A PRELIMINARY NOTE ON THE SEED CONSTITUENTS OF CAESALPINIA BONDUCELLA

M. Qudrat-i-Khuda, M. Erfan Ali and M. Siddiqullah

Natural Products Research Divisoin, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

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The powdered kernel of bonducella nuts has been extracted successively with light petroleum (b.p. 40-60°C.), chloroform, rectified spirit and water at room temperature. The petroleum extract gives a fatty oil in ca. 20% yield, having an iodine value of 112; the oil deposits a jelly-like substance on long standing. From the chloroform extract, two new highly crystalline bitter substances α-caesalpin, m.p. 187° and β-caesalpin, m.p. 239-240° have been separated, while the alcoholic extracts yield sucrose in two crystalline forms, one cube and the other rectangular bar, besides some reducing sugars. A water soluble protein has also been obtained from the aqueous extract.

Introduction

Caesalpinia bonducella, or the commonly called fever-nut, grows wild all over the Indo-Pakistan sub-continent, especially in West Bengal, East Pakistan and South India, and is known locally by different names (Hindi, Karanjava; Bengali, nata or nata-karanja; Persian, khayae-i-iblis; Arabic, kitmakit). The common name for the seed is bonduc seed (bonduc in Arabic means a little ball or filbert). Every part of the plant is used medicinally, but in the present communication only the constituents of the seed have been discussed. The seeds are said to possess well-marked anti-periodic, antipyretic and tonic properties and were largely used by the local people as a substitute for quinine. They are considered by some to be useful for alleviating swellings and restraining hoemorrhage. Their application in leprosy and as an anthelmentic is also known.

Heckel and Schlagdenhauffen[1] isolated a non-alkaloidal bitter principle as a white amorphous powder from bonducella seeds, and assigned the name 'bonducin' to it. Bacon[2] said that bonducin was not a glucoside but a mixture of complex resinous bodies. In 1912, Bhaduri[3] reported to have isolated an alkaloid, 'natin' from the seeds. In an attempt to isolate the natin, Godbole et al.,[4] found that the bitter principle was not an alkaloid. He said that it was a sulphur-containing glucoside. Tummin Katty[5] obtained bonducin as an amorphous powder of indefinite melting point and concluded that the bitter principle was a complex non-alkaloidal and non-glucosidic mixture of resins; and Ghatak[6] stated that the bitter principle, bonducin, was a non-crystalline glucoside, $C_{20}H_{28}O_8$, m.p. 119-120°C., and it was neither an alkaloid nor a sulphur-containing glucoside. These varied opinions, as regards the nature of the active principle, are due to the fact that none of the workers was able to obtain the active principle in a pure and crystalline condition.

In view of the importance of bonducella nuts in the Ayurvedic system of medicine, and because of the very divergent views expressed by the earlier workers about the nature of its bitter constituent, a re-investigation of the seeds appeared to be desirable to find out the exact physiologically active component. This report consists of a complete preliminary account of the constituents of the fruit.

The fruit consists of a hard greenish grey shell, which has a white kernel. In immature fruit the colour of the kernel is brown and the constituents vary considerably. For the present investigation, only matured kernels have been used.

The kernel showed the presence of some fixed oil and was first treated to separate this oily constituent with a solvent. The amount of oil appears to be about one-fifth of its weight and is a very bitter substance. The bitterness can be attributed to the partial dissolution of the main bitter principle, mentioned below, in the oil. With the help of either hot rectified spirit or better in a charcoal column, this bitterness could be removed and the oil obtained as a clear colourless liquid mass, where the untreated oil was rather dark brown in colour. The oil has a fairly high iodine value and on standing for some time deposits a jelly-like product. The constituents of the oil are now under examination.

The defatted pulp was extracted with chloroform when a fairly neat sample of an extremely bitter substance came out. It was not one pure compound but appeared to be a mixture, m.p. 140-148°C., which was free from nitrogen and sulphur. Two bitter substances, one melting at 187°C., and the other at 239-240°C. have been separated from this mixture by fractional crystallisation from methanol. We have called the former α-caesalpin and the latter β-caesalpin; both of these are well-defined crystals. The quantities of the products are, however, rather small; these are

being accumulated and their detailed account will follow subsequently.

The debittered powdered kernel was next extracted in a percolator with rectified spirit, which removed a fairly large quantity of a sweet viscid mass. This on treatment with methanol, gave a well-defined crop of cubical crsytals, while another crop of crystals was obtained from the mother liquor as stout rectangular bars. Both the crops have been identified as sucrose, although literature records only one form of crystals (cubical) for the same. Besides this crystalline component, a syrup remained behind which reduced Fehling's solution and gave an osazone similar to glucosazone. So far it has resisted separation into crystalline components. However, besides the reducing sugar, it also contains a high proportion of non-reducing sugar, and other foreign materials which might have contributed greatly to the difficulty in the separation. Further work in this direction is in progress and the results will be communicated in due course.

