PAKISTAN JOURNAL
OF
SCIENTIFIC AND INDUSTRIAL RESEARCH

Vol. 8, No. 4 October 1965

CHEMICAL EXAMINATION OF ROOT BARK OF ALANGIUM LAMARCKII THWAITES

SALIMUZZAMAN SIDDIQUI, M. AMJAD ALI
AND VIQAR UDDIN AHMAD

Drugs and Pharmaceutical Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received January 13, 1965)

From the root bark of the plant two new alkaloids, marckine, $C_{28}H_{35}O_3N_3$, and marckidine, $C_{28}H_{35}O_3N_3$, have been isolated. The former has been characterised through the preparation of its several salts and drivatives; the absorption spectra of both the alkaloids have also been studied. From the same source a new sterol, alangiosterol, $C_{29}H_{48}O$, has also been isolated, and characterised through its derivatives.

Alangium lamarckii Thwaites, an important medicinal plant of the Indo-Pakistan subcontinent, has been subjected to chemical examination by various groups of workers, and the isolation of several alkaloids and sterols [1–7] from it has been reported. More recently, the alkaloid alamarckine, isolated by Subbaratnam and Siddiqui [6] from the seed kernels of this plant after methylation of the total base, has been identified as N-methyl cephaeline by Pakrashi and coworkers [8] who have also reported the isolation of cephaeline, emetine and psychoterine from both seeds[8] and root bark [9] of the plant.

Working on the fresh undried root bark of *Alangium lamarckii*, the present authors succeeded in the isolation of a new alkaloid marckine which has been reported earlier in a short communication.[10] In the present paper, the method of isolation of marckine and its physical and chemical properties are described in detail. It further deals with the isolation of another alkaloid, closely related to marckine and provisionally named as marckidine, as well as of a new sterol which does not appear to have been recorded in literature.

According to the procedure described in detail in the experimental, the two bases marckine and marckidine were isolated from the non-phenolic fraction of the total alkaloids obtained from the root bark on removal of the solvent from its alcoholic percolates and thereafter liberating the base from the water-soluble portion of the residue through sodium carbonate. The major base, namely marckine (yield, 0.25 percent on dry weight basis), forms prismatic rods, m.p. 281°, $[\alpha]_D^{22} = -68°$ (c=3, pyridine), pKa=7.3 (aqueous dimethyl formamide), analyses for $C_{28}H_{35}O_3N_3$, and contains two methoxyls and one C-methyl but no N-methyl group.

The IR spectrum of marckine (Fig. 1) shows a strong peak of NH at 3380 cm^{-1} but no hydroxyl or carbonyl absorption. In the UV spectrum there are maxima at 226 mμ (log ε, 4.47) and 281 mμ (log ε, 4.05) and a minimum at 252 mμ (log ε, 3.50) which is typical of unconjugated indole alkaloids. The conclusion that marckine is an indole alkaloid is also supported by the colour reactions, discussed in the experimental, and from the results of the degradative experiments which will be published in a subsequent communication. On potentiometric titration of marckine with dilute hydrochloric acid, a sharp fall of pH is obtained only when two moles of the acid have been consumed following which the pH of the solution remains almost constant. This indicates that out of the three nitrogen atoms of the alkaloid, two are basic and of equal strength. Moreover, marckine forms a crystalline dipicrate, m.p. 192° (decomp.) and a monochloroplatinate. Other salts of marckine have not so far been obtained in a crystalline state. On acetylation, marckine yields a monoacetyl derivative, $C_{30}H_{37}N_3O_4$, m.p. 225-8° (decomp.) which is a monoacidic base, as evidenced by potentiometric titration and the formation of a monopicrate, m.p. 224-5° (decomp.). This shows that in acetyl marckine one of the basic groups of marckine has been acetylated. Acetyl marckine still shows the NH

peak in the infra-red spectrum, though in a considerably reduced intensity. With methyl iodide, marckine yields dimethyl marckine dimethiodide, $C_{28}H_{33}N_3O_3$ $(CH_3)_2$ $(CH_3I)_2$, H_2O, m.p. 270° (decomp.) while acetyl marckine gives, with the same reagent, acetyl methyl marckine methiodide, $C_{30}H_{36}N_3O_4$ (CH_3) (CH_3I), H_2O, m.p. 265° (decomp.).

