

A NOTE ON THE PREPARATION AND REACTIONS OF KARANJIN

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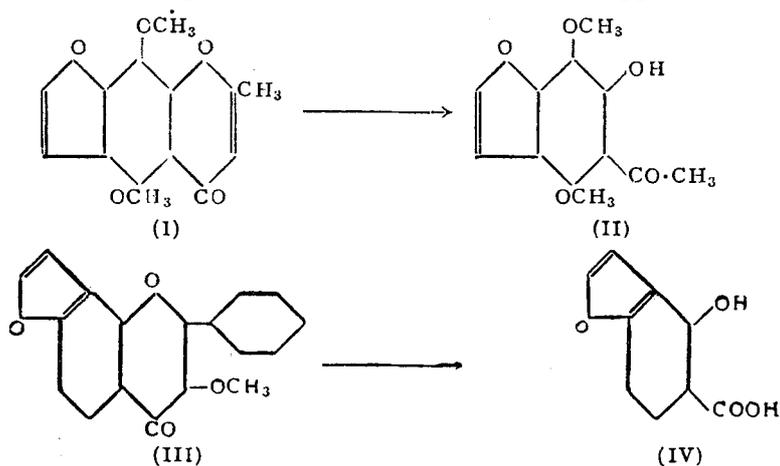
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KARANJIN, the bitter principle of the Pongamia oil, was first isolated by Limaye¹ by shaking the oil with alcohol. In our attempts to get large quantities of the substance for clinical use, we have examined different methods and found that extraction using industrial methylated spirits and the apparatus described in the experimental portion for continuous extraction is the most effective and economical. Karanjin was obtained in a yield of 0.9% ; but it should be pointed out that this good yield was obtained from the oil pressed from the seeds collected round about Waltair and that the yield varied with samples obtained from elsewhere. In one case there was very little Karanjin present. It has now been found that Karanjin is the active principle responsible for the curative action of the oil on leucoderma. When applied as a paste with any vegetable oil and rubbed, it stimulates pigmentation of the depigmented skin successfully. Karanjin does not taste bitter when the solid is placed straightaway on the tongue owing to the insolubility of the substance. But when a few drops of dilute alcoholic or aqueous alcoholic solution are employed, the taste is bitter and is associated with a peculiar tingling effect. The bitterness is quite mild as compared with the taste of Psoralen, Isopsoralen and Naringin.

The alkaline hydrolysis of Karanjin has been shown by Limaye² to give rise to four fission products : (1) Benzoic acid, (2) Karanjic acid (4-hydroxy-coumarone-5-carboxylic acid), (3) C-acetyl karanjol (4-hydroxy-5-acetyl-coumarone) and (4) Karanjol (4-hydroxy-coumarone). But the experimental details regarding the hydrolysis have not been published by him. We had occasion to examine the different methods of hydrolysis but meanwhile Manjunath *et al.*³ reported that by using alcoholic potash a mixture of (2) and (3) are obtained, C-acetyl karanjol being the main product, whereas, Karanjic acid was obtained in very poor yields. Our results agree with this observation. Further, we noticed that fusion with potash produced considerable destruction of the substance and only benzoic acid could be detected amongst the products. By employing boiling aqueous alkali for

the hydrolysis, Karanjic acid was easily obtained as the only product along with benzoic acid. But the action of dilute aqueous alkali was very slow and a large portion of Karanjin remained unaffected even after boiling for several hours. The reaction was considerably expedited by adding some alcohol in order to increase the dissolving power.

Working with Kellins, a chromono-furan having the constitution (I) Späth and Gruber⁴ found that one per cent. aqueous potash reacts smoothly in the course of half an hour to give rise to a good yield of kellinone, the corresponding ketone (II). The same effect was observed by Fantl and Salem⁵ when they used aqueous barium hydroxide for the hydrolysis of kellins. Adopting exactly the same procedure and excluding air as much as possible, we found that Karanjin (III) undergoes decomposition in a different way and produces only Karanjic acid (IV) in addition to benzoic acid and the ketonic product is not produced in any appreciable amounts.



