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# STUDIES ON ABRUS PRECATORIUS LINN.

## Part I.—Isolation and Toxic Properties of "Abrulin" a Protein Fraction from the Seeds

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By fractional precipitation with ammonium sulphate, an active fraction, named Abrulin, has been obtained. The ultraviolet spectrum of this fraction resembles that of a typical portein with a minimum at 250 mμ and a maximum at 278 mμ. Abrulin is toxic towards albino mice in an amount as small as 1 μg. The toxicity of abrulin against four other laboratory animals, i.e., rat, rabbit, guinea pig and cat, has also been studied. Its activity is generally found to be much higher than previously reported.

### Introduction

*Abrus precatorius* or jequirity, as it is called in English, (*gunja* in Sanskrit, *ratti* in Urdu and *kunch* in Bengali ) is a perennial twiner belonging to the natural order *Leguminosae*. The seeds are usually bright scarlet, with a black spot on the top and are highly polished. There are three varieties of the seeds—scarlet, white and black— and the findings of the present paper are on the scarlet variety.

Sattler, Cornil and Berlioz[1] observed that the irritant action of jequirity was due to a special bacillus which grew in the infusion of the seeds. Warden and Waddell[2] gave the name 'abrin' to the poisonous principle of jequirity, and showed that it was proteinous in character. Martin[3] later succeeded in isolating a globulin and an albumin from the kernels of the seeds, although no quantitative account has been given by him. Both the proteins were found by him to be toxic, globulin being more potent than the albumin.

Ghatak and Kaul[4] while attempting to isolate the toxin, isloated a non-toxic crystalline compound named 'abrine' which was later on identified by Toshio Hoshino[5] as β-(β-indolyl)-α-methylaminopropionic acid. A few more papers dealing with the haemagglutination properties of a watery infusion of the seeds of jequirity have also appeared.[6,7]

The above account, to the knowledge of the authors, represents the work hitherto done on the isolation of the toxin from the seeds of *Abrus precatorius*. In the experiments detailed as follows, a procedure has been devised for the isolation of a very potent fraction from the seeds. Due to certain experimental difficulties it has not been possible, at present, to completely ascertain the homogeneity of this fraction. It has, however, been shown that the toxin is a typical protein, cf. Fig. 2, and is lethal for mice, rats, rabbits and guinea pigs in a dose of 0.04 mg. per kilo body weight.

### Experimental

*Material and Method.*—The scarlet variety of the seeds was obtained from the local market. The red coat was removed by coarsely crushing the seeds in a grinding machine. The hard yellow kernels were first roughly ground in an electrical machine and then down to 30 mesh in a hand mill to avoid exposure to higher temperature.

Due to certain limitations, only 100 g. of the powdered seeds were used at a time. The powder