The minor base marckidine was isolated from the ethyl acetate mother-liquors of marckine through careful fractional crystallisations. This base also analyses for the molecular formula, $C_{28}H_{35}O_3N_3$, but, in contrast to marckine, it contains two N-methyl and two C-methyl groups. The IR spectra of the two alkaloids (Figs. 1 and 2) are similar but not identical, and moreover, marckidine is more eardily soluble in organic solvents than marckine. The potentiometric titration curve of marckidine is different from that of marckine, because the former shows two turning points at the points of consumption of one and two moles of the acid (pKa 7.9 and 7.15; aqueous dimethyl formamide) indicating that it is also a diacidic base but, unlike marckine, the two basic centres in it are distinctly different in their strength.

Both marckine and marckidine appear to be new bases as none of the alkaloids so far obtained from this plant has been found to contain three nitrogen atoms. Moreover, the characteristics of marckine and marckidine do not compare with those of any of the alkaloids reported in literature.

The non-alkaloidal fatty portion of the alcoholic extractive of the root bark was saponified and the unsaponifiable fraction, which seemed to contain a mixture of sterols, ultimately yielded, on several recrystallisation from petroleum ether, a pure sterol, m.p. 162°, $[\alpha]_D^{25} = -58°$ (c=1, chloroform). This sterol appears to be new and has been provisionally named as alangiosterol.

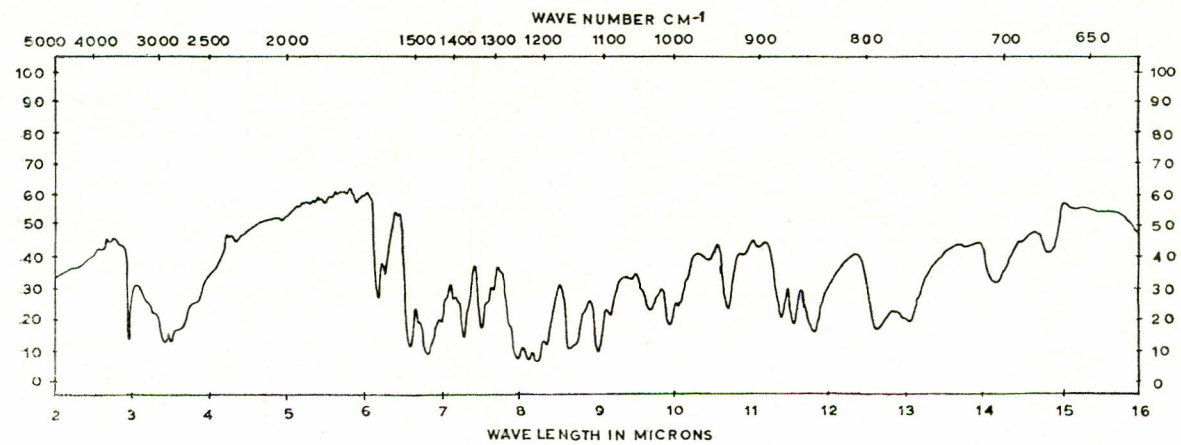

Fig. 1.—Infra-red spectrum of marckine in KBr.