Desai, Sudborough and Watson⁶ obtained a deep yellow precipitate possessing the properties of proteins when Pongamia oil was treated with 2% of a mixture of 2 parts of sulphuric acid and one of water. It was reported to have turned white on washing with water and to have been incapable of being crystallised. On repeating the experiment, it was noticed by us that no amorphous substance could be obtained and that a crystalline precipitate of potassium sulphate was formed instead. That potassium was present in some form in the oil was confirmed by slowly ashing the oil and detecting potassium in the ash. The metal was further proved to be present in the ash of the seed kernel.

Karanjin undergoes mercuration with mercuric acetate in methyl alcoholic solution to form a sparingly soluble product. It is noticed that

two acetoxy-mercury groups have entered into combination but their position in the Karanjin molecule has not yet been ascertained.

Experimental

The seed of *Pongamia glabra*, as it is obtained fresh, consists of an external woody shell within which is lodged the kernel. After drying, the outer shell could be easily removed by gentle hitting with a hammer. The following are the results of proximate analysis of air-dried kernels. The methods employed are those adopted by the Association of the Official Agricultural Chemists.

	%
Moisture ..	19
Oil ..	27.5
Starch ..	6.6
Crude fibre ..	7.3
Proteins ..	17.4
Ash ..	2.4

Potassium sulphate from the oil.—

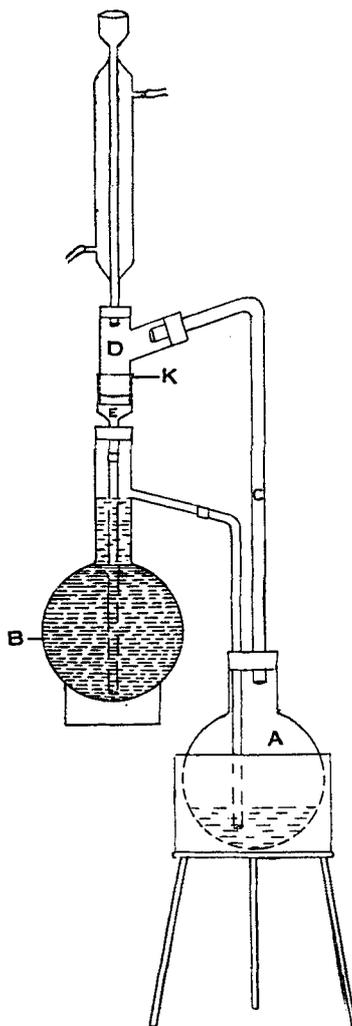
50 g. of the oil were shaken well with 4 c.c. of a mixture of 2 parts of concentrated sulphuric acid and one of water. The oil assumed a bright orange colour and a solid was observed to deposit slowly. The mixture was set aside for 24 hours to ensure complete precipitation of the solid. It was filtered and the residue on the filter washed with petroleum ether and then with benzene. The solid was then recrystallised from water when rhombic crystals of potassium sulphate were obtained. It gave a lilac violet colouration when introduced into the flame on the platinum wire, a precipitate with perchloric acid, and with sodium cobaltinitrite. No protein, as reported by Desai, Sudborough and Watson, could be isolated.

With a view to confirm the presence of potassium in the oil, the ash of the oil was prepared. It formed 0.25% on the weight of the oil and contained potassium and iron.

Isolation of Karanjin.—

Pongamia oil obtained by pressing the kernels in a country-press was filtered to remove suspended impurities and used for the isolation of Karanjin with the apparatus given in the accompanying diagram. It is a modification of the apparatus found in Gattermann's *Laboratory Methods of Organic Chemistry* and it makes the extraction less tedious and more efficient. The chief feature to be noticed is that the tube C conveying the vapours of the solvent (alcohol) from flask A is made as short as possible by connecting it

to the bottom of the reflux condenser instead of to the top and consequently the solvent in a rather warm condition extracts the oil kept in the flask B. D and E are glass three-way tube and adapter respectively which are



connected to each other by means of a rubber cone K. This prevents loss of the solvent by evaporation. The other parts of the arrangement are easily understood.