The residue after alcohol extraction gave a considerable portion of it in water. When the aqueous solution was heated this portion coagulated into a white mass. It was found to be a water soluble protein while the rest of the solid material consisted of several other types of porteins which are now under examination and a report about it will follow later.

Experimental

Separation of Fixed Oil.—The sun-dried kernels of the matured seeds were ground to a fine powder, and 1000 g. of the same was percolated slowly for 5 days at room temperature with light petroleum (b.p. 40-60°C., 4000 ml.). Further percolation gave practically colourless oil-free extract. The combined percolates (3200 ml.) on removal of the solvent gave a brown oil (192 g., 19.2%) which was bitter to taste.

Purification of the Oil.—(i) The crude oil (200 g.) was vigorously shaken with hot rectified spirit (150 ml.) in a separating funnel. The mixture formed an emulsion which was set aside overnight whereupon two layers separated. The oily layer was treated twice in a similar manner, whereby it became completely bitterless but still contained an appreciable amount of colouring matter.

The alcoholic layers were combined and the solvent was removed under reduced pressure. The viscous residue after having been washed with light petroleum (40-60°C. 20 ml.) was dissolved in ether (10 ml.) and the solution kept in the refrigerator for 48 hours. The crystalline deposit (0.07 g.), m.p. 167°C., was collected and recrystallised from methanol, when an extremely bitter substance, m.p. 183°C., was obtained. Its mixed m.p. with the bitter principle of m.p. 187°C. was 186°C.

(ii) The petroleum extract (1200 ml.) from 500 g. of the kernel was diluted with the solvent (1000 ml.) and was then passed through a column (16 × 3 cm.) of animal charcoal (E. Merck), the rate of flow being regulated at 30 drops per minute. The solution emerged clear and colourless from the column . The adhering oil in the column was washed down with a little solvent. The combined solution and the washings were evaporated to a colourless and bitterless oil (113 g.). It showed saponification value of 190, and iodine value (Hanus) of 112.

The used charcoal was eluted with boiling rectified spirit (3 × 100 ml.) The eluate was freed from the solvent and the residual viscous mass was washed with a little light petroleum (b.p. 40-60°C.). Crystallisation of the semi-solid mass from ether (5 ml.) in the cold, followed by re-crystallisation from methanol, as before, gave the bitter (0.05 g.), having m.p. and mixed m.p. of 187°C.

Isolation of Bitter Principles.—The defatted kernel (originally 1000 g.) after removal of the last traces of petroleum ether by a water pump, was soaked with chloroform (1500 ml.) in a percolator and left at room temperature overnight. The extract was then run out of the percolator drop by drop during the whole day. The residual mass was again treated with fresh chloroform (900 c.c.) and the extraction operations were similarly carried out. This process of extraction was then repeated three times, after which only a bitterless and colourless percolate appeared to come out. The combined extracts (4200 ml.) were made free from the solvent by distillation under reduced pressure. The viscous residue was triturated with light petroleum (b.p. 40-60°C., 150 ml.) and the mixture was left in the cooler overnight. The solid residue (9.2 g.) was collected and washed with a little of the solvent. This was then dissolved in ether (40 ml.) and kept in a refrigerator for 48 hours whereupon a crystalline substance (0.7 g.; 0.07% on wt. of the kernel) deposited. It was collected on the filter, washed with a little ether and recystallised from benzene. It melted at 140-148°C.(not sharp) and gave negative test for nitrogen and sulphur. The substance was extremely bitter, readily soluble in methanol, ethanol, chloroform and acetic acid, but was very sparingly soluble in ether and petroleum ether and was moderately soluble in benzene. This was fractionally crystallised from

