Pakistan J. Sci. Ind. Res., Vol. 14, Nos. 4–5, August–October 1971

CHEMICAL INVESTIGATIONS OF SEEDS OF ABRUS PRECATORIUS LINN

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(Received July 1, 1970; revised October 2, 1970)

In the present work petroleum ether, 70% alcoholic, and ammonium oxalate extracts of scarlet seeds of *Abrus precatorius* were studied. The oil obtained was saponified and the chemical compositions of saponifiable and unsaponifiable portions were studied. Carbohydrates and amino acids present in the alcoholic extract were separated and the quantities were determined. Polysaccharides present in the ammonium oxalate extract were hydrolysed and the sugars obtained were separated and identified on paper. The uronic acid anhydride content of polysaccharides was determined.

Abrus precatorius Linn, is a shrub of the natural order Leguminacae growing wild in the plains of India, Burma and other tropical countries. There are three varieties of it namely white or white with black spot, black and scarlet.

The seeds are physiologically active and are usefull in affections of nervous system, in skin diseases, ulcer and leprosy. Seeds contain a substance that produces local inflammation of conjunctiva.[1] The oil from the seeds acts as an active antifertility agent.[2]

Ghatak and Kaul[3] reported that oil from it contained low percentages of free fatty acids, unsaturated acids and unsaponifiable matter but no hydroxy acid. The results obtained by Mandiratta and Dutt[4] are in good agreement with those reported by Ghatak *et al.*, however they reported higher percentages of free fatty acids and presence of hydroxy acids, Lefar *et al.*[5] studied the oil portion in detail by gas–liquid chromatographic method.

The qualitative studies of the free amino acid have been made by Riaz and Khan.[6] Recently Sharma *et al.*[7] have studied the amino acid contents of seed protein.

The pectic substances of *Abrus precatorius* seeds were studied by Roudier and Orillard.[8]

In the present work qualitative and quantitative estimations of free amino acids and carbohydrates present in the scarlet variety have been made and an attempt to isolate the biologically active component/components by simple method has also been made. The work on the active oil portions (four) is in progress in collaboration with the Physiology Deptartment, University of Karachi, Karachi, and will be reported later.

Experimental and Results

The scarlet variety of seeds of *Abrus precatorius* Linn., 450 g, was extracted successively with petroleum ether, 80% alcohol and ammonium oxalate. An oil (sp. gr. 0.88), was obtained on removal of petroleum ether from its extract and phospho- or sulpho-lipids, mono-, di- and triglycerides, alcohols, sterols, free fatty acids and sterol esters were present. The oil was saponified and separated by repeatedly extracting the diluted mixture with ether. All ethereal extracts were combined, washed with water and dried (Na_2SO_4). After evaporating the solvent, the residue was dissolved in alcohol and subjected to fractional crystallisation when four products were isolated with m.p. 136–38, 122–24, 134–36, and 129–21°C. These gave positive tests for sterols and their IR spectra were studied. At least one of these products is expected to be biologically active. The amounts obtained were small and the structural work is now in progress with larger quantities.

Saponifiable Portion.—From saponofiable portion free fatty acids were liberated on acidification with HCl. These were taken in alcohol and separated further by fractional crystallization: and were identified to be stearic (2.72%), palmitic (4.92%) and behenic (1.78%) acids.

Alcoholic Extract.—The solvent was removed *in vacuo* and the material obtained (11.33 g) was treated with distilled water and heated up to 50°C when a soluble S_I (9.92 g) and insoluble SR_I (1.41 g) were obtained. On keeping in refrigerator soluble portion S_I threw out a small quantity of insoluble material which was separated by centrifugation, when a soluble S_Ia and a residual portion SR_I were obtained. The soluble portion S_Ia was subjected to ion-exchange chromatography for separation of amino acids and carbohydrates.

The column was packed with activated resin Amberlite-IR120. Soluble portion was slowly applied on column and eluted with distilled water in order to separate carbohydrates from amino acids. Fractions of 10 ml each were collected at a rate of 50 ml/hr, using an automatic fraction collector. Forty-three such fractions were collected and treated for carbohydrates. Fractions 3–15 gave positive tests for carbohydrates and their optical densities were observed at 365 nm. These carbohydrate fractions are concentrated and marked CF_I (Fig. 1).

After separation of carbohydrates elution was made with 1% ammonia. Ten ml fractions were collected at the same rate. The concentration

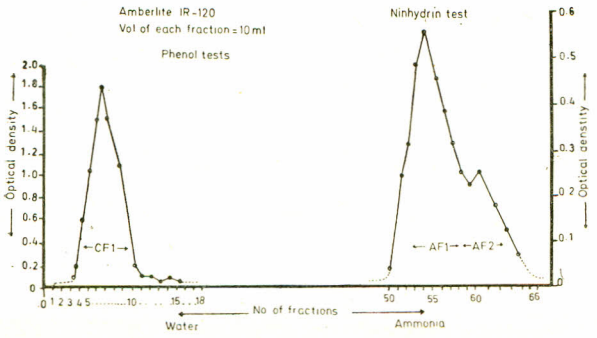

Fig.1

of ammonia was raised to 2% after 75 fractions. Seventy such fractions were collected, of which fractions 50–63 gave positive tests for amino acids. Figure 1 shows the optical densities of carbohydrates and amino acids against fractions. There are two peaks for amino acids i.e. fractions 50–58 and 60–66. These were combined and labelled AF_1 and AF_2 both these fractions were concentrated and further separated by paper chromatography.