Using a litre distilling flask as a container for the oil (900 g.), extraction was carried on for 30 hours, when a good yield of Karanjin was obtained. The alcoholic extract was coloured brown and a small quantity of the oil was also carried over along with Karanjin into the flask A. At the end of

the experiment, a crystalline solid settled down when the alcoholic extract was allowed to cool. However, the solution was concentrated by distilling away most of the solvent and then set aside for crystallisation. The crystalline solid was filtered, washed with a little petroleum ether and finally recrystallised from alcohol. Karanjin was thereby obtained as colourless rectangular plates melting at 158–9° C. The yield was 8.0 g. (about 0.9%). (Found: C, 73.5; H, 4.2%, $C_9H_6O_2$ requires C, 74.0; H, 4.1%.) The molecular weight of the substance was determined by Rast's method using camphor and the value obtained was 283 (compare Manjunath *et al.*, 285). This value therefore agrees with the molecular formula $C_{18}H_{12}O_4$, the required figure being 292.

Karanjin is easily soluble in ethyl and methyl alcohols, benzene and petroleum ether. Concentrated sulphuric acid dissolved it forming a yellow solution which turned red and finally green when heated to 100° C. On dilution with water, Karanjin was produced unaltered.

The oil left after treatment with alcohol as described above became quite transparent. It was noticed that this contained very little of unsaponifiable matter obviously due to their removal by alcohol.

Hydrolysis of Karanjin.—

Preparation of Karanjic acid.—Karanjin (2 g.) was treated with 3% aqueous alcoholic potash (alcohol 100 c.c., water 150 c.c., potash 7.5 g.) in a litre flask and after displacing all the air with nitrogen boiled under reflux, whereby a reddish yellow solution was produced. All the Karanjin went into solution within the first half hour. At the end of 3 hours, as much alcohol as possible was distilled off and the aqueous solution acidified with dilute sulphuric acid (200 c.c. of 4% acid). The colourless precipitate that was obtained was filtered and dried in air (yield 0.9 g.). It crystallised from 50% alcohol as rectangular rods and melted at 216–18° C. with decomposition. (Found: C, 60.9; H, 3.2%. $C_9H_6O_4$ requires C, 60.7; H, 3.4%.) The substance gave a blue colouration with ferric chloride and dissolved in both sodium carbonate and bicarbonate solutions. It agreed with the description of karanjic acid given by Manjunath *et al.*

The aqueous filtrate left after the removal of crude Karanjic acid was repeatedly extracted with ether and the ether extract evaporated to dryness. The residue was dissolved in aqueous sodium carbonate and by extracting this with a little ether, a very small quantity of a brown oily liquid was obtained. It was, however, in too small a quantity to be identified. The sodium carbonate solution was then strongly acidified with

hydrochloric acid, when a grey solid melting at 115° C. was obtained. When this was boiled with water a small quantity was left undissolved. This melted at 210° C. with decomposition and was obviously karanjic acid. By ether extracting the aqueous solution and removing the ether from the extract, a colourless crystalline solid melting at 120° C. was obtained and this was identified as benzoic acid.

Trials using 1% aqueous potash and otherwise observing the above conditions gave karanjic acid in a yield of 0.4 g. about half the quantity of Karanjin being left unaffected. Employing 2% and 3% alkali solutions the hydrolysis was better but still 30% and 20% respectively of the original Karanjin was left unaffected.

Mercuration of Karanjin.—

Karanjin (0.5 g.) was dissolved in anhydrous methyl alcohol (50 c.c.) and added to a solution of mercuric acetate (7.5 g.) in anhydrous methyl alcohol (120 c.c.) and boiled under reflux for 20 hours. The contents of the flask were filtered to separate the yellow mercurated product which was washed repeatedly with methyl alcohol. It was then dried and analysed. The yield was 0.4 g. (Found: Hg, 50.1%. $C_{13}H_{10}O_4$ (Hg.O.CO.CH₃). or $C_{22}H_{16}O_8$ Hg₂ requires Hg, 49.6%.) The product obtained was therefore diacetoxy-mercuri-karanjin.

Summary

A method of extracting Karanjin in good yield from Pongamia oil is described. Karanjin is only faintly bitter and is found to be useful for the treatment of leucoderma. A method of hydrolysis of Karanjin giving rise to a good yield of Karanjic acid has been found. It has been shown that Pongamia oil contains potassium in some form of chemical combination. By the action of mercuric acetate, Karanjin yields a di-acetoxy-mercuri compound.

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