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THE TOXIC PRINCIPLE OF *COURBONIA VIRGATA*: ITS ISOLATION AND IDENTIFICATION AS A TETRAMETHYLAMMONIUM SALT

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A tuberous root submitted for examination from the Southern Sudan as having been concerned in the death of two women and since identified at Kew as *Courbonia virgata A. Brongn.*, a member of the *Capparidaceae*, has been found to contain a toxic principle of an unusual nature.

The highly toxic nature of the root was immediately confirmed by oral administration of aqueous extracts to rabbits, but preliminary examination for alkaloids yielded negative results and no evidence could be found for the presence of a glucoside. Purification of the aqueous extract by treatment with basic lead acetate was found, by toxicity tests, to leave the toxic principle in solution, and it was observed that the purified solution, after removal of excess lead with hydrogen sulphide, gave an immediate precipitate of well-formed dark green crystals with a metallic lustre on addition of Wagner's reagent (iodine in potassium iodide). This crystalline precipitate contained free iodine, and was evidently the periodide of a base. On treatment with hot water it slowly dissolved with liberation of free iodine, which could ultimately be driven off completely. After repeated evaporation of the resulting solution to dryness, baking at 100° C. and taking up with water, followed by fractional crystallization, the iodide of the base was finally obtained in pure form as colourless, well-formed crystals. Injection of an aqueous solution of this salt into rabbits and mice showed it to be highly toxic and to produce symptoms closely similar to those produced by the aqueous infusion of the root which had been originally tested. The above treatment of the material had therefore successfully isolated, in the form of its iodide, the main toxic principle present in the root. Analysis of the periodide, re-formed from the purified iodide, has shown it to approximate to the formula $BI.I_6$.

In conformity with the results obtained on the original root extract, it was shown that the base is not precipitated on alkalinization of a solution of the iodide with ammonia or caustic soda, and is not extracted from alkaline solution by the usual organic solvents; this indicated a base of an unusual type, most probably containing a quaternary nitrogen atom. Moreover the action of silver hydroxide on the iodide produced a strongly alkaline solution the conductivity of which was as high as that of a solution of caustic soda of equivalent concentration, and which, on evaporation to dryness, yielded a very hygroscopic solid.

The iodide is very stable to heat. Up to about 400° C. there was little sign of melting or sublimation, but on further heating an oily sublimate was produced. An aqueous solution of the iodide was neutral, optically inactive, non-fluorescent, odourless, and appeared to be quite stable. Analysis of the iodide was not possible here owing to lack of facilities for carrying out combustions. Even a satisfactory estimation of nitrogen could not be carried out, as the compound either shows an extraordinary resistance in the Kjeldahl analysis (even with a mercury-selenium catalyst) or loses nitrogen during the digestion. In consequence, a specimen of the salt was submitted to Dr. Harold King of the National Institute for Medical Research, Hampstead, and I am deeply indebted to him for its identification as tetramethylammonium iodide (Found: C, 24.4; H, 6.0; N, 6.8; I, 62.6. Calc. for $C_4H_{12}NI$: C, 23.9; H, 6.0; N, 7.0; I, 63.1 per cent). Its picrate melted with decomposition at 315° C. as did an authentic specimen, and a mixture of the two showed an identical behaviour.

This is the first occasion on which a salt of tetramethylammonium hydroxide has been found in the vegetable kingdom. Ackermann, Holtz, and

Reinwein (1923) found it in the animal kingdom in a sea-anemone (*Actinia equina*) and gave it the name tetramine.

As a qualitative test for the toxic principle of *C. virgata* (tetramine) the crystalline precipitate which it produces with Wagner's reagent can be used. From warm, dilute solutions, either acid or neutral, the periodide rapidly separates as well-shaped rhombic crystals which are readily recognizable under the microscope. By this means it has been shown that tetramine also occurs in the thick, scaly shoots of the superstructure of the plant, and in the leaves of the subsidiary shoots. Its presence in the shoots and leaves of the specimen sent to Kew was confirmed in the same way. Another specimen of leaves submitted from the South for identification purposes, and reputed to be of the same species as that involved in the fatalities, was identified at Kew as being *Osyris compressa* (Berg.) A. DC., " . . . absurdly like *C. virgata* in the sterile state and could very easily be confused with it in the field." A purified extract of these leaves failed to give crystals of any sort with Wagner's reagent. From the incomplete data at present available it is estimated that the fresh root of *C. virgata* contains about 0.2 per cent of the toxic base, and that about 0.25 g. of the base, taken orally, has proved a lethal dose for adult human beings, death occurring within an hour. The victims " . . . did not vomit but appeared drunk. They threw their arms about, babbled incoherently, and were difficult to control." Subcutaneous injection into mice showed the lethal dose of the iodide, by this route, to be 0.5–1.0 mg. per 25 g. mouse, the observed symptoms being convulsive spasms, collapse, and death within 30 minutes. Intravenous injection of 8 mg. of the iodide into a rabbit caused death within two minutes. With rabbits some contraction of the pupils has been observed prior to death. Daily sub-lethal doses (0.25 mg.), during which 5 mg. were administered subcutaneously, failed to confer any immunity on a mouse or to reveal any cumulative action of the poison, as, at the end of the series of injections, the mouse was badly shocked by injection of 1 mg. and four days later was killed within two minutes by injection of 2 mg.

From these results it is evident that *C. virgata* is to be classified as a relatively highly toxic plant, and from the toxicologist's point of view failure of the toxic principle to be extracted from alkaline solution by organic solvents is important. Unless its presence is suspected, and its properties known, it might easily be overlooked. After addition of 0.02 g. of the iodide to 200 g. of macerated liver the normal Stas-Otto procedure for recovery of alkaloids from such material yielded an aqueous solution of the toxic principle which, after further purification with basic lead acetate, gave an excellent positive result with Wagner's reagent. The liver of the rabbit killed by intravenous injection of 8 mg. of the iodide failed to give a definite positive result on application of this procedure. The toxic properties of *C. virgata* appear to be well known to the natives of the areas where the plant occurs (e.g., it is said to be used for poisoning hyenas), so that its toxicological aspect, about which nothing was previously known, is likely to prove important in such regions. The plant is known to occur in northern Uganda, north-eastern Kenya, and parts of French Equatorial Africa, as well as in the Southern Sudan.

A solution of tetramine iodide (1 part per 1,000) was tested for bacteriostatic action against *Staphylococcus aureus, S. typhosus, Proteus proteus, Ps. pyocyaneus,* and *B. subtilis,* with negative results in each case. It has not been possible to have trypanocidal tests carried out here, but preliminary insecticidal tests show that it appears to be an effective stomach poison for house-flies.

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