

sequence, by absorption in appropriate reagents. In the other case, the total volume (V_2) of I and II is first measured by absorption in syrupy phosphoric acid saturated with P_2O_5 (phosphoric acid is used since it does not absorb IV while 96% H_2SO_4 does to some extent, though both absorb I and II quantitatively) and then IV by absorption in KOH solution, followed by V and III as before. The volume of II is obtained by subtracting IV of the second analysis from V_1 of the first as also by subtracting I of the first analysis from V_2 of the second. These two values are found to agree within 4%, indicating the reliability of the method. The data of Table I show the effectiveness of the dimedone reagent, compared to concentrated H_2SO_4 , in removing methacrolein from gaseous mixtures.

TABLE I

Comparison of methacrolein estimation by absorption in concentrated H_2SO_4 and dimedone solution

Percentage of methacrolein in gas mixture		% Deviation
96% H_2SO_4	Dimedone solution	
9.64	9.45	2.0
7.27	7.12	2.1
6.04	5.90	2.3
4.16	4.05	2.6
1.50	1.45	3.3

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COMPONENTS OF THE SEED COATS OF *PHASEOLUS MUNGO* AND *PHASEOLUS* *RADIATUS*

THOUGH a good amount of work has been done on the amino acids, proteins, enzymes and carbohydrates of *P. mungo* (green gram) and *P. radiatus* (black gram) seeds and their nutrient value, no work has so far been reported on the

polyphenolic and related components. Information about them was needed for biological studies.

Miyamichi *et al.*¹⁻² reported the isolation of a saponin, m.p. 202°, from the acid portion of the seed extract of *P. radiatus*; its constitution has not yet been completely established. The acid portion also contained palmitic, stearic, arachidic, linolic, linolenic and oleic acids.

For the present work, the seed coats (husk) were obtained as follows: (1) the seeds were soaked in water for 4-5 hr and then rubbed with the hand. While the kernel submerged, the seed coats floated. They were collected and dried in the shade. (2) The seed coat was separated after milling the seeds in a small stone mill. In this connection a special observation was made. Samples of these seeds had been kept in a place accessible to rats; the black gram was left untouched and the green gram was dehusked and the kernels eaten. Explanation of the behaviour of the rats is not clear. Can the presence of polyphenols in the husk be responsible? The cleanly separated seed husk was used in the study. The samples obtained by all these methods gave the same result.

The hot alcohol extract of both *P. mungo* and *P. radiatus*, husks was separated into 2 fractions using ether.

(1) Ether insoluble fraction of both on elution with methanol : chloroform (15 : 85) from a column of silica gel, yielded the same yellow crystalline substance, m.p. 265-66° (decomp). Identity of the samples was proved by TLC, paper chromatography and m.m.p.

The compound gave brown colour with alcoholic $FeCl_3$, positive Mg/HCl and negative Zn/HCl tests. Though it gave positive Molisch's test, it was not hydrolysed by Kiliani mixture or 7% sulphuric acid. Hence the compound is a flavonoid C-glycoside. The u.v. data³ suggested the compound to be vitexin and a comparison with an authentic sample of vitexin (TLC, paper chromatography, m.m.p. and IR) confirmed the identity.

The ether insoluble portion of *P. mungo* did not yield any other flavonoid; however that of *P. radiatus* contained another phenolic component eluted by methanol : chloroform (1 : 4) from the column, in very minor amounts, highly contaminated with sugars. Apart from these, this fraction of both husks contained considerable amount of sugars and therefore the husks have some nutritive value.

(2) The ether soluble portion of *P. mungo* and *P. radiatus* gave a hydrocarbon, m.p. 85-87°, by column chromatography. Another compound, m.p.

135°, was identified as β -sitosterol. Apart from these, this contained many minor components none of which were phenolic in nature.

The presence of the C-glucoside, vitexin, in both *P. mungo* and *P. radiatus* may be of value with regard to the chemotaxonomy of pulses and the protection offered by the seed coat from the invading pathogens.