Separation of the Residual Mass.—The residue was found to be soluble in acetate buffer, pH 1.85. The carbohydrate and amino acids were separated employing the same technique but eluted with acetate buffer. Fifty fractions of 10 ml each were collected at the same rate and tested for amino acids and carbohydrates. Fractions 14–23 gave positive test for carbohydrates. These were combined and labelled CF_2. Fractions from 24–35 contained amino acids and were combined and marked AF_3.

Paper Chromatography for Separation of Amino Acids.—Samples of amino acids were spotted on Whatman filter paper No. 1 and eluted by the descending method. Two solvent systems were used for elution: (1) *n*-butanol–acetic acid–water (4:1:5); (2) *n*-butanol–pyridine–water (5:5:2).

After elution paper chromatograms were taken out, air dried and sprayed with cadmium ninhydrin solution (5 g of cadmium acetate was dissolved in 100 ml of glacial acetic acid and 500 ml of distilled water (solution 1). To 0.5 g ninhydrin was added 50 ml of acetone and 6 ml of solution 1).

The amino acids separated corresponded most closely to the R_f values of standard amino acids and identified as shown in Table 1.

Quantitative Examinations of Amino Acids.—After the development of colour with cadmium ninhydrin paper was dried and left in tank containing H_2SO_4 for 3 hr for complete development of colour. The spots were taken out from the paper and cut out into small pieces. Their colour was taken out in 5 ml of methanol by keeping for 2 hr with occasional shaking. Optical density measurements were made at 500 nm. From the calibration curve of standards the different amino acids were estimated quantatively (Table1).

TABLE 1

Amino acids	Khan *et al.*6	Present work*			%
		AF_1	AF_2	AF_3	
Asp	+		2.0	1.36	0.8
Ser	+			5.52	1.4
Glu	+	5.13	0.81	0.52	1.6
Pro		**	**	6.32	1.6
Gly	+		0.87	5.52	1.6
Ala	+	9.8		6.64	4.1
Vat	+			2.72	0.68
Ile				1.2	0.3
Leu				2.08	0.51
Tyr				1.20	0.3
Phe	+	4.37	1.52	0.90	1.7
Lys	+			2.16	0.54
Cys	+				

* Amount represented in μ-moles
** Present but not measured
\+ Present

Paper Chromatographic Examination of Carbohydrates

Mono- and Oligo- saccharides.—Two solvent systems were used for the separation of carbohydrates: (1) ethyl acetate–pyridine–water (10:4:3); (2) n-butanol–ethanol–water (4:1:5).

In the first case time of flow was 8–10 hr while in the later the paper was run for 24 hr Whatman filter paper No. 1 (56 × 22 cm) was spotted with samples alongwith standards and run with solvents by descending method. The chromatogram after drying in air were developed with aniline phthalate (aniline 0.9 ml and phthalic acid 1.6 g were mixed together and dissolved in 100 ml saturated butanol) and were dried at 80°C water for 15 min. A single spot was observed and identified to be sucrose.

For the quantitative estimation of sucrose, Smith's phenol–sulphuric acid method was used.[9] From the calibration curve quantity of sucrose was determined and was found to be 7.59×10^{-4} with respect to the weight of the seeds.

Ammonium Oxalate Extract.—From this extract polysaccharides were precipitated with acetone (1:1). The precipitates were centrifuged and dried with ether. Out of 3.05 g of dried precipitates obtained 0.05 g was hydrolysed in a sealed tube containing 4 ml of 1N H_2SO_4 for 18 hr. After hydrolysis excess of acid was neutralised with barium carbonate. This was centrifuged and deionised by passing it through a column of Amberlite-IR120 and IR45 respectively. The deionised solution was concentrated in thin film rotary evaporator at 40–45°C. The sugars present in this concentrated mass were separated by partition paper chromatography. The following solvent systems were used: (1) ethyl acetate–pyridine–water (10:4:3); (2) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (3) butanol-ethanol–water (4:1:5).

TABLE 2

Carbohydrates	Solvent system 1	Solvent system 2	Solvent system 3
Galacturonic acid	+	+	+
Galactose	+	+	+
Arabinose	+	+	+
Xylose	+	+	+
Rhamnose	+	+	

Samples and sugars were spotted on Whatman filter paper No. 1. and run with different solvents. The chromatograms were air dried and developed with aniline phthalate. The sugars identified are shown in Table 2.

Uronic Acid Anhydride Contents of Polysaccharides.—The uronic acid anhydride content was determined by Anderson decarboxylation method[10] and found to be 84.35%.

Acknowledgement.—The authors record their thanks to Professor Salimuzzaman Siddiqi, F.R.S., for providing facilities and keen interest in this work. Thanks are also due to Professor A. Robson, Department of Fibre Science, University of Leeds, Leeds, for analysis of fraction AF_3 and Dr. Mahboob Uddin for helpful discussions.

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