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CHEMICAL INVESTIGATIONS OF THE PAEONIA EMODI TUBERS

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The tubers of *Paeonia emodi* have been shown to contain salicylaldehyde, a fixed oil, starch, sucrose, fructose, glucose, benzoic acid and an unidentified substance giving different coloration at different pH. β-Sitosterol is the major constituent of the non-saponifiable matter of the fixed oil, while the saponifiable portion contains unsaturated fatty acids of C_{16} and C_{18} series.

Introduction

Paeonia emodi (family *Renunculaceae*) is an erect herb and is commonly found in the upper Hazara District and the Malakand, Khyber and Kurram Agencies. The tubers of the herb are medicinally used in uterine and nervous diseases and its seeds are used as purgative and emetic. A study of chemical literature shows that, so far, the chemical composition of the tubers has remained unknown. Nevertheless, work has been reported on the oil and the seed of *Paeonia officinalis*[1] and *P. peregrina*.[2] Characteristics, physical as well as chemical, for these oils have been ascertained, while the separation of peonin[3] and other components[4] from *P. albiflora* has been mentioned. Takaishi[5] has shown the presence of different glycosidic components in *P. suffructiocosa*.

Investigations have now been carried out on *P. emodi* with reference to its chemical constituents and the present paper sums up studies that have so far been completed. Fresh tubers of the herb as collected from Naran, Kagan Valley and Hazara District were dried in shade and ground to a powder (20 mesh).

The fractionation scheme of this material and the yields of various compounds isolated from it are shown in Chart 1.

Experimental

Steam-distillation Products: *Recovery of an Essential Oil.*—The powder was subjected to steam-distillation in an all-glass apparatus. An essential oil, amounting to 0.15%, was obtained. (i) The oil gave characteristic smell of salicylaldehyde. The b.p. of both the oil as well as that of salicylaldehyde was 197°C. (ii) Both the oil as well as the known sample of the aldehyde gave an identical violet coloration with ferric chloride. (iii) With strong solution of sodium hydroxide both gave a similar yellow coloration. (iv) The oil gave a 2,4, dinitrophenylhydrazone, m.p. 248°C, which is identical to the hydrazone obtained from an authentic sample of salicylaldehyde and 2, 4, dinitrophenylhydrozone. Mixed m.p. of both the hydrazones was also the same. It was, therefore, concluded that the oil was salicylaldehyde.

Petroleum Ether Extractive.—A new and fresh lot of the powder was extracted with petroleum ether 60–80°C in a Soxhlet apparatus. A fixed oil and an acid were separated from the extractive by means of partition in minimum quantities of cold petroleum ether. The acid was identified as benzoic acid through various tests which have been described elsewhere in the text.