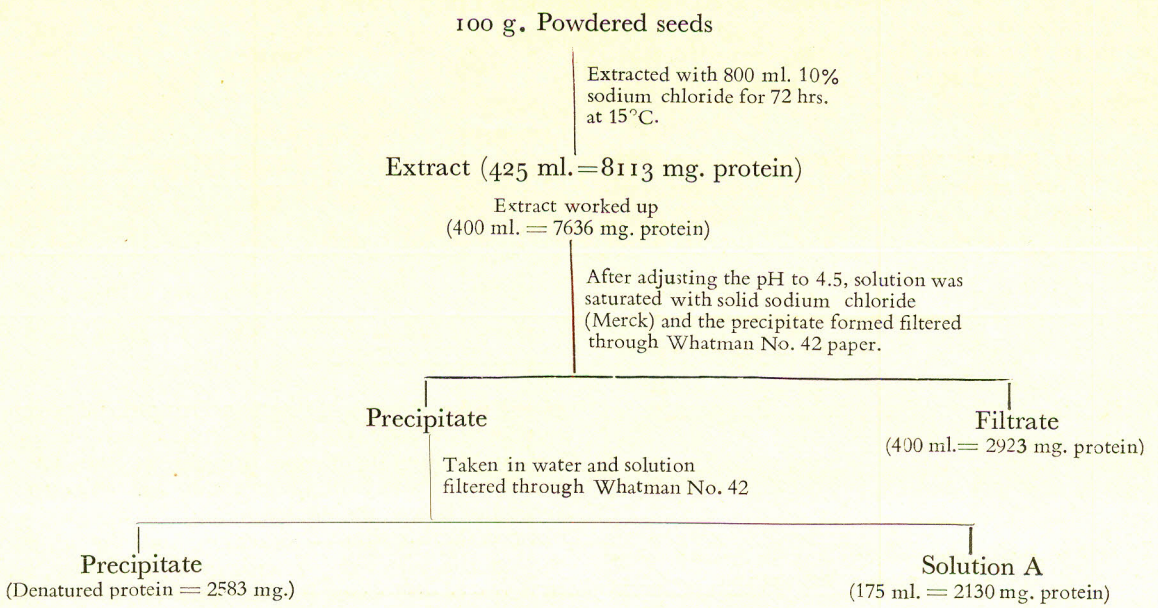


Fig. 1.—The precipitation of toxin with sodium chloride.

was extracted with 800 ml. of 10% sodium chloride at 15°C. for 72 hours. The extract (425 ml.) had a brownish tinge, and its pH was around 5.5. The extract was very slightly turbid. The proteins in the extract were determined both by micro-Kjeldahl and by Lowry's method[8] using Versatol (General Diagnostics Division, Warner Chilcott Div., Morris Plains, N.J.) as the standard. Four hundred ml. of the extract was worked up for the isolation of the toxin, as shown in Fig. 1.

The toxin was isolated from Solution A by fractional precipitation with ammonium sulphate as follows. After adjusting the pH of the solution to 4.5, it was stirred very gently with a glass rod and then 87.5 ml. of saturated ammonium sulphate solution was added to it very slowly. The precipitate (Fraction $B_1$) was centrifuged off and the clear supernatant was treated with an additional 87.5 ml. of saturated ammonium sulphate solution. The precipitate (Fraction $B_2$) was centrifuged off and the turbid supernatant was marked Fraction $B_3$.

Fraction $B_1$ was taken up in 50 ml. of 10% sodium chloride and placed at 10°C. for 24 hours to remove any insoluble material. This fraction was reprecipitated by the addition of 25 ml. saturated ammonium sulphate solution at 10°C. The protein left in the supernatant was designated Fraction $B_1$. Fraction $B_1$ has been designated 'abrulin' by the present authors to distinguish it from abrine, the crystalline non-toxic compound isolated by Ghatak and Kaul.

*The Protein Nature of Abrulin.*—The ultraviolet

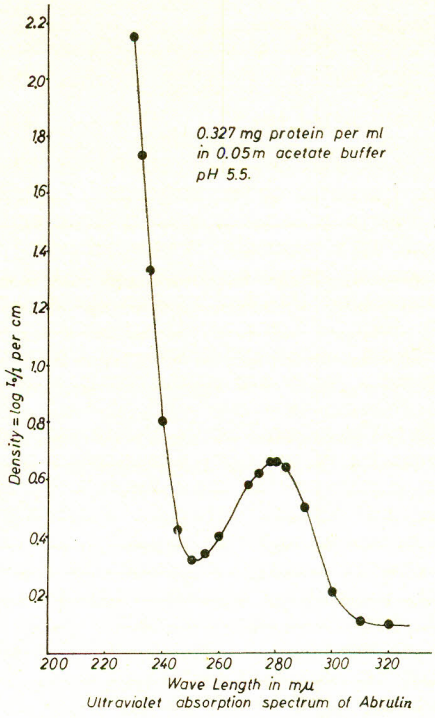


Fig. 2

absorption spectrum (Fig. 2) of abrulin resembles that of other proteins with a maximum absorption at a wavelength of 278 mμ and a minimum at 250 mμ. The toxin was dialysed overnight against the buffer before its ultraviolet spectrum was taken.

The toxin was found to be completly denatured with alcohol and could be precipitated by 10% trichloroacetic acid.

*Toxicity towards Mice.*—Fraction $B_2$ and $B_3$ were not further purified. The yields of the various fractions and their toxicity for mice are given in Table 1, which shows that most of the toxin was concentrated in Fraction $B_1$.