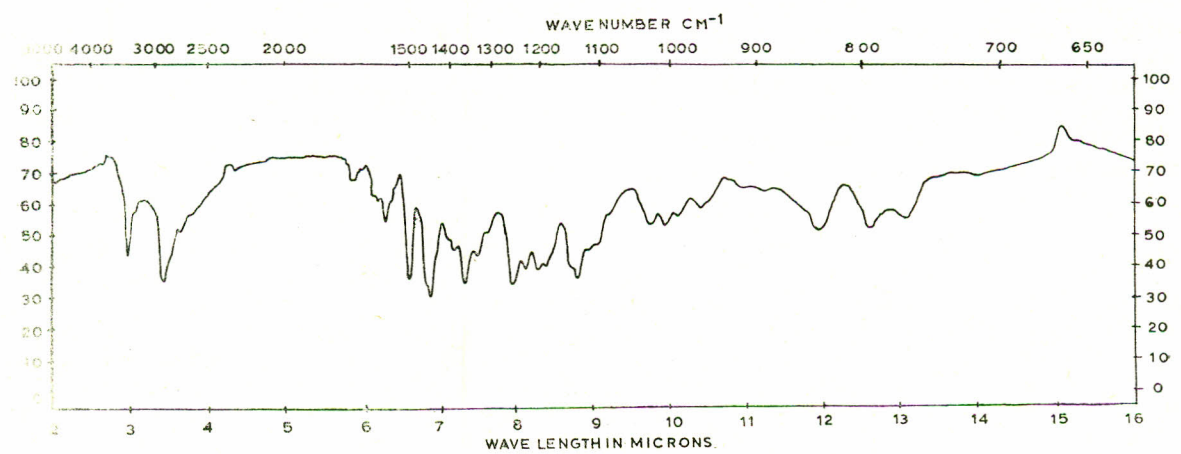

Fig. 2.—Infra-red spectrum of marckidine in KBr.

It analyses for the molecular formula, $C_{29}H_{48}O$, and gives monoacetyl ($C_{31}H_{50}O_2$, m. p. 140°), monobenzoyl ($C_{36}H_{52}O_2$, m. p. 159-160°) and phenylurethane drivatives ($C_{36}H_{53}O_2N$, m. p. 178°) showing the presence of a hydroxyl group. Catalytic microhydrogenation indicates the presence of two double bonds in it. With bromine in glacial acetic acid or chloroform, the sterol produces a green colour (Tortelli-Jaffe test) which may be due to the presence or formation by isomerisation of a double bond between two tertiary carbon atoms in a bridge head.[11]

Further studies in the constitution of marckine are in progress and the results will be communicated in a subsequent paper.

Experimental

Isolation and Characterisation of Marckine

17 kg. of fresh root bark of *Alangium lamarckii* Thwaites (equivalent to 5.9 kg. dry weight) obtained from Dacca, East Pakistan, was chopped into small pieces and percolated six times with alcohol at room temperature. The solvent from the combined reddish percolate was removed under reduced pressure below 40°.* The dark red viscous residue was taken up in water and shaken out exhaustively with ethyl acetate. The ethyl acetate extracts were worked up for the isolation of sterol, and a small quantity of darkish insoluble material at the interface was cottoned off. The red coloured aqueous solution was freed of the dissolved organic solvent and then basified with a 10% solution of sodium carbonate. The voluminous brick-red precipitate was filtered under suction and the filtrate shaken out with ethyl acetate. The aqueous layer gave a positive test with Dragendorff reagent even after repeatedly shaking out with ethyl acetate but, for the time, it was not pursued further. The undried precipitate was exhaustively extracted with large quantities of hot ethyl acetate. The insoluble residue was dissolved in dilute acetic acid, charcoa'ed and filtered, and the base re-precipitated from the filtrate with sodium carbonate was again shaken out with ethyl acetate.

The combined ethyl acetate extract (10 litres) was washed once with water, concentrated to about one third of its volume and shaken out with 1%, 2% and finally with 5% sodium hydroxide solutions. The alkali soluble phenolic bases recovered from the aqueous layers were set aside for subsequent work. The ethyl acetate solution of the alkali insoluble, non-phenolic bases, was repeatedly washed with water, dried over anhydrous sodium sulphate and filtered. On concentration of the filtrate to a small volume, a sizeable quantity of the crystallisate was obtained which increased on keeping in the cold. A further crop of crystals was obtained on concentrating the filtrate, making for a total yield of 15 g. (0.25% on dry weight). On repeated crystallisation from a mixture of methanol and benzene (1:2) and finally from methanol alone, the crystallisate which initially melted at 270° (decomp.) finally gave aggregates of prismatic rods and elongated plates showing m.p. 281° (decomp.) † $-[\alpha]_D^{22}=-68°$ (c=3, pyridine), pKa=7.3 (aqueous dimethyl formamide). Found after drying at 100° *in vacuo*: C, 73.08, 72.83; H, 8.01, 8.15; N, 8.90, 8.93; O, 10.54, 10.56; OCH_3, 13.35, 13.32; C-CH_3, 2.70, 2.59%; Mol. Wt. 473, 461. Calculated for $C_{28}H_{35}O_3N_3$: C, 72.85; H, 7.64; O, 10.40; N, 9.10; $2\times OCH_3$, 13.44; $1\times$C-CH_3, 3.25%; Mol. Wt. 461.