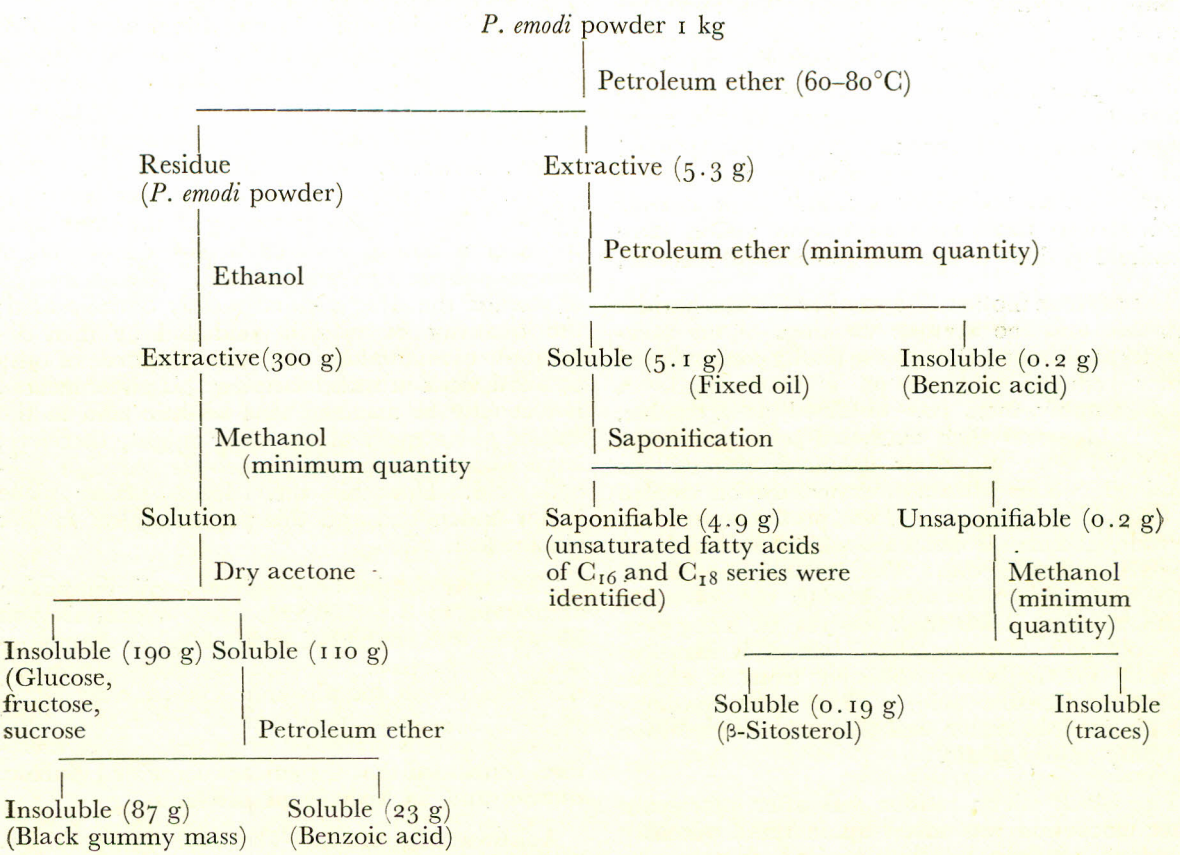
The amount of the oil obtained from the drug was 0.51%. Some of the characteristics of the oil are as follows:

Refractive index, 1.4920 at 17.2°C; setting temperature, 13–10°C; specific gravity 0.943 at 27°C; tint, 21 red and 9.9 yellow units on the Lovibond tintometer; acid no. 61.1; iodine value, 107.54 (Wij's 1 hr); ester no. 27.28; maleic anhydride value, 89.66; hexabromide value 0.56%; unsaponifiable matter 3.6%.

The oil was saponified and resolved into the saponifiable and non-saponifiable fractions according to Hilditch.[6] The fatty acids as recovered from the saponifiable fraction were esterified with diazomethane and the esters subjected to examination by gas chromatography using Griffen and George gas chromatograph Model-S18-762. The column was packed with 5% silicon elastomer, E-301, on celite 30–80 mesh and its temperature was 220°C, bridge current 125 ma and nitrogen flow 1.25 l/hr. Column inlet and outlet pressures were 52 and 46 cm respectively. Chart speed was 12 in/hr and recorder sensitivity XI. The esters gave peaks which were identical to the methyl esters of palmitic and stearic acids thus confirming that the oil contains acids of C_{16} and C_{18} series.

From the unsaponifiable matter a sterol was isolated and identified. The unsaponifiable matter was resolved into methanol soluble and insoluble portions.

CHART 1



The methanol-soluble portion, on concentration, yielded a substance having appearance of leaflets, m.p. 140–141°C. It gave positive Liebermann-Burchard test.[7] This test, therefore, indicated that the substance was a sterol. That the sterol is β-sitosterol was established by recording (1) the m.ps of the sterol (140°C) and those of its derivatives, *viz.*, acetate (127°C) and benzoate (147°C), (2) mixed m.ps of the sterol and its acetate and benzoate with an authentic sample of β-sitosterol and its acetate and benzoate derivatives respectively and (3) the IR spectrum of the sterol which was identical with that of the known β-sitosterol.

Ethanol Extractive.—The powder was extracted, by percolation, with redistilled ethanol (95%). The extract was concentrated under reduced pressure (40–50°C) and the concentrated mass was treated with diethyl ether in order to remove ether-soluble matter. The residue was dissolved in a minimum quantity of methanol and to it was added dry acetone when a precipitate settled down. The precipitate was filtered, redissolved in methanol and precipitated again with acetone. This procedure was repeated till the precipitate obtained was milky white in colour. The precipitate was finally crystallised from methanol when white cubic crystals were obtained. These crystals were identified to be those of sucrose by the following tests:

(i) They gave blue solution with $CuSO_4$ and sodium hydroxide.

