THE PHYTOCHEMISTRY OF THE BARK OF *TABERNAEMONTANA CORONARIA* BR.

By A. N. RATNAGIRISWARAN and K. VENKATACHALAM

(*From the Medical College, Madras*)

Received 14th May, 1939

In the search for an Indian source of a cardiac drug a preliminary examination was made of some plants of the natural order Apocynaceae, a family that is known to comprise the largest number of plants characterised by a high content of cardiac glycosides. The observation was made that all parts of the indigenous plant *Tabernaemontana coronaria* contained, instead of glycosides, alkaloids which, nevertheless, were active in showing a digitalis-like action on a frog's heart. The root bark was the richest source of total alkaloids; the stem bark, flowers and leaves contained smaller amounts of the alkaloids in the order mentioned.

Tabernaemontana coronaria is an evergreen, glabrous, shrub attaining a height of 6 to 8 feet. It is mostly cultivated in gardens on account of the beautiful white flowers it bears. In the wild state it is met with in the forests adjoining the Western Ghats, and the Bellary and Vizagapatam districts of the Madras Presidency. Some of the local vernacular names for the plant are:—Tamil-Nandiavattam, Telugu-Nandivardhana, Malayalam-Nandiarvattam, Canarese-Nandibatta.

The root is used as a local anodyne and is chewed for relieving toothache. Rubbed with water into a thin paste it is administered as an anthelmintic. The milky juice of the flowers and tender stems are applied to the eye in inflammation of the cornea.

The root of the plant was examined by Pillay[1] and found to contain, besides fatty acids and phytosterols, some easily oxidisable amorphous bases. Analysis showed that the different parts of the plant contained the following percentages of total alkaloids calculated on the weight of the air-dried material: root bark 1·28, stem bark 0·90, flowers 0·35, leaves 0·33. A colourless crystalline alkaloid, m.pt. 208°–209° C. (decomp.), which, in accordance with usual practice, has been named tabernaemontanine, and a yellow crystalline alkaloid, m.pt. 196°–198° C. (decomp.) for which the name coronarine is proposed, have been isolated from the fraction of the total alkaloids soluble in light petroleum (b.pt. 40° to

60° C.). Considerable difficulty was experienced in separating the alkaloids on account of their sensitiveness to heat and light.

The portion of the total alkaloids that remained undissolved in the light petroleum was a buff-coloured powder decomposing to a dark liquid at about 255° C. from which it has not been possible yet to obtain any crystalline substance. Further attempts in this direction are in progress.

Tabernaemontanine was obtained as colourless, prismatic needles by dissolving the dried amorphous base, precipitated by ammonia, in methyl alcohol and allowing the solvent to evaporate spontaneously. The alkaloid was very sensitive to heat and light and this might have been the reason for the very poor yield obtained. Only 0·05 gm. of the crystalline alkaloid could be isolated by working up 17 kgm. of the bark, and with this amount no detailed examination was possible. The quantity available was sent for microanalysis to Dr. Ing. A. Schoeller, of Berlin, and the results reported by him correspond to the formula $C_{20}H_{26}N_2O_3$, which should, however, be taken only tentatively until more of the substance is isolated for a confirmatory analysis. Unfortunately, this has not been possible of accomplishment owing to difficulties encountered in procuring a supply of the bark.

Coronarine was obtained as thin, yellowish plates, from a solution of the substance in methyl alcohol by the careful addition of warm water until turbidity was just produced. Analysis of the dried substance by Dr. Schoeller gave results that conformed to the formula $C_{44}H_{56}N_4O_6$. The alkaloid gave a characteristic greenish fluorescence in solutions in alcohol, ether and chloroform.

The colour reactions given by the alkaloids are described in a later section.

The alkaloids showed a definite slowing of the rate and an increase in the amplitude of the beats when applied to a frog's heart *in situ*. Details of the work will form the subject of a separate communication.

The other constituents identified in the alcoholic extract of the bark were: fatty matter, yielding on saponification palmitic, cerotic and oleic acids, resin acids which were not subjected to any detailed examination, a colourless, crystalline substance which appeared to be a resin alcohol with the molecular formula $C_{17}H_{32}O_4$ and m.pt. 180°–181° C., caoutchouc, resins, sugars and potassium salts.

