A NEW SEPARATION OF THE COMPONENTS OF *PSORALEA CORYLIFOLIA*, LINN.

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These seeds (more correctly fruits) have been greatly valued for their pleasant aroma as will be evident from the Sanskrit name *sugandha* and have been widely used in medicine. Internally they are said to be useful as laxative, diuretic and anthelmintic and externally as cure for leucoderma and leprosy. They are frequently employed in several toilet preparations. Regarding chemical work on this material done in the past reference should be made to Dymock,[1] Menon,[2] Chopra and Chatterjee,[3] Chopra[4] and Jois, Manjunath and Venkatarao.[5] A sample of the seeds was sent from Trichinopoly to one of us (T. R. S.) when he was working in the Agricultural Research Institute, Coimbatore, in 1932 with a view to explore possibilities of industrial utilisation. The chemical investigation that was then begun could not be continued. Early in 1933 the paper of Jois, Manjunath and Venkatarao appeared embodying the results of a detailed chemical study. Besides examining the fixed oil of the seeds they were able to isolate two crystalline solids psoralen and isopsoralen. The latter has been identified as angelicin (Jois and Manjunath[6]) and the former has been successfully synthesised recently by Spath, Manjunath, Jois and Pailer.[7] Since however our method of study is different from those of others and it enables the components to be separated more effectively in greater purity and yield and at the same time gives information regarding the parts of the seeds in which they occur, it has been thought desirable to record our results.

A simple inspection of the seed (fruit) showed that it consisted of a sticky, oily pericarp which seemed to contain all the odour, a hard seed coat and a kernel which had no smell, contained a fixed oil and a bitter substance. It was found that it was rather difficult to powder the whole seed owing to the sticky pericarp. A few exploratory experiments indicated that almost all the pericarp could be dissolved in ether by cold percolation leaving the seed coat and kernel in tact. The ether extract contained a steam volatile essential oil, a caustic alkali soluble resin and a terpenoid liquid insoluble in

caustic alkali. From the ether-extracted material, the remaining portion of the pericarp (skin) could be easily rubbed off and the substance powdered. On extracting this powder in a Soxlet apparatus using light petroleum (sp. gr. 0·64 to 0·66) all the fixed oil was removed and along with this a colourless crystalline solid was deposited in the receiver. The essential oil, the resin and the terpenoid oil were present in the pericarp only and not in the kernel. The crystalline solid obtained during the extraction with petrol was a mixture of psoralen and isopsoralen and the fixed oil finally obtained was pale brown and devoid of any prominent odour. It was reasonably pure consisting almost completely of glycerides. A small unsaponifiable portion of the fixed oil was found to contain a sterol which appeared to be phytosterol. These were therefore components of the kernel. Besides these it contained nitrogenous and mineral matter.

All previous workers ground up the whole seed and extracted the powdered material and hence did not get pure fractions and the examination of the products was difficult. Amongst the advantages of the present method may be mentioned (1) the possibility of obtaining the components of the pericarp in three fractions, (2) a purer fixed oil free from much colour and uncontaminated with resin and (3) a better yield of the psoralen mixture which is the cause of the bitter taste of the seed. These fractions can be used conveniently to satisfy different requirements as for example where the perfume and resin are wanted the pericarp fractions could be used. A note on the physiological properties of the fractions is given towards the end of this paper.

Experimental.

Components of the pericarp. Aromatic principles and resin.—The material available in the local market was employed. The entire seed was kept soaked in ordinary ether for 12 hours, the ether extract decanted and the process repeated once again. The extraction was then practically complete and all but a thin skin was removed. In a test experiment it was found that this extraction removed about 11·7 per cent. of the whole seed. It was also noted that the whole extract was soluble in alcohol and that a portion of it was soluble in aqueous sodium hydroxide. The ether solution was therefore extracted repeatedly with 5 per cent. aqueous sodium hydroxide solution till no more was removed. The brown alkaline solution was once extracted with pure ether and then acidified with hydrochloric acid. A dark brown semi-solid resin separated (8·6 per cent. by weight of the seed). It did not possess any definite smell or taste. It slowly solidified to a brittle solid on keeping. The ether solution

