2% hydrochloric acid. The acid solution was filtered from the insoluble resin, then extracted twice with chloroform to remove a small quantity of resinous matter. It was then made alkaline with ammonium hydroxide and filtered under suction to separate the precipitate (E) from the alkaline solution (F).

The alkaline solution (F) was extracted with chloroform three times. The chloroform extract yielded a brown pasty mass (G, 1.562 Gm.) on removal of solvent. The remaining aqueous solution, which was still positive to Mayer's reagent, was mixed with solution (D) and extracted again three times with chloroform. The chloroform extract yielded a brown residue (H, 0.562 Gm.).

The precipitate (E) was dried on a porous plate kept in a vacuum desiccator. On drying, a deep brown powder (7.9 Gm.) was obtained. The powder was put in a thimble and extracted in a Soxhlet extractor with chloroform, then absolute alcohol. The chloroform extract yielded on evaporation a deep brown pasty mass (I, 3.064 Gm.). The alcoholic extract yielded a brown powder (J, 2.92 Gm.).

Isolation of the Alkaloid, Ankorine.—The chloroform soluble tertiary alkaloids (G, 1.562 Gm., and I, 3.064 Gm.) were mixed together and almost dissolved in benzene by repeated addition of benzene, warming, and decanting. The benzene solution was chromatographed over Brockmann aluminum oxide (80 Gm.), suspended in benzene in a column. Elution was continued with benzene, benzene–chloroform mixtures of varying proportions, chloroform, then chloroform–methanol mixtures. On removal of the solvent, fractions from benzene, benzene–chloroform mixtures, and chloroform eluants yielded a brown gummy mass with embedded solid in varying proportions. They were all combined with chloroform. The chloroform solution, on removal of solvent, yielded a brown gummy mass (2.630 Gm.). Chloroform–methanol fractions showed no sign of crystalline materials in the residues. The above gummy mass (2.630 Gm.) was dissolved in 2% hydrochloric acid (about 100 ml.), filtered from sticky gummy mass, and the brown acid filtrate was made alkaline with ammonium hydroxide when a precipitate was obtained. The solution with the precipitate was extracted with chloroform. The aqueous solution after extraction with chloroform was slightly positive to Mayer's reagent and was rejected. The chloroform extract was washed with water and dried over calcium chloride. On removal of chloroform, a brown residue (2.435 Gm.) was obtained; it was dissolved in benzene by repeatedly boiling with fresh quantities of benzene, cooling, and decanting. The total benzene solution was chromatographed over Brockmann aluminium oxide (20 Gm.), suspended in benzene in a column. Elution was continued with benzene, benzene–chloroform mixtures in varying proportions, chloroform, then chloroform containing increasing amounts of methanol. Residues from different eluants were combined on the basis of their paper chromatographic pattern and crystallized from a mixture of benzene and petroleum ether (40–60°) when crude ankorine (0.83 Gm.; yield, 0.033%) was obtained in the form of yellow solid, m.p. 160–168°. This was crystallized again from benzene, then from acetone. Recrystallization from acetone yielded colorless shiny flakes of ankorine (0.273 Gm.), m.p. 174–176°; $[\alpha]_D^{20°}$ −53.11° ($CHCl_3$).

Anal.—Calcd. for $C_{19}H_{29}NO_4$: C, 68.03; H, 8.71; N, 4.18; NCH_3(one), 8.66; OCH_3(one), 9.85; mol. wt., 335.43. Found: C, 68.64; H, 8.61; N, 4.04; NCH_3, 7.70; OCH_3, 11.60; mol. wt., 290 (Rast).

The alkaloid is a one spot material on paper chromatogram. Paper chromatography was conducted on Whatman No. 4 filter paper, which was treated with phosphate–citric acid buffer (pH 3.5) using as eluant the organic phase of a mixture of *n*-butanol–*n*-butyl acetate–water (25 : 10 : 10 by volume) with formic acid (1 by volume) to the separated organic layer. After drying, a chloroform solution of bromophenol blue was sprayed to detect the alkaloids. Ankorine is a phenolic alkaloid because it dissolves quickly and completely in 2% caustic potash solution. It gives a stable red (toward pink) color instantaneously with concentrated nitric acid, but no color with concentrated sulfuric acid. An alcoholic solution of the base gives no color change with ferric chloride solution. Ankorine gives a negative methylenedioxy test with concentrated sulfuric acid and gallic acid.

The brown residue (H, 0.562 Gm.) was dissolved in benzene and chromatographed on aluminium oxide, eluting with benzene, benzene–chloroform mixtures, chloroform, and ethanol. No crystalline compound could be isolated from any of the fractions.

The brown powder (J, 2.92 Gm.) is being worked out.

Preparation of Salts of Ankorine.—*Hydrochloride.*—To a solution of ankorine (50 mg.) in absolute alcohol (2–3 ml.) a few drops of absolute alcohol saturated with dry HCl gas were added until the solution was acidic. On addition of ether, clusters of plates separated. Crystallization from alcohol–ether mixture afforded colorless crystals of hydrochloride (0.0405 Gm.), m.p. 233–234°.