EXPERIMENTAL

The material for the investigation was an authentic sample of the bark of the stem and root which was supplied by Mr. S. N. De, Calcutta, to whom our thanks are due.

Alkaloid.—Examination for alkaloid by extraction with Prollius's fluid gave marked reactions with the usual reagents.

Volatile substances.—500 gm. of the powdered bark was extracted with alcohol (95 per cent.) in a Soxhlet apparatus and the residue left after completely driving off the solvent was subjected to steam distillation. The distillate, which was acid to litmus, was shaken up with ether. The residue from ether was practically negligible in amount and had no characteristic odour. The aqueous liquid which had been shaken with ether was warmed to drive off the dissolved ether, neutralised with barium hydroxide and the acid converted into the silver salt. Analysis of the silver salt showed that the acid in the steam distillate was probably acetic acid. Ag found: 63·8 per cent.; CH_3COOAg requires: Ag : 64·66 per cent.

Preliminary Examination

To ascertain the amounts of material extractable by different solvents, 20 gm. of the powdered bark was extracted in a Soxhlet apparatus with the solvents mentioned below in succession, the residues dried at 105° C. and weighed.

Petroleum ether (b.pt. 40° to 60° C.)	extracted	2·45 per cent.
Ether	,,	2·40 ,,
Chloroform	,,	0·05 ,,
Dehydrated alcohol	,,	3·50 ,,
Alcohol (90 per cent.)	,,	2·50 ,,

For the purpose of a systematic examination, 600 gm. of the disintegrated bark was extracted in a continuous extraction apparatus with different solvents in succession and the residues obtained from each of them examined separately.

The *petroleum ether extract* contained alkaloid, fatty matter, a crystalline substance which appeared to be a resin alcohol, and caoutchouc. The *ether extract* was a dark brown resinous mass and contained alkaloid and resins. The *chloroform extract* also contained alkaloid and dark coloured resins insoluble in caustic alkalis. The *alcoholic extract* was found to contain alkaloid, resin, a trace of saponin, sugars, potassium nitrate and potassium chloride.

For a detailed investigation, 17 kgm. of the powdered bark was extracted with alcohol (90 per cent.) and the solvent evaporated under reduced pressure at a temperature not exceeding 60° C. The residue (1340 gm.) was repeatedly shaken with a 1 per cent. aqueous solution of hydrogen chloride in a mechanical shaker till the acid extract gave no reaction for alkaloid. The

insoluble resinous mass (A) was filtered under suction from the aqueous acid solution (B), washed free from acid and dried.

Examination of the Resinous Mass (A)

The dried mass was boiled under a reflux condenser with an excess of 2N alcoholic potassium hydroxide for about six hours and then some of the alcohol was distilled off. The solution was cooled and diluted with twice its volume of water. The alkaline solution was repeatedly shaken with ether to remove all the unsaponifiable matter. The ether extract was carefully washed with distilled water to remove the alkali, dried over anhydrous sodium sulphate and the ether was distilled off.

Isolation of a resin alcohol.—The residue from ether was crystallised several times from alcohol, and finally from ethyl acetate, when glistening needles, m.pt. 180° to 181° C. were obtained.

Found C: 68·63, 68·45 per cent.; H: 10·35, 10·50 per cent.; mol.wt. 279·8, 278·6. $C_{17}H_{32}O_4$ requires C; 68·00: H; 10·67; mol.wt. 300.

$[\alpha]_D^{28°C}$, 87·2 (0·69 per cent. solution in benzene) and 82·87 (2·24 per cent. solution in chloroform).

The benzoyl derivative was prepared by the action of benzoyl chloride on a solution of the substance in pyridine, and on being crystallised first from pentane and then from ligroin melted at 192° to 194° C. The acetyl derivative was obtained by heating the substance under a reflux condenser with acetic anhydride and fused sodium acetate and after crystallisation from alcohol (95 per cent.) melted at 193° to 195° C.

With acetic anhydride and concentrated sulphuric acid, and also in the Liebermann–Burchard reaction, the resin alcohol gave a beautiful violet colour which was stable and changed into green only on standing for more than ten hours. It dissolved in concentrated sulphuric acid and gave a lemon-yellow solution. In Hesse's reaction the chloroform layer was light yellow in colour and the sulphuric acid layer orange coloured. The substance did not give a precipitate with an alcoholic solution of digitonin.