containing the alkali insoluble matter was distilled to remove the solvent. The residue was a viscous brown-red oil possessing all the odour of the seed (3·1 per cent.). On passing steam through it an almost colourless essential oil came over (0·05 per cent.). It was obtained from the distillate by ether extraction. It smelt strongly of the seed and had the refractive index 1·4808 at 30°C. The fraction in the distilling flask had still some odour of the seed. For purposes of further examination it was taken up in ether, dried over calcium chloride and the ether removed. The residual brown viscous oil when distilled under reduced pressure underwent decomposition to a thicker oil. Some of its properties were as below :

Specific gravity	0·9759
Refractive index	1·5310
Saponification value ..	21·0
Unsaponifiable matter ..	87·5
Iodine value	92·2

It was completely soluble in petroleum ether and seemed to consist mostly of complex terpenoid compounds. The pericarp gave tests for the presence of a pigment of the nature of hydroxy flavone giving yellow colour with alkali. But since the quantity present was very small it was not further investigated.

Components of the Kernel. Psoralen-Isopsoralen mixture and the fixed oil.—The ether-extracted seed was cleaned by rubbing, powdered and extracted with light petroleum (sp. gr. 0·64 to 0·66) in a Soxlet apparatus for about 12 hours. Vigorous extraction for this period effected complete extraction. After about three hours, a crystalline deposit was invariably noticed in the receiver and this increased in bulk as the extraction progressed. The extract was then set aside for a few days, filtered under suction and the solid washed with small quantities of light petroleum in order to remove all the adhering oil. After drying, the solid was obtained in a yield of 1·1 per cent. of the crushed seed or 0·97 per cent. of the fresh seed (Jois, Manjunath and Venkatarao, 0·27 per cent.). It was bitter to the taste, was moderately soluble in boiling water, easily in alcohol or chloroform and sparingly in ether and petroleum ether. It melted at about 110–115° C. and it could be separated into psoralen (m.p. 168–69°C.) and isopsoralen (m.p. 141–42°C.) by fractional crystallisation as already outlined by Jois, Manjunath and Venkatarao. The former is the less soluble of the two and always comes down as large hard crystals whereas the latter crystallises as finer and softer needles.

When the light petroleum extract was distilled on a water-bath to remove the solvent, a fixed oil was obtained with a pale brown colour. The solvent was completely removed by passing a current of nitrogen. The

yield was then 11 per cent. of the powdered seed or about 10 per cent. of the fresh seed. An attempt was made to get a colourless oil by soaking the ether-extracted seed in water, rubbing very well, drying in the sun and then powdering and extracting. But this did not effect any appreciable change. The oil was rather viscous and had still a bitter taste. With a view to estimate if any considerable amount of psoralen or isopsoralen was present in it, about 40 g. of the oil was saponified and the unsaponifiable fraction removed by ether extraction of the alkaline solution. The acids were then liberated by strongly acidifying the solution and the mixture was then warmed. During this process the bitter principles which are coumarins would have been first dissolved in the alkali undergoing hydrolysis and subsequently been reformed on acidifying and heating. Then they would be insoluble in aqueous sodium carbonate. In the collected solid the portion that was insoluble in sodium carbonate was very small showing that the whole solid was composed almost completely of fatty acids and the bitter substances were present only in very small quantities.

The fixed oil obtained was examined for some of its properties with a view to note how far it differed from the previous specimens and the physical constants are given below :

	Our sample	Sample of Jois, Manjunath and Venkatarao
Specific gravity	0·9283 at 30° C.	0·9692 at 25° C.
Refractive index	1·4739 at 30° C.	1·5132 at 25° C.
Acid value	8·01	19·9
Saponification value	194·7	117·2
Iodine value	96·4	96·9
Insoluble acids (fatty and resin acids)	90·5	69·0
Unsaponifiable matter ..	1·72	27·0

The figures clearly indicate that our sample is free from resins and terpenoid hydrocarbons which obviously contaminated the samples of previous workers. The high value for unsaponifiable matter was due to the presence of alkali-insoluble terpenoid compounds which we have separated from the pericarp. High acid value and low saponification value may be due to the presence of resins and hydrocarbons. The high percentage of insoluble acids in our sample along with other figures shows that it is almost pure glyceride. Further since our sample was not coloured there was no difficulty in the determination of the saponification and acid values.