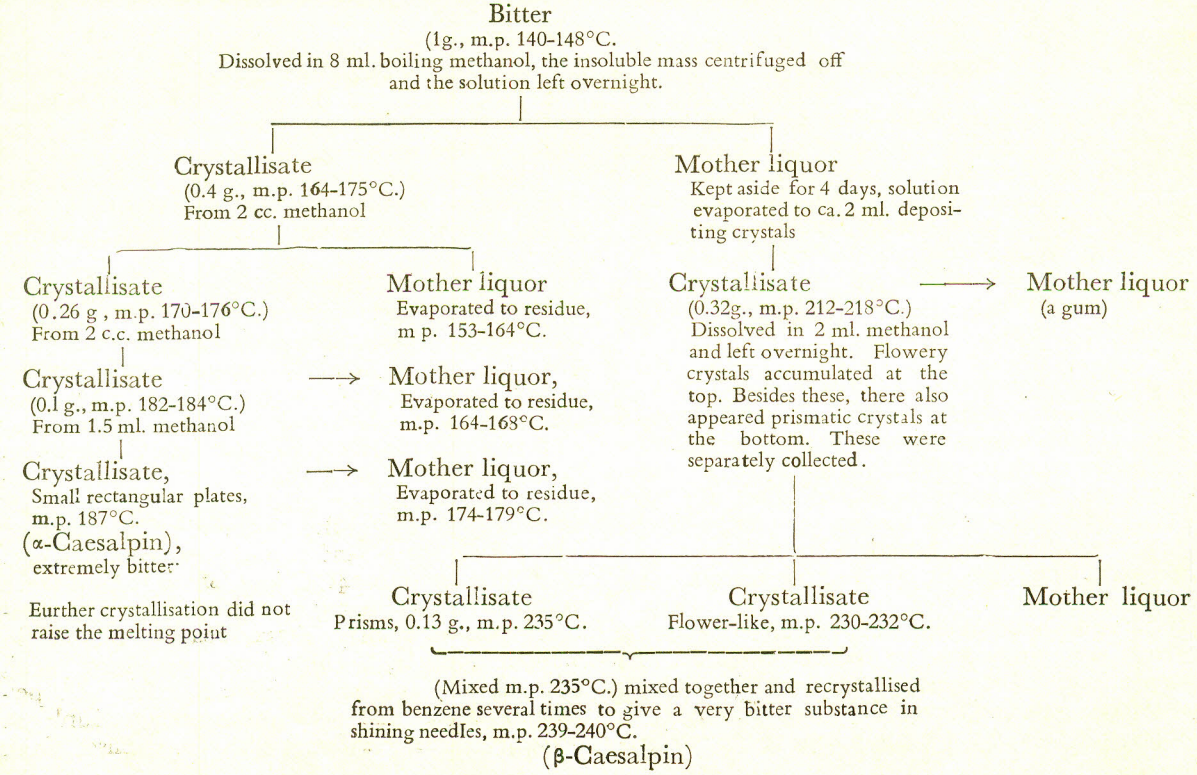
methanol as schematically shown in the chart. The above ethereal mother liquor was evaporated to an amorphous solid residue (7.3 g.) which could not be crystallised from different solvents or mixed solvents. It melted at 104-120°C. and was also extremely bitter to taste.

Isolation of Sugars.—The defatted and debittered residue of the kernel, after the above treatment was percolated with rectified spirit (7,600 ml.) at room temperature. The solvent from the percolates was removed by distillation first at ordinary pressure and then under vacuum. The viscous residue (73.5 g.) was then triturated once with absolute alcohol; a semi-solid mass was thus obtained. This was refluxed for several hours with methanol (300 ml.). The mass mostly went into solution leaving behind some crystalline solid. The mixture was allowed to cool and the crystalline solid (0.82 g.) was collected. This was dissolved in methanol (40 ml.) by boiling and the solution was filtered from the impurities. The clear solution on standing overnight deposited sucrose in small cubes, m.p. and mixed m.p. with a genuine sample of pure sucrose was 187°C., $[\alpha]_D^{32} = +66.69°$. Octa-acetate was prepared by the method of Linstead[7] when it melted at 88-89°C. Its mixed melting point with an authentic sample, prepared from pure sucrose, was not depressed. Thus the identity of sucrose is confirmed.

The methanolic extract (of the crude sugar) as obtained above was concentrated and allowed to stand, whereby some crystals in the shape of rectangular bars appeared. These were collected, and had m.p. 187°C. Its mixed m.p. with cane sugar was undepressed. They had $[\alpha]_D^{31} = 66.07°$ and gave an octa-acetate, m.p. 88-89°C.; mixed m.p. of this octa-acetate with sucrose octa-acetate was undepressed.

The methanolic mother liquor on further standing did not give any more crystalline solid. It was evaporated to a viscous mass which reduced Fehling's solution and also gave a small amount of an osazone, prepared by the method of Vogel,[8] melting at 205°C.; an admixture with an authentic sample of glucosazone did not depress the melting point. So far the sugar itself could not be crystallised and no crystalline acetyl derivative or *p*-nitrophenyl hydrazone has been obtained. It is therefore not possible at this stage to report its identity but more work on it is expected to give interesting results.

Estimation of Reducing and Non-reducing Sugars in the Non-Crystallisable Portion of the Alcohol Extract.—This estimation was carried out according to the procedure of H.K. Archbold and E. M. Widowson[9] involving oxidation with potassium hypoiodite before and after hydrolysis of the sample. It was



found to contain 11.6% reducing sugar; calculated as glucose, and 46.17% of a non-reducing sugar, presumably sucrose.

Separation of Proteins.—The residue (25 g.) left after alcohol extraction was exhaustively extracted with water, 10% sodium chloride, 10% sodium hydroxide and 10% hydrochloric acid, successively. The water extract was heated on the steam bath for about an hour. The coagulates were collected and dried to give the water soluble protein, 19.7 g.; or 79% of the alcohol treated residue.

The sodium chloride extract, on being saturated with ammonium sulphate, yielded 1.75 g. or 7.02% of a protein on alcohol treated residue. The alkaline and the acidic extracts on neutralisation similarly gave 1.09 g. or 4.37% and 0.04 g. or 0.16% of proteins, respectively.

The residue, 1.86 g. or 7.42% on the alcohol treated residue, was very tough and appeared to be cellulosic in character. More detailed examination of the above four types of proteins is in progress.

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