The base, thus obtained and named as marckine, is fairly soluble in 1:2 mixture of methanol-benzene, moderately soluble in pyridine, sparingly in ethanol and methanol, and in the crystalline state it is nearly insoluble in other common organic solvents. It is insoluble in dilute alkali and gives no colour with alcoholic ferric chloride.

Colour Reactions of Marckine

	Reagent	*Colour*
1.	Concentrated H_2SO_4	Golden yellow
2.	Concentrated HNO_3	Dull reddish violet
3.	Concentrated $H_2SO_4+MnO_2$	Light to deep blue
4.	Concentrated $H_2SO_4+K_2Cr_2O_7$	Deep greenish blue
5.	Kellers reagent	Violet
6.	Marquis reagent	Blue changing rapidly to dirty red
7.	Ceric sulphate in 10% H_2SO_4	Violet
8.	Ehrlich reagent in concentrated H_2SO_4	Green
9.	Nitrous acid	Red
10.	Frohde's reagent	Deep blue slowly changing to green

Marckine Picrate.—A saturated aqueous solution of picric acid was added to a solution of marckine (0.10 g.) in dilute acetic acid. The yellow precipitate was filtered, washed well with water and redissolved in water with the help of a little alcohol. On slow evaporation, it was obtained as aggre-

*In all subsequent operations, the removal of solvents was done under these conditions unless otherwise stated.

†All melting points are uncorrected.

gates of orange-red spike shaped crystals, m.p. 192° (decomp.), 0.10 g. Found after drying at 100° *in vacuo*: C, 52.00; H, 4.75; N, 14.15; O, 30.04%. $C_{28}H_{35}O_3N_3$, 2 $C_6H_3N_3O_7$ requires: C, 52.23; H, 4.45; N, 13.71; O, 29.59%.

Marckine Chloroplatinate.—A 3% aqueous solution of chloroplatinic acid was added to a solution of marckine (0.05 g.) in dilute acetic acid. The yellowish orange precipitate was filtered, washed well with water and dried over silica gel. Found after drying at 100° *in vacuo*: C, 38.93; H, 4.52; N, 4.80; Pt., 21.49%. Calculated for $C_{28}H_{35}O_3N_3$, H_2PtCl_6: C, 38.57; H, 4.25; N, 4.82; Pt., 22.39%. $C_{29}H_{37}O_3N_3$, H_2 $PtCl_6$ requires: C, 39.31; H, 4.17; N, 4.72; Pt., 22.05%.*

Monoacetyl Marckine.—Marckine (0.5 g.) was dissolved in anhydrous pyridine (10 ml.), freshly distilled acetic anhydride (5 ml.) was added to it and the mixture left overnight at room temperature. The solvent was then removed *in vacuo* and the residue taken up in a small quantity of water, cooled and basified with ammonia whereby a thick precipitate was obtained. The precipitate was extracted with ethyl acetate and the extract washed with water, dried (Na_2SO_4), filtered and freed of the solvent and adhering pyridine by distilling repeatedly with alcohol under reduced pressure. It was then dissolved in a little benzene, chromatographed over a column of alumina (Brockmann, activity 1, 30 g.) and eluted with benzene-methanol (99:1). The middle fractions crystallised from dilute alcohol in the form of long needles (0.3 g.), m.p., 225-8° (decomp.) with previous sintering. Found after drying at 100° *in vacuo:* C, 71.93; H, 7.37; O, 12.40; OCH_3, 11.92%. Calculated for $C_{30}H_{37}O_4N_3$: C, 71.54; H, 7.41; N, 8.34; O, 12.71; OCH_3, 12.32%.

Monoacetyl Marckine Picrate.—Monoacetyl marckine (50 mg.) was dissolved in ether (15 ml.) and treated with an ethereal solution of picric acid. The yellow microcrystalline precipitate of monoacetyl marckine picrate was filtered, repeatedly washed with ether and dried, when it melted with decomposition at 224-5°. Found after drying at 60° *in vacuo:* C, 58.79; H, 5.72; N, 11.80; O, 23.72%. Calculated for $C_{30}H_{37}O_4N_3$, $C_6H_3N_3O_7$: C, 59.01; H, 5.46; N, 11.47; O, 24.04%.