Fatty acids.—About 10 g. of the oil was saponified with alcoholic potash, diluted with water and after removing by ether extraction the unsaponifiable fraction, acidified. The insoluble fatty acids thereby precipitated were extracted with light petroleum, dried over anhydrous calcium chloride and the solvent removed. The yield of the mixed fatty acids was 90·5 per cent. of the oil (Jois *et al*, 69 per cent.). It consisted of a mixture of some colourless crystalline solid and a faint brown liquid. The following properties of the mixture were noted :

		Jois *et al*
Iodine value	113·1	108·4
Mean molecular weight ..	302·3	378·6
Resin acids (Twitchell's volumetric method)	1·01%	21·5%

The separation of the saturated and unsaturated fatty acids by Twitchell's lead salt method did not offer any difficulty since the percentage of the resin acids was very small. In an estimation the value for the saturated acids was found to be 22·4 per cent. the rest being taken as undsaturated acids. The properties of the two fractions are given below :

	Satured		Unsatured	
	Our value	Jois *et al*	Ours	Jois *et al*
Percentage yield from oil ..	20·05	13·0	69·5	41·3
Iodine value	0·42	3·5	136·2	157·5
Mean molecular weight ..	311·5	294·5	316·0	320·0

Unsaponifiable matter. Isolation of a sterol acetate.—From the unsaponifiable matter a small quantity of a sterol was isolated. It could not be crystallised though it gave all the characteristic tests satisfactorily. It was therefore acetylated with acetic anhydride and sodium acetate (boiling for 3 hours), poured into a large volume of water and the solid crystallised from 95 per cent. alcohol. It was obtained as colourless plates melting at 126-28°C. and hence the sterol seemed to be phytosterol.

The cake.—The cake left behind after the removal of the oil was slightly bitter to the taste and this bitterness could be removed by boiling it with a little water. This taste was possibly due to a small amount of psoralen and isopsoralen. With a view to examine if there was much left, the cake was extracted twice by boiling with alcohol for 6 hours and the solvent distilled off. A very small quantity of solid was obtained thereby showing that almost all had been extracted by the petroleum.

The cake contained about 6·7 per cent. nitrogen (Kjeldahl) and about

7·75 per cent. of ash. The main components of the mineral matter were calcium, potassium, phosphates and silicates; iron, aluminium, magnesium, chloride and sulphate were present in small quantities only.

Physiological properties of the various fractions.—The utility of these for skin affections has been examined in the King George Hospital, Vizagapatam. The alkali-soluble resin had no effect and the alkali-insoluble terpenoid oil including the volatile oil had a pronounced depilatory property and no other influence. The volatile oil was not separately tried since it was very small in quantity. The fatty oil produced strong reaction on the skin particularly in cases of leucoderma and so also the bitter solids (psoralen and isopsoralen) when applied as a paste with lanolin. However, the work is still in progress and no definite statement could be made at this stage. We thank Dr. V. Iswariah for kindly undertaking this part of investigation.

Summary.

The components of the seeds have been obtained in a greater number of fractions which are fairly homogeneous by using a new method of analysis. It consists in extracting the entire seed with ether in the cold thereby removing all the components of the pericarp. This portion has been separated as the volatile essential oil, non-volatile terpenoid oil and alkali-soluble resin. The rest of the seed is crushed and extracted with petroleum ether. This gives rise to a good yield of the bitter principles as crystalline solids and the fixed oil in a pure condition. The physical and chemical properties of the pure fixed oil and its component acids and certain properties of the terpenoid oil have been studied in detail. A sterol (Phytosterol) in the form of its acetate has been isolated from the unsaponifiable portion of the fixed oil. The residual cake contains only traces of the bitter solid, a good percentage of proteins and mineral matter and is suitable as a feeding stuff and manure. Some of the fractions have definite action on the skin.

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