EXPERIMENTAL

Extraction and Separation.—120 g of air-dried seed coats of *P. mungo* and *P. radiatus* (from 1 Kg seeds of each) were extracted separately exhaustively with hot 95% ethanol (300 ml \times 6) and concentrated under reduced pressure. The concentrate was dried over P_2O_5 in a vacuum desiccator. The dry concentrate was macerated well with ether (50 ml \times 4) and separated into ether insoluble and ether soluble portions.

Ether insoluble portion of *P. mungo*.—The dried residue was subjected to column chromatography over silica gel (NCL) and methanol : chloroform (15 : 85) eluate gave compound A (30 mg), m.p. 265–66° (decomp.) after crystallization from methanol as yellow prisms.

Ether insoluble portion of *P. radiatus*.—The dried residue was subjected to column chromatography over silica gel (NCL) and methanol : chloroform (15 : 85) eluate gave compound A', 80 mg, m.p. 265–66° (decomp.), crystallized from methanol as yellow prisms.

Comparison of A and A'.—Both A and A' had the same R_f 0.5 in TLC (methanol : chloroform (1 : 4) and paper chromatography R_f 0.3 (15% aqueous acetic acid) and both melted at 265–66°, (decomp.). The m.m.p. of A and A' was undepressed.

Hydrolysis of A and A'.—A and A' were separately subjected to hydrolysis with Kiliani mixture (HCl : HOAc : H_2O 1 : 4 : 5) and 7% aqueous H_2SO_4 . The ether extract in each case after 4 hr of the reaction over hot water-bath was tested for flavonoids and were not detected.

U.V. and visible spectra of A and A'.—Both A and A' had the same absorption values as vitexin in methanol and in the presence of added reagents³.

Comparison with vitexin.—A and A' were compared with an authentic sample of vitexin by paper chromatography (15% aqueous acetic acid, R_f 0.3 for all). The m.m.p. of A and A' individually with the authentic sample of vitexin was undepressed.

I.R. spectra of A and A'.—IR spectra (in KBr) of A and A' were identical and compared well with the reported IR data of vitexin⁴.

Ether soluble fraction of *P. mungo*.—Benzene : petroleum ether (2 : 5) eluate (NCL silica gel) gave a compound B, crystallizing as white flakes from methanol, 30 mg, m.p. 135°. It gave positive Libermann-Burchard reaction for steroids and compared with an authentic sample of β -sitosterol (TLC m.m.p.).

Ether soluble portion of *P. radiatus*.—Column chromatography and thin layer chromatography indicated the presence of the above compound, apart other compounds in very minor amounts.

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TRITERPENOID CONSTITUENTS OF *ALSTONIA VENENATA* FRUIT PODS

Alstonia venenata R. Br. (Fam. Apocynaceae) is a shrub which grows in the hilly regions of South India—in the hills of Ganjam up to a height of 2,000 ft and in the Western Ghats and Nilgiris up to a height of 6,000 ft. The plant is used as tonic, antiperiodic and anthelmintic. Ripe fruits of the plant find use in the treatment of syphilis, insanity and epilepsy in the Indian system of medicine¹.

Like other *Alstonia* species, *A. venenata* is quite rich in indole alkaloids^{2–8} but it differs from other species in elaboration of alkaloids of vincadifformine and refractin skeletons^{5–7}. In addition to indole bases, fruits of *A. venenata* have been found to contain monoterpene alkaloid^{9–10}. No work on the non-nitrogenous principles of the fruits has yet been reported and the present communication describes the isolation and characterisation of β -amyrin and ursolic acid from the fruit pods of this plant.

The coarsely powdered and air-dried fruit pods of *A. venenata* were successively extracted with petroleum ether (60–80°) and rectified spirit. The petrol extract was freed from alkaloids by treatment with aqueous citric acid and the acid-insoluble yellowish gum was subjected to chromatographic resolution over Brockmann alumina. Elution of the column with petrol-benzene (95 : 5) mixture furnished a crystalline triterpene alcohol, m.p. 192–93° (M⁺, 426). With acetic anhydride and triethylamine, it formed an acetate, m.p. 241–42°.