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Chemical investigation of oil from *Psoralea corylifolia* Linn.

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We report here the analysis of the oil obtained from the seeds of *Psoralea corylifolia* by identifying the individual saturated fatty acids namely Behenic, Palmitic, Stearic, Lignoceric while the unsaturated were Oleic, Linolenic and Linoleic. The overall percentage of each was also analysed with saturated being on 38.5% and unsaturated 61.4% respectively. The unsaponifiable material showed the presence of a sterol stigmasterol.

Psoralea corylifolia Linn. (Family : Leguminosae) is an important medicinal plant and has been widely exploited since ages for its magical effect against several skin diseases like Psoriasis, Leucoderma and Leprosy. Each part of the plant i.e. roots, leaves, fruits and seeds have a broad spectrum of biological activity. Bavachinin isolated from the seeds has remarkable anti-inflammatory, antipyretic and analgesic properties. Furanocoumarins i.e. Psoralen and Isopsoralen again isolated from the seeds are photosensitizers. Oil extracts from the seeds and fruits have shown antirepellant, antibacterial and antifungal activities[1-7].

The fatty acid composition and physical constants of the oil have been studied by Jois and coworkers together with Sheshadri and Venkata Rao[8,9] where they have separated the free fatty acids and the unsaponifiable matter. In another study by Shahina *et al.*[10] separation of the oil into triglycerides, diglycerides and monoglycerides has been done. Now we report the separation and individual identification of the free fatty acids from the hexane extract of the seeds.

Results and discussion

Physical constants of the oil :

Refractive index 1.4744 at 30°C; specific gravity : 0.9382 at 30°C; viscosity : 400 CP at 26.2°C; saponification value : 194.906; iodine value : 94.505; acid value : 10.467.

Percentage composition of saturated and unsaturated fatty acids by Twitchell's lead salt method :

(i) Percentage of saturated fatty acid : 37.82%; iodine value : 0.62. (ii) Percentage of unsaturated fatty acids : 58.69; iodine value : 130.28.

The TLC analysis of the free fatty acid mixture showed the presence of 6 major spots in hexane, diethyl ether, acetic acid (8 : 2 : 0.1) solvent system which corresponded to the individual free fatty acids estimated by GLC. The individual free fatty acids were identified by comparing their retention time (R_T) with R_T of the authentic samples. The percentage compositions were estimated by calculating the area of the respective peaks.

The percentage composition by GLC were found to be : saturated fatty acids 38.5%; unsaturated fatty acids 61.4%.

The major saturated fatty acids were found to be Behenic and Palmitic acids with 18% and 15% respectively while stearic acid was present in a smaller amount i.e. 5% followed by lignoceric with only 0.49%. Among the unsaturated acids oleic acid was found to be in greater amount i.e. 49.5%, while the other two i.e. linolenic and linoleic were present in smaller quantity i.e. 7.6% and 4.2%.

The results indicate that the percentage composition of the saturated and unsaturated fatty acids by Twitchell's lead salt method were comparable and in accordance to that obtained by GLC.

The unsaponifiable matter obtained by ether extraction gave positive colour test for sterols[14]. The sterol fraction could not be purified by repeated crystallization. Therefore, a tetrabromo acetyl derivative of the sterol was prepared. It was separated from other compounds on the basis of varying solubilities. The tetrabromoacetyl derivative was sparingly soluble in acetic acid, acetone and was separated from the other more soluble fractions. The tetrabromo acetate (m.p. 200°C) obtained was comparable with the tetrabromoacetate of stigmasterol (lit. m.p. 205°C), m.m.p. and Co-TLC. Debromination was followed by hydrolysis and the sterol obtained was purified by crystallization; using methanol-chloroform mixture. This sterol was identified further as stigmasterol on the basis of m.p. 168–169°C (lit. 169°C) m.m.p. and Co-TLC with the authentic sample[15,16].

Experimental

Refractive index was measured by ABBE-SPENCER refractometer (Range nD : 1.300–1.710) and specific gravity by pycnometer. Viscosity measurement was done by BROOK FIELD Programmable Viscometer : DV-II+ while GLC was carried out on a column injection using SE_{30} column with dimension 25 × 0.25 mm (i.d.). Nitrogen used as a carrier gas with flow rate 1 ml/min^{-1}. Temperature programming : 60° to 400°C (increase at the rate of 20°C/min). Nitrogen pressure : 20 psi and detector used : Flame Ionisation Detector (FID).

The seeds of *Psoralea corylifolia* were dried, cleaned and ground into a powder (2 kg). The powdered seeds were extracted with hexane 3–4 times at room temperature and then by heating up to 40°C on an electric mantle. For each extraction the solvent was distilled off and the remaining concentrate was left undisturbed overnight. It showed some crystalline deposit at the bottom, which was removed carefully by filteration and washed with hexane to remove the adhering oil. The remaining filtrate was vacuum distilled to get a dark red coloured viscous oil, weighing about 250 g (11% approx. of the weight of seed). 100 g of the oil was saponified, extracted with ether to separate the non-saponifiable matter.

Determination of physical constants of the oil :

Refractive index, specific gravity and viscosity were measured by the instruments given earlier. Saponification value, iodine value and acid value[11,12] of the oil were determined by reported methods.

Saponification of fixed oil :

Approximately 30 g of oil was taken in a round bottom flask fitted with a reflux condenser. To it 5 g of KOH pellets and 200 ml of 95% ethyl alcohol was added. The solution was refluxed for 2–3 h on a water bath.