Dimethyl Marckine Dimethiodide.—Marckine (0.10 g.) was suspended in methyl iodide, (0.5 ml.) and methanol was added drop by drop with vigorous shaking until the base just went into solution. The solution was kept at room temperature for some time when an oil began to separate which slowly crystallised. When recrystallised from methanol, it formed rectangular prisms (60 mg.), m.p., 270° (decomp.). Found after drying at 60° *in vacuo*: C, 48.57; H, 6.33; N, 5.17; O, 8.05; I, 31.98; OCH_3, 7.42%. $C_{32}H_{47}O_4N_3I_2$ ($C_{28}H_{33}O_3N_3$ $(CH_3)_2$ $(CH_3I)_2$, H_2O) requires: C, 48.54; H, 5.94; N, 5.30; O, 8.09; I, 32.11; $2\times OCH_3$, 7.83%.

Acetyl Methyl Marckine Methiodide.—Acetyl marckine (0.05 g.) was dissolved in dry chloroform (1 ml.) and methyl iodide (0.5 ml.) was added to it. A colourless microcrystalline precipitate appeared gradually. After keeping for an hour at room temperature, the solvent was completely removed. The residue (0.06 g.) crystallised from methanol in the form of prismatic rods, m.p. 270° (decomp.). Found after drying at 60° *in vacuo:* C, 56.58; H, 6.54; N, 6.34; O, 12.02; I, 18.63; OCH_3, 9.05%. Calculated for $C_{32}H_{44}O_5N_3I$ [$C_{30}H_{36}O_4N_3$ (CH_3) (CH_3I),H_2O]: C, 56.72; H, 6.49; N, 6.20; O, 11.81; I, 18.75; $2\times OCH_3$, 9.15%.

ISOLATION OF MARCKIDINE

The ethyl acetate mother-liquors of marckine deposited a microcrystalline precipitate on standing for several days which, on repeated crystallisation from moist ethyl acetate, finally gave the base, marckidine, in cluster of rods, m.p. 228°, $[\alpha]_D^{25}$ =-84° (pyridine). Found after drying at 100° *in vacuo:* C, 72.93, 73.07; H, 7.97, 7.92; N, 8.80, 8.93; O, 10.44, 10.43; OCH_3, 13.83; N-CH_3, 7.02; C-CH_3, 6.12%. Calculated for $C_{28}H_{35}O_3N_3$: C, 72.85; H, 7.64; N, 9.10; O, 10.40; $2\times OCH_3$, 13.44; $2\times NCH_3$, 6.46; C-CH_3, 6.46%. The base is comparatively much more soluble than marckine in common organic solvents but like the latter it is insoluble in dilute sodium hydroxide solution and gives no colour with alcoholic ferric chloride.

ISOLATION AND CHARACTERISATION OF ALANGIOSTEROL

The ethyl acetate extract containing the fatty portion, referred to at the outset, was completely freed of the solvent and the residue extracted with petroleum ether. The petroleum ether-soluble portion was saponified by refluxing with 10 percent sodium hydroxide in 70 percent alcohol for four hours. The reaction mixture was freed of the solvent and the residue was taken up in water and exhaustively extracted out with ether. The ethereal extract was

*The analyses of the chloroplatinate would seem to favour the $C_{29}H_{37}O_3N_3$ formulation for marckine.

washed with water, dried over anhydrous sodium sulphate, filtered and freed of the solvent. The dark coloured crystalline residue was crystallised twice from alcohol, when a colourless mixture of sterols melting around 145° was obtained (yield 5 g.). On repeated fractional crystallisation from petroleum ether (b.p. 60-80°), alangiosterol was obtained in fine needles, m.p. 162° (yield 0.5 g.), the melting point remaining constant on further crystallisations from the same solvent.

Alangiosterol has $[\alpha]_D^{25}$ =-58° (c=1, chloroform) and analyses for the formula, $C_{29}H_{48}O$. Found after drying at 100°: C, 84.28; H, 12.08; O, 4.38%; Mol. Wt., 412; H_2 absorption/mole, 1.93, 1.83 moles. $C_{29}H_{48}O$ requires: C, 84.40; H, 11.72; O, 3.88%; Mol. Wt., 412.

