

# COLOURING MATTER OF TAMBUL SEEDS

## Part I. Tambuletin

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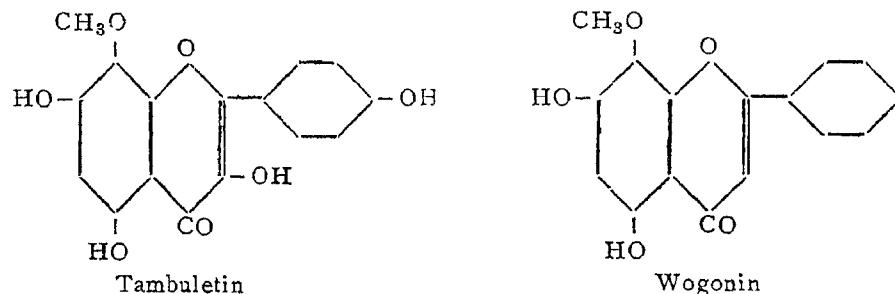
THE new flavonol herbacetin was first isolated from the Indian cotton flowers.<sup>1</sup> In these it occurs along with gossypetin, quercetin and also kæmpferol (populnetin). Later it was found to be present in the flowers of *Thespasia populnea* along with kæmpferol.<sup>2</sup> Its isolation from these sources is difficult since it involves fractionation and separation of mixtures and since the flowers vary very markedly in composition. The discovery by Bose and Bose<sup>3</sup> of tambulin, a trimethyl ether of herbacetin in the fruits of *Zanthoxylum acanthopodium* (tambul seeds) unaccompanied by any other flavonol seemed to suggest that this may prove to be a convenient source of natural herbacetin when required in small quantities. We had occasion to examine these seeds with this object in view.

The seeds were obtained from Calcutta and when extracted by the original method of the above authors using boiling alcohol or by an improved method using cold acetone surprisingly did not yield tambulin, but gave another crystalline compound which we have now named tambuletin, the yield being about 0·03%. A number of samples were procured and extracted at different times during the past two years and the same results were repeated.

Bose and Bose<sup>3</sup> reported that tambulin melted at 205° and formed an acetate melting at 160–61°. It had the composition  $C_{18}H_{16}O_7$  and had three methoxyl groups in the molecule. On further methylation it yielded the pentamethyl ether of herbacetin. Tambuletin on the other hand is now found to melt at 269–71° and to yield an acetate melting at 140–42°. Its composition is represented by the formula  $C_{16}H_{12}O_7$  and it has only one methoxyl. However like tambulin it yields O-pentamethyl herbacetin on complete methylation. Further on demethylation herbacetin itself is obtained. Consequently it should be considered to be a monomethyl ether of herbacetin and thus the tambul seeds could be used as a handy source of natural herbacetin.

The properties and colour reactions of tambuletin provide some information about the location of the methyl ether group. They resemble closely those of kæmpferol. Particularly significant are (1) the ferric chloride colour, (2) colour in sodium hydroxide solution and (3) lack of reaction

with *p*-benzoquinone. These indicate that one of the two hydroxyl groups in the 5- and 8-positions is not free. Since the 5-hydroxyl is difficult to methylate and will be the last one to be affected in hydroxy flavones, it could be concluded that the methoxyl is situated in the 8-position and that tambuletin is another example of the rare type of 8-methyl ethers analogous to wogonin. Work is in progress in order to confirm this constitution.



Bose and Bose reported the isolation in a very small yield of a second substance named tambulol melting at 265-67°. Though its melting point is very near that of tambuletin its composition and properties are very different.

## EXPERIMENTAL

### *Isolation of Tambuletin:*

For the extraction of the fruits of *Zanthoxylum acanthopodium* Bose and Bose<sup>3</sup> employed hot rectified spirit. Cold acetone is now found to be better. The fruits (3 kg.) were crushed to a coarse powder and extracted with acetone by cold percolation. Each time the solvent was allowed to be in contact with the material for 24 hours and the extract drained off; this was repeated three times. The solvent was distilled off as far as possible on a water-bath and the concentrated extract (600 c.c.) was thoroughly mixed with petroleum ether (2 litres). It was set aside for a fortnight when a bright yellow solid along with some brown resin separated out. The supernatant layer of petroleum ether (A) was filtered off and the residue stirred up with a little cold alcohol and filtered. A yellow solid was thus obtained and this was washed free from the accompanying resinous material with a little more alcohol. The alcohol solution was marked (B). The yellow substance melted at 254–56°. Taking advantage of the fact that it dissolves freely in aqueous sodium hydroxide and can be recovered unchanged by acidifying the solution, the crude material was purified in the following manner. It was dissolved in aqueous alkali and the solution was quickly filtered thus removing all suspended impurities. The clear solution was acidified with hydrochloric acid and the precipitated solid was recovered by filtration; ether extraction of the aqueous filtrate did not yield any more of it. It was further purified by crystallisation from glacial acetic