Anal.—Calcd. for $C_{19}H_{29}NO_4$, HCl, H_2O: C, 58.53; H, 8.27; N, 3.59. Found: C, 57.91, 57.98; H, 8.56, 8.72; N, 4.36.

Hydrobromide.—Hydrobromic acid (40%) was added dropwise to a solution of ankorine (50 mg.) in absolute alcohol (2–3 ml.) until the solution was acidic. Addition of ether to turbidity and standing yielded fine needles of hydrobromide (0.0463 Gm.), m.p. 220–222° dec.

Oxalate.—A saturated solution of oxalic acid in absolute alcohol was added dropwise to a solution of ankorine (50 mg.) in absolute alcohol (2–3 ml.). Addition of ether to turbidity and standing afforded plates of oxalate (0.0503 Gm.), m.p. 190–191° dec.

Sulfate.—To a solution of ankorine (50 mg.) in absolute alcohol (2–3 ml.) 1 microdrop of concentrated sulfuric acid was added with ice-cooling. Addition of ether to turbidity and standing yielded clusters of rods and needles of sulfate (0.0255 Gm.), m.p. 286° dec.

Picrate.—Picrate of the base could not be prepared either from (*a*) alcoholic solution of the base and alcoholic picric acid followed by ether or from (*b*) aqueous HCl solution of the base and aqueous picric acid.

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Preparation of Digilanide-A

By OLE GISVOLD and AHYAN ULUBELEN

A method for the convenient isolation of digilanide-A from *Digitalis mertonensis* is described.

PREVIOUS INVESTIGATIONS (1) on the isolation of acetyl digitoxin from *Digitalis mertonensis* revealed that it was the chief desglucoglycoside. Preliminary studies also revealed that the precursor to acetyl digitoxin was digilanide-A. Because this species of digitalis appeared to contain such minor quantities of other glycosides, it suggested the opportunity to use this plant as a possible convenient source of digilanide-A. The experience previously gained in the isolation of the desglucoglycosides was utilized to develop effective methods for the isolation of digilanide-A. Although alcohol and methanol in high concentrations effectively inhibit the enzyme that cleaves the terminal glucose residue of many of the native cardioactive glycosides, they also extract large amounts of chlorophyll and its degradation products. These pigments introduce problems in the subsequent isolation of the native glycosides. Although concentrations of methanol less than 60% have significant inhibitory activity on the glycosidase, concentrations from 60 to 70% are more effective without undue solubilization of chlorophyll. This is true of both the dried and fresh leaves. The pH of aqueous or low hydroalcoholic extracts of fresh leaves of *D. mertonensis* is about 6, and the glycosidase activity is rapid and complete. It also has been shown (2) that this glycosidase is active at a pH of 1. During the drying of the leaves of the various species of digitalis, minimal glycosidase activity is obtained when the leaves are dried rapidly at about 50°. Even on a small scale, it is doubtful that complete glycosidase activity has been arrested. Larger-scale operations prove considerably less effective.

It will be noted under *Experimental* that in the latter steps of the isolation of digilanide-A anhydrous ether was used to extract this glycoside from its dispersion on filter cell together with other substances. In view of the published solubilities of digilanide-A, one might not expect anhydrous ether to dissolve significant quantities of this glycoside, even though it was dispersed on filter cell. Nevertheless, the rate of dissolution is sufficiently great to use this solvent effectively, especially by continued extraction in a Soxhlet extractor. The low boiling point of ether precludes significant decomposition of the glycoside.

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EXPERIMENTAL

The details of the paper chromatographic techniques used in these studies have been described previously (3). Solvent system II was used most extensively for the development of the paper chromatograms. The ascending technique was satisfactory for rapid preliminary screening studies. Decending techniques gave more effective resolution where such was desired. The Raymond reagent was used most extensively to detect the glycosides. Certain flavones gave a yellow color with this reagent. Some unknown impurities gave a brown spot, and still others gave a pink color. The latter might be the digitenolides (4) that are related to diginin, etc., and are physiologically inactive.

Preparation of Digilanide-A

From Dried D. mertonensis.—The leaves of *D. mertonensis* collected in the fall of 1962 were rapidly dried at 50° with the aid of a fan and subsequently powdered and stored in well-sealed glass containers. In preparing a primary extract in which enzymatic activity was inhibited it was shown that 60 to 70% aqueous methanol proved to be quite suitable. The powdered leaves, 400 Gm., were macerated overnight with 1 L. of 65% methanol. The next day they were packed in a percolator, percolated slowly with 65% methanol, and 1300 ml. of precolate was collected. The methanol concentration was reduced to 33% by means of vacuum distillation and the resultant preparation extracted twice with 125 to 150 ml. each of methylisoamyl