(ii) They gave no reaction with Fehling's solution. On hydrolysis with dilute hydrochloric acid they, however, reduced the solution.

The crystals were further shown to be those of sucrose by subjecting them to mild acid hydrolysis and by paper-chromatography confirming the presence of glucose and fructose in the hydrolysate.

The acetone mother liquor was carefully concentrated to a minimum volume. It resulted in the precipitation of most of the saccharides.

The saccharide precipitate was washed with dry acetone. The insoluble matter was examined chromatographically using the descending paper

chromatographic technique with Whatman No. 1 filter paper and n-butanol:acetic acid: water= (40:10:22 v/v, upper layer) as the developing solvent.[8] The matter was dissolved in 10% (v/v) isopropyl alcohol and allowed to run simultaneously with the known sugars on the same paper. The dried chromatograph was sprayed with *p*-anisidine phosphate solution and placed in an oven at 100°C for 5 min.[9] The matter resolved into three significant spots which were identified as those of sucrose, glucose and fructose.

The acetone mother liquor, after the saccharides removal, and the acetone washings of the saccharides as obtained above were finally concentrated under reduced pressure on a water bath. A black gummy mass was obtained as a residue. This residue was then extracted with petroleum ether whereby a yellow coloured extract was obtained. This extract on concentration yielded a crystalline product. The product was recrystallised from alcohol when white needle-shaped crystals separated out. The crystals were found to be those of benzoic acid having the following characteristics: Their m.p. was 121°C. They gave *p*-nitrobenzyl ester, m.p. 89°C, which is identical to that of the ester obtained from a known sample of benzoic acid. They were sparingly soluble in cold water and their aqueous solution was only mildly acidic.

The black sticky residue left after petroleum ether extraction was taken up in small quantity of water. Strong solution of lead acetate was added and a dark-coloured precipitate, obtained as a result of this addition, was filtered out. The metal was removed from the filtrate by its precipitation with hydrogen sulphide. The solution was filtered and the filtrate thus obtained was concentrated to dryness. A light red-coloured powder was obtained. Its aqueous solutions were green at alkaline pH and red in acidic medium. The substance did not produce any coloration with ferric chloride solution. The exact nature of this product remains to be investigated.

Detection and Determination of Starch in the Tubers.—*P. emodi* tubers were ground to 50 mesh and extracted with 80% ethyl alcohol to remove sugar. The sugar-free sample was further ground to 100 mesh and subjected to analysis, with respect to the estimation and separation of starch according to the method suggested by Sullivan.[10] The drug was found to contain 6.7% of starch. Confirmation that the separated material according to this method was, in actual effect, starch was obtained by hydrolysing the material in question and analysing it by means of paper chromatography.[8,9] It contained glucose only which confirmed the parent material as starch.

Discussion

To begin with, these investigations were carried out with tubers which had been under storage for more than a year. In this sample, salicylaldehyde was virtually absent and the amount of benzoic acid was rather high. The fresh samples of the tubers, however, gave salicylaldehyde. The amount of benzoic acid in this sample was negligible. But drying and storing of the same drug for over a month resulted in the conversion of salicylaldehyde into salicylic acid. After 6 months of storage the drug yielded mostly benzoic acid; the quantity of salicylic acid had by then decreased considerably. In the absence of any detailed work in understanding this phenomenon, it can only be assumed that benzoic acid in the earlier old sample of the drug might, therefore, have formed from salicylaldehyde through salicylic acid. How this conversion is taking place is not understood and this point requires further elaboration.

The sugar obtained through ethanolic extraction amounted to 8.3% which after hydrolysis was identified and confirmed through paper chromatography as sucrose. As such the glucose contents of the tubers in the alcoholic extract were found to be 5.3%, after hydrolysis of the extract total glucose contents increased to 14.8%. It is, therefore, concluded that the extract contained glucose, sucrose and its hydrolysed products.

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