Examination of the alkaline solution for acids.—The alkaline solution after removal of the unsaponifiable matter was acidified, when a bulky precipitate was thrown down. This mixture of fatty and resin acids was filtered, washed well, and dried. The dry powder was then thoroughly extracted with light petroleum to dissolve out the fatty acids. The resin acids left undissolved were not subjected to further examination.

Saturated fatty acids.—The fatty acids were separated in the usual manner by Twitchell's method. After repeated crystallisation from alcohol an acid m.pt. 76° to 77° C. having a mean mol.wt. of 396·5, and another acid m.pt. 62° C. and of a mean mol.wt. of 257·8, were obtained. These would appear to be dentical with cerotic and palmitic acids respectively.

Unsaturated fatty acids.—The residue of the crude acids was oxidised with alkaline potassium permanganate in the usual manner. The oxidised product that was obtained melted at 135° C. and had a mean mol.wt. of 316·4. The substance was evidently dihydroxystearic acid and the original acid should, therefore, have been oleic acid.

Examination of the aqueous solution (*B*).—The solution was concentrated under reduced pressure to about 2 litres and repeatedly shaken up with ether and chloroform. The residues from these solutions were dark coloured resins from which no crystalline substance could be isolated. To the acid solution sufficient solution of ammonia was added to make it just faintly acid, and the slimy precipitate was filtered off. The filtrate was made alkaline with a 20 per cent. solution of ammonia and shaken with chloroform until the residue from the latter gave no reaction for alkaloid.

Isolation of tabernaemontanine.—The chloroform solution after washing and drying was poured into twice its volume of light petroleum (40° to 60° C.) when a waxy yellowish precipitate, which did not contain any significant amount of alkaloid, was thrown down. The filtrate was shaken with 2N acetic acid to remove the alkaloids, and nitrogen was passed through the acid extract to drive out the dissolved solvent. To the filtered acid solution concentrated solution of ammonia was added drop by drop until the solution was faintly acid to litmus. The brownish slimy precipitate was filtered off and to the clear yellow filtrate 5 per cent. sodium hydroxide solution was added in slight excess to precipitate all the alkaloids.

The yellowish precipitate was filtered rapidly under suction, washed thoroughly with distilled water and dried in a vacuum desiccator over concentrated sulphuric acid (72 gm.). The crude alkaloids so obtained were dissolved in ether and the ether solution poured with constant stirring into ten times its volume of light petroleum (40° to 60° C.). The mixture was kept overnight in the ice chest and on the following morning the faint yellow solution was filtered from the coloured precipitate that had settled down. The light petroleum solution was shaken three times with 5 per cent. tartaric acid solution to remove the coronarine and then again with 2 per cent. hydrogen chloride solution to dissolve out

the tabernaemontanine which, being feebly basic, had remained unextracted by the tartaric acid solution.

The hydrochloric acid extract was cooled, rendered alkaline with 5 per cent. solution of sodium hydroxide, and the curdy precipitate was shaken with light petroleum. The petroleum solution was washed and again extracted with 5 per cent. acetic acid solution. The acid extract was cooled in ice and the precipitate obtained on adding excess of 20 per cent. solution of ammonia was filtered rapidly, washed and dried in a vacuum desiccator over sulphuric acid.

The amorphous dried powder so obtained was dissolved in the minimum quantity of methyl alcohol and the solution kept in a cool, dark place. After three days prismatic needles were deposited. These were collected and recrystallised from the same solvent (0·05 gm.). On heating, the crystals sintered at 203° C. and melted to a brown liquid at 208° to 210° C.

Found: C: 70·65 per cent.; H: 7·25 per cent.; N: 7·79 per cent.; mol.wt.: 336, 340. $C_{20}H_{26}N_2O_3$ requires C: 70·13 per cent.; H: 7·66 per cent.; N: 8·18 per cent.; mol.wt.: 342.