Acetylation of Alangiosterol.—To a solution of alangiosterol (100 mg.) in dry pyridine (2 ml.), acetic anhydride (1 ml.) was added and the mixture left overnight at room temperature. On working up the reaction product in the usual manner, the acetate was obtained through crystallisation from alcohol in plates, m.p. 140°. Found after drying at 100° *in vacuo*: C, 81.67; H, 10.97; O, 7.36%. $C_{31}H_{50}O_2$ requires: C, 81.88; H, 11.08; O, 7.04%.

Benzoylation of Alangiosterol.—Alangiosterol (100 mg.) was dissolved in pyridine (2 ml.), and after the addition of benzoyl chloride (1 ml.) the mixture was left overnight at room temperature. The reaction mixture was poured into ice, stirred well and then extracted with ether. The ethereal layer was washed with 5 percent HCl, water, 5 percent ammonia and then again with water. On removal of the solvent, a crystalline residue was obtained which, on recrystallisation from ethanol, gave rectangular plates of the benzoate, m.p. 159-60°. Found after drying at 100° *in vacuo*: C, 83.94; H, 10.09; O, 6.00%. $C_{36}H_{52}O_2$ requires: C, 83.66; H, 10.14; O, 6.19%.

Phenylurethane of Alangiosterol.—Alangiosterol (100 mg.) was dissolved in dry benzene (5 ml.), and phenyl isocyanate (0.5 ml.) was added to it. The mixture was left for a week at room temperature and the supernatant liquid decanted from a small quantity of the carbanilide formed. The residue left on careful removal of the solvent from the decantate, was extracted with hot petroleum ether, rejecting the insoluble carbanilide. The petroleum ether extract, on concentration, yielded the phenylurethane of alangiosterol in a crystalline form. On recrystallisation from petroleum ether (b.p. 60-80°), it gave needles, m.p. 178°. Found after drying at 100° *in vacuo*: C, 81.38; H, 10.07; N, 2.62; O, 6.11%. $C_{36}H_{53}O_2N$ requires: C, 81.30; H, 10.03; N, 2.63; O, 6.02%.

Acknowledgement.—The microanalyses were carried out by Dr. A. Bernhardt, 433, Mulheim (Ruhr), W. Germany, and by the Microanalytical Section of the Central Laboratories, P.C.S.I.R. The authors wish to express their sincere thanks to Dr. S. Hedayetullah, East Regional Laboratories, P.C.S.I.R., Dacca, for the supply of the drug.

References

1. A. Lakshminarasimhaiah, B. L. Manjunath and B.S. Nagaraj, J. Mysore University, Section B, **3,** 113 (1942); C. A., **37,** (1439).
2. P.N. Bhargava and S. Dutt, Proc. Indian Acad. Sci., **16A,** 328 (1942).
3. D.B. Parihar and S. Dutt, Proc. Indian Acad. Sci., **23A,** 325 (1946).
4. N.K. Basu, N.S. Nair and N.N. Bhattacharya, Indian J. Pharm., **12,** 93 (1950).
5. M.P. Singh and J.D. Tewari, Proc. Natl. Acad. Sci., India, **17A,** 1 (1948).
6. A.V. Subbaratnam and S. Siddiqui, J. Sci. Ind. Res. (India), **15B,** 432 (1956).
7. D.S. Bhakuni, M.M. Dhar and M.L. Dhar, J. Sci Ind. Res. (India), **19B,** 8 (1960).
8. H. Budzikiewicz, S.C. Pakrashi and H. Vorbruggen, Tetrahedron, **20,** 399 (1964).
9. S.C. Pakrashi and P.P. Ghosh-Dastidar, Indian J. Chem., **2,** 379 (1964).
10. S. Siddiqui, M.A. Ali and V.U. Ahmad, Pakistan J. Sci. Ind. Res., **7,** 144 (1964).
11. E.H. Rodd, *Chemistry of Carbon Compounds* (Elsevier Publishing Co., Amsterdam), Vol. II B, p. 836.