acid when it was obtained as bright yellow needles melting at 269–71°. Even without the alkali treatment the crude material could be purified by repeated crystallisation from glacial acetic acid to give the above melting point (Found: C, 60·4; H, 4·0; OCH<sub>3</sub>, 9·4; C<sub>16</sub>H<sub>12</sub>O<sub>7</sub> requires C, 60·7; H, 3·8; OCH<sub>3</sub> 9·8%).

The petroleum ether extract (A) was examined for the existence of any crystalline matter. When the solvent was distilled off, there remained a very small quantity of a brown sticky solid. This was extracted with aqueous alkali and the solution acidified. The same coloured and sticky substance was obtained and all attempts to get a crystalline substance from it using different solvents failed.

Similarly the alcohol washings (B) also were examined. The solvent was removed by distillation and the residue was obtained as a brown pasty mass. When it was shaken with petroleum ether, some more tambuletin was precipitated. From the remaining amorphous portion no other crystalline substance could be obtained even after repeated effort.

Thus only tambuletin melting at 269–71° could be isolated from the total acetone extract and the presence of no other crystalline compound could be detected.

Tambuletin is a bright yellow solid which is readily soluble in hot glacial acetic acid but more sparingly in hot ethyl acetate, acetone and alcohol. When its alcoholic solution is treated with concentrated hydrochloric acid and magnesium powder the solution which is initially coloured bright yellow turns bright orange-red. With ferric chloride in alcoholic solution it gives a dull green colour and this colour is stable and does not change to brown with excess of the reagent. When an alcoholic solution of the substance is treated with neutral lead acetate in the same solvent an orange coloured precipitate is thrown down. With concentrated sulphuric acid the crystals of the substance immediately develop a bright red colour and go into solution later; the solution is coloured brownish-yellow and does not exhibit any fluorescence. Tambuletin dissolves easily in aqueous alkali to form a bright yellow solution; the colour of the alkali solution is stable for 12 hours and the substance could be recovered by acidification after this period. In absolute alcoholic solution tambuletin does not react with *p*-benzoquinone.

#### *Methyl tambuletin*

Tambuletin (0·2 g.) was dissolved in dry acetone (50 c.c.) and anhydrous potassium carbonate (5 g.) and dimethyl sulphate (0·5 c.c.) were added. The contents were refluxed for 30 hours and the hot acetone solution filtered

from the potassium salts which were finally washed with hot acetone. On distilling the combined acetone solution to remove the solvent, the crude methyl ether was obtained. It was crystallised twice from dry ethyl acetate when it melted at 156-57°. The mixed melting point with herbacetin-penta-methyl-ether was undepressed.

*Nor-tambuletin*

Tambuletin (0.5 g.) was dissolved in hot acetic anhydride (1 c.c.) and the solution cooled. To this was added hydriodic acid (d., 1.7; 5 c.c.) with cooling under the tap. The contents were boiled for one hour, cooled and treated with water containing sulphur dioxide. The precipitated yellow solid was filtered off and crystallised from dry ethyl acetate when it was obtained as bright yellow flat needles melting at 279-80°. The mixed melting point with herbacetin was undepressed. The identity was established by comparing the reactions of nortambuletin and herbacetin (1) with alkaline buffer solutions, (2) with *p*-benzoquinone and (3) with ferric chloride. Both gave identical colour changes with (1), the brownish-red quinone with (2) and with (3) the colour was olive green changing to reddish-brown with excess of the reagent.

*Acetyl-tambuletin*

Tambuletin (0.5 g.) was treated with acetic anhydride (10 c.c.) and a few drops of pyridine. The contents were refluxed gently for one hour and poured into ice-cold water. The reaction mixture was stirred well and set aside for some time. The separated solid was filtered off, dried and crystallised from dry ethyl acetate when it was obtained in the form of colourless needles melting at 140-42°.

SUMMARY

Extraction of the fruits of *Zanthoxylum acanthopodium* has yielded a new crystalline component and it is named tambuletin. It seems to occur in the place of tambulin reported by earlier workers. It has the composition  $C_{16}H_{12}O_7$  and is a monomethyl ether. Experiments on methylation and demethylation prove that it is a monomethyl ether of herbacetin and its reactions suggest that the methoxyl group is in the 8-position.

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