Isolation of Coronarine

The mother liquors from the crystallisation of tabernaemontanine were freed from the solvent under reduced pressure and the residue taken up in 5 per cent. solution of tartaric acid. This solution was combined with the tartaric acid extract of coronarine obtained in the course of isolation of tabernaemontanine and to the combined solution dilute solution of ammonia was added drop by drop till the solution was faintly acid to litmus. The dirty coloured precipitate that separated was filtered off, excess of 20 per cent. solution of ammonia was added to the filtrate, which was then shaken with ether. The alkaloid was extracted from the ether solution with 5 per cent. tartaric acid solution and precipitated by strong solution of ammonia. The light yellow precipitate was filtered under suction, thoroughly washed, and dried in a vacuum desiccator over phosphorus pentoxide. The dried base was dissolved in methyl alcohol and warm water carefully added till the solution was just turbid. On slightly warming the solution and keeping it overnight in the ice chest, thin plates of a golden yellow colour separated out, which were filtered, washed with 80 per cent. methyl alcohol, and dried on a porous plate (1·8 gm.). The crystals sintered at 183° C. and melted with frothing to a dark red liquid at 196° to 198° C.

On heating *in vacuo* over phosphorus pentoxide at 100° C., the crystals suffered a loss of weight of 5·60 per cent.

$C_{44}H_{56}N_4O_6$, $2\frac{1}{2}$ H_2O requires a loss of weight of 5·76 per cent.

The dried alkaloid was sent for analysis to Dr. Ing. A. Schoeller, of Berlin, and the following results were reported by him. C: 70·72 per cent.; H: 7·45 per cent.; N: 7·46 per cent.; mol.wt.: 667, 695. $C_{44}H_{56}N_4O_6$ requires C: 71·67 per cent.; H: 7·67 per cent.; N: 7·61 per cent.; mol.wt.: 736.

Coronarine was easily soluble in ether, chloroform, ethyl alcohol, methyl alcohol, and acetone, but only sparingly in light petroleum. Solutions in the above solvents showed a characteristic greenish fluorescence. The hydriodide and the nitrate were obtained as granular precipitates by adding concentrated solutions of the respective potassium salts to faintly acid solutions of the alkaloid in acetic acid. These salts could be obtained only as amorphous powders with no definite melting-points. On heating they charred at about 200° C.

Colour Reactions of the Alkaloids

	Tabernaemontanine	Coronarine
Concentrated nitric acid ..	Light yellow	Evanescent purple changing to cherry-red
Concentrated sulphuric acid	Light green	Reddish-brown
Froehde's reagent	Green, becoming darker on standing	Orange-red, turning green after some time
Erdmann's reagent ..	Leaf green	Yellowish-green, turning darker on standing
Potassium dichromate and sulphuric acid	Dark green	Orange-red, changing to green

The alkaline aqueous solution left after removal of the alkaloids was neutralised with acetic acid and mixed with excess of lead acetate solution. The small amount of precipitate that separated was filtered, washed, suspended in water and decomposed with hydrogen sulphide. On filtering off the lead sulphide a brownish liquid was obtained which produced frothing, due probably to the presence of a saponin, but no definite substance could be separated. The filtrate from the lead acetate precipitate was freed from lead and on examination was found to contain non-reducing sugars, potassium nitrate and potassium chloride.

Summary

1. The bark of the stem and root of *Tabernaemontana coronaria* has been found to contain the following constituents: fatty matter yielding on saponification palmitic, cerotic and oleic acids, a crystalline substance melting at 180° to 181° C. with properties resembling those of a resin alcohol, caoutchouc, resins, sugars, potassium nitrate, potassium chloride and alkaloids.

2. Two alkaloids, which have been named tabernaemontanine and coronarine, have been isolated in the pure state. Results of micro-analyses indicate the formula $C_{20}H_{26}N_2O_3$ for tabernaemontanine and $C_{44}H_{56}N_4O_6$ for coronarine. Preliminary experiments show that the alkaloids are pharmacologically active bodies.

The authors are indebted to Lt.-Col. R. N. Chopra and Dr. S. Ghosh, of the School of Tropical Medicine, Calcutta, for having suggested the investigation and for valuable advice and criticism. Grateful acknowledgment is also made to Dr. S. Rajagopal Naidu, Chemical Examiner to the Madras Government, for the analysis of the resin alcohol which was carried out in his laboratory.

Reference

1. Pillay, *Proc. Ind. Sci. Cong.*, 1938, *Abstracts*, III, 76.