TABLE 1.—FRACTIONATION OF SOLUTION A BY $(NH_4)_2SO_4$ SOLUTION.

| | *Fraction* $B_1$ | *Fraction* $B_1'$ | *Fraction* $B_2$ | *Fraction* $B_3$ |
|---|---|---|---|---|
| Yield (mg. protein) | 694.75 | 17.69 | 72.83 | 116.66 |
| | Subcutaneous toxicity for mice quoted as no. of deaths within 5 days to the no. of mice injected. | | | |
| Protein injected µg | | | | |
| 0.1 | 1/2 5 days | — | — | — |
| 1.0 | 2/2 <96 hrs.* | 2/2 <48 hrs. | 1/2 <72hrs. | 1/2 <48 hrs. |
| 10.0 | 2/2 <48 hrs. | Not tested | Not tested | Not tested |
| 100.0 | 2/2 <24 hrs. | 2/2 <24 hrs. | 2/2 <24 hrs. | 2/2 <24 hrs. |

*Longest life period of any mouse which succumbed.

The toxicity of the fractions was estimated by the subcutaneous injection of 0.1 ml. of ten fold seriai dilutions of solutions standardised on the basis of protein content. The injections were given on the hind legs (gluteal region) of the animals. Female albino mice weighing 20 to 25 g. were used, two being injected with each dilution. Death or survival for five days was taken as the end point.

*Toxicity towards other Laboratory Animals.*—The susceptibility of several other laboratory animals, e.g., rats, guinea pigs, cats and rabbits, was also determined by injecting the toxin in 0.04, 0.4 and 4.0 mg. protein per kilogram body weight of the animal. It is evident from Table 2 that at higher concentrations all the animals are susceptible to the same extent but as the concentration is reduced their tolerance towards the toxin is found to vary. Cats show the least susceptibility to the toxin.

*General Action on the Body.*—In all the animals against which the toxicity of abrulin was tested, exactly similar symptoms were observed. An area of about 0.5 - 1.5 cm. in diameter around the point of injection became red and oedematous, and a slight rise in temperature was noticed at the site of injection. Even when the toxin was injected in sublethal doses, the oedema and ecchymosis was noticed at the site of injection and the animals moved the injected leg with great difficulty, and in most of the cases it was observed to be paralysed. Twelve hours after the injection (4.0 mg./kg.) the animals became drowsy, lethargic, cold and clammy, and responded very sluggishly to external stimulii. The eyes were kept half open and the movements were very restricted. The animals stopped taking food and passed loose bulky motions, in which however no blood could be observed. The rate of respiration increased and this state continued till 5 - 30 minutes before death, when the respiration rate gradually declined to complete cessation. Two to four hours before death, the animals became extremely weak and were unable to maintain the normal posture of the body.

TABLE 2

| Concentration | Guinea pig | Rabbit | Cat | Rat |
|---|---|---|---|---|
| 0.004 mg./Kg. | — | — | — | — |
| 0.04 mg./Kg. | 2/2 120 hrs. | 1/1 96 hrs. | — | 2/2 80 hrs. |
| 0.4 mg./Kg. | 2/2 48 hrs. | 1/1 48 hrs. | 1/1 48 hrs. | 2/2 36 hrs. |
| 4.0 mg./Kg. | 2/2 24 hrs. | 1/1 24 hrs. | 1/1 24 hrs. | 2/2 24 hrs. |

*Post-mortem Findings.*—In general, post-mortem examination was done immediately after death. The body of each animal was opened by a single straight incision from the middle point of the lower border of the mandible to the symphysis pubis. The following are the main changes observed in (all) the animals examined.

1. The site of injection was oedematous, congested and ecchymosed. There was slight exudation of blood tinged fluid.

2. Respiratory system. Numerous purpuric haemorrhagic spots were seen on the pleura and on the surface and interior of both the lungs. Minute haemorrhagic spots were also seen on the internal surface of trachea.

3. Circulatory system. Signs of congestion were present on the pericardium and the heart. Several purpuric haemorrhagic spots were seen on the outer surface of the heart. On opening the

larger blood vessels, a few scattered haemorrhagic spots were seen.

4. Gastro-intestinal system. Mucous membrane of the stomach, and small and large intestines were highly congested. Haemorrhagic spots were seen on the peritoneal surface of these organs and also on the peritoneum. No abnormality was observed in the pancreas.

5. Liver and spleen. Numerous haemorrhagic patches were seen on the surface as well as in the interior of these organs.

6. Kidneys. Haemorrhagic spots were seen on the surface of both the kidneys.

7. Nervous system. Haemorrhagic spots were seen on the spinal cord and in the brain.

Taken together with the toxic symptoms before death, these findings indicate that death is caused by the affection of the nervous system and widespread haemorrhages.

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