TRANSACTIONS

OF THE

NEW ZEALAND INSTITUTE.

ART. I.—*On the Toxicity of Tutu Fruit and Seed.**

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[*Read before the Otago Institute, 10th September, 1918; received by Editors, 13th September, 1918; issued separately, 14th May, 1919.*]

ATTENTION has frequently been drawn to the remarkable fact, discovered by the Maoris, that the juice of the ripe tutu-berry is harmless, while the seed is intensely toxic.

It says much for the intelligence and powers of observation of that race that such a discovery should have been made. Perhaps the possession of subjects of experiment in the form of prisoners of war played a part in establishing the fact. As the writer had already reported some experiments on the toxicity of shoots and leaves of *Coriaria ruscifolia* and *C. angustissima*, the opportunity was taken last summer of collecting some of the fruit in order to test the degree of toxicity of the seed of *C. ruscifolia*, and at the same time to examine the juice. The material was obtained by stripping the so-called "berries" off the stalks of the racemes (sample I), and in another case (sample II) by simply shaking twigs laden with fruit inside the calico collecting-bag. In the latter case only the fully ripe berries dropped off.

The juice was expressed by simple pressure on the bag, and the seed was obtained from the remainder by washing and kneading the bag till the strainings were almost colourless. By suspension in water it was then comparatively easy to separate the seed from other debris, for the latter remained suspended for a longer time than the seed. A considerable proportion of the seeds rose at once to the top and floated there, but the bulk of them sank rapidly to the bottom. The seed was dried in the air, and thus preserved for future use.

THE JUICE.

A known quantity of the juice as first expressed from the bag was evaporated down on a slow fire. The reaction remained acid during the

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* The expenses incurred in this research were defrayed out of a Government grant made through the New Zealand Institute.

evaporation. It did not seem to form a jelly at any stage in the evaporation, and merely thickened to a stiff syrup. In this state it did not grow moulds, although weaker concentrations readily did so and also readily underwent fermentation with brewers' yeast.

After standing some weeks the syrup became crystalline, but the crystals were mingled with so much precipitated material and pigment, and recrystallization was so slow, that it was difficult to obtain a quantity of the purified crystalline substance. The small quantity that was obtained evidently consisted of glucose, as shown by its physical appearance and its osazone crystals. That laevulose was also present in the crude syrup was, however, clearly shown by the following observation: The syrup was extracted with hot alcohol, clarified with charcoal, and examined in the polarimeter in watery solution; the result was laevorotation; and the solution gave very distinctly Seliwanoff's test for laevulose. That this solution contained a mixture of dextro- and laevorotatory sugars was proved by the fact that in a clear solution containing 15·5 per cent. reducing-sugar as estimated by Allihn's method the rotation corresponded to only 2·3 per cent. laevulose.

The syrup was also tested for galactose by the mucic-acid test, but with negative results, so that no evidence was found of the presence of raffinose.

The ash of the juice was found to contain a considerable amount of iron salts.

Two experiments were made in order to test whether the concentrated juice contained any of the poisonous properties of the other parts of the plant. In one a known amount of the crude syrup was diluted and administered by stomach-tube to a rabbit. No symptoms followed. The dose corresponded to about 54 grammes of the juice as expressed from the ripe fruit. It would correspond to about 2 lb. to a human adult.

In the other an attempt was made to extract any tutin that might be present. The quantity used would correspond to about 1 kilogram (2·2 lb.) of the original juice. It was covered with acetone in a stoppered bottle, and left for three weeks, with frequent stirring and shaking. The extract so obtained was heated to drive off the acetone, dissolved in water, and administered to a rabbit. No symptoms resulted which could be ascribed to tutin. The animal became practically anaesthetized and unconscious, but recovered fully in a few hours. The symptoms were probably due to some acetone or acetone compounds which had not been completely removed by the heating.

From these experiments it seems extremely unlikely that the juice contains any tutin.

Toxicity of the Seed.

After trying the hypodermic injection of solutions obtained by various methods of extracting the tutin, the conclusion was arrived at that oral administration of the seed would be the best in this case, as it resembled more closely the natural way in which poisoning might occur. Accordingly, since rabbits could not be induced to swallow the amount of seed required, a watery extract containing suspended matter was made by grinding the dose of seed in a coffee-mill, adding successive small quantities of water, and straining the extracts through cheesecloth. In this way the pulverizable part of the seed was separated from the husk, and a muddy-looking suspension was obtained which could be administered to the rabbit by stomach-tube. The residue left on the straining-cloth was considerable; in several cases where it was collected and dried it

amounted to 50 to 60 per cent. of the whole dose of crushed seed. In order to test whether such residues contained any appreciable amount of tutin, a fairly large amount was dried, extracted with ether, and the ether-soluble material administered to a rabbit in watery suspension by stomach-tube. It produced no symptoms whatever in a dose equal to 7·5 grm. of "husk" per kilogram body weight, so that the bulk of the tutin may be supposed to have been present in the watery suspension. When the seed was administered in this way the results shown in the table were obtained.

TOXICITY OF SEED

No.	Material used.	Dose per Kilogram, in Grammes.	Result.	Estimated Percentage of Tutin in Seed.
256c	Sample I	3·7	Death in three hours	0·16
267	Sample II	3·7	Slight, if any, symptoms ..	..
270	"	4·0	Distinct minor symptoms	0·14
*295	"	4·3	Death in about three hours ..	0·16
272	"	4·5	Symptoms more marked than in exp. 270	0·12
274	"	5·0	Severe symptoms, but recovered	0 11
282	"	6·5	Death in three hours	0·10
296	Green seed	6·0	Death in one hour and a half ..	0·125

* This animal had been used for exp. 274, and had then had its thyroid gland removed, about four weeks before being used for exp. 295

The percentages of tutin in these experiments have been calculated from the results of previous work by Fitchett and other experiments by the writer. The standards adopted for rabbits were that a dose of 5 milligrams per kilogram produces no marked symptoms; 6 milligrams produces symptoms in one hour and a half, and is ultimately fatal; 7·5 milligrams produces symptoms in about half an hour