The alcohol was then distilled to half of its original volume and the soap obtained was extracted with petroleum ether 2–3 times after dilution.

Isolation of free fatty acid mixture :

The free fatty acid mixture was obtained by the hydrolysis of the soap with 25% sulphuric acid followed by ether extraction. The etherial extracts were finally washed with water, dried over anhydrous sodium sulphate and distilled to get a mixture of free fatty acids which were stored at 0°C under nitrogen atmosphere in a well stoppered flask.

Esterification of free fatty acid mixture :

About 10 g of the free fatty acid mixture was taken in a 100 ml round bottom flask and approximately 5 times w/v of methanol containing 1% by weight of concentrated sulphuric acid was added. The mixture was refluxed for 1–2 h, after which about half of the alcohol was distilled off and the esters were extracted with ether 2–3 times. The ether extracts were combined together, washed with water to remove any traces of the mineral acid and then with 10% aqueous caustic potash solution to remove any unesterified acids. It was washed thoroughly with water and treated with anhydrous magnesium sulphate. The solvent was removed by distillation and the last traces of solvent and moisture were removed by heating at 100°C under reduced pressure. The esters were stored under nitrogen at 0°C.

GLC of methyl ester of free fatty acids mixture :

The constitution and percentage composition of mixed free fatty acids were determined by subjecting their methyl esters on to GLC[13] by injestion using SE_{30} column. The fatty acids were identified by comparing their retention time (R_T) with the authentic samples (Table 1).

Table 1. Identification of fatty acids

Sl. no.	Area (%)	R_T	% Composition	Acids identified
1.	15.062	16.71	15.00	$C_{16:0}$ (Palmitic)
2.	49.527	20.54	49.50	$C_{18:1}$ (Oleic)
3.	4.203	21.33	4.20	$C_{18:2}$ (Linoleic)
4.	7.672	21.35	7.60	$C_{18:3}$ (Linolenic)
5.	5.025	23.80	5.00	$C_{18:0}$ (Stearic)
6.	18.018	24.90	18.00	$C_{22:0}$ (Behenic)
7.	0.493	25.56	0.49	$C_{22:0}$ (Lignoceric)

Separation of saturated and unsaturated fatty acids from mixed fatty acids :

Twitchell's lead salt method[12] :

Approximately 5 g of fatty acid mixture was dissolved in 25 ml of 95% alcohol heated and to it was added a boiling solution of alcoholic lead acetate solution (3.5 g of lead acetate is 25 ml of 95% alcohol) and the contents were refluxed for 1 h in 0.75 ml of glacial acetic acid. The solution was left overnight. The deposited lead salts of the saturated acids obtained were filtered off and recrystallized with 95% alcohol and glacial acetic acid.

The insoluble lead salts were treated with sufficient hydrochloric acid to decompose the lead salts and the mixture warmed until a clear layer of fatty acids floated on the aqueous mineral acid solution. On cooling, the aqueous mineral acid solution was decanted from the flask into a separating funnel and extracted with ether. The combined ether extracts were washed with water to free it from the acid and lead chloride. The solvent of combined ether ex-

tracts was distilled off and the solid acids were finally freed from the last traces of solvent and water by heating at 100°C under reduced pressure.

The soluble lead salts after distillation of the alcohol were dissolved in ether and washed 2–3 times with water.

The ethereal solution of acids were washed with dilute HCl to decompose any residual soluble lead salts and then with water until free from mineral acid. Excess ether was distilled off and the last traces of solvent and water were removed by heating at 100°C under reduced pressure. The unsaturated fatty acids thus obtained were stored at 0°C in an atmosphere of nitrogen.

Isolation of unsaponifiable matter :

The unsaponifiable material obtained as a dark brown coloured residue was dissolved in 50 ml petroleum ether (b.p. 40–60°C), and steam was passed into the solution until the saturation point was reached. The solution was allowed to stand overnight to get a precipitate consisting of colourless crystals melting at 138–144°C.

Acetylation :

The crude material was acetylated with 20 ml acetic anhydride by refluxing for 1–1½ h. The mixture was then cooled for one hour at 20°C and the crude acetates were filtered.

Bromination :

1 g of the crude acetates were dissolved in 10 ml ethyl ether and to this solution 12 ml bromine-acetic acid solution (5 g bromine in 100 ml glacial acetic acid) was added. After cooling, the insoluble tetrabromides were filtered and washed with cold ethyl ether. Recrystallisation was done with chloroform-methanol mixture (1 : 2), m.p. 200°C.

Debromination :

To a solution of 300 mg recrystallized bromides in 4 ml glacial acetic acid, 300 mg zinc dust was added. The mixture was refluxed for 1–1½ h, filtered hot, diluted with 10 ml water and extracted with ethyl ether. The ethereal solution was washed with dilute aqueous sodium sulfite solution, then with water and ether was removed by evaporation. The product weighed about 150 mg. It was recrystallized from ethanol and then from methanol-chloroform (2 : 1), m.p. 139–140°C.

Hydrolysis :

100 mg of the debrominated product was hydrolysed for 1 h with about 10 ml 10% alcoholic KOH. About 10 ml water was then added and the mixture was extracted with ethyl ether. The ethereal solution was washed with dilute aqueous sodium carbonate and then with water. After removal of ether, the residue was recrystallized from 95% ethanol. About 30 mg of the product was obtained and its identity was confirmed by Co-TLC and m.m.p. with the authentic sample, which came undepressed at 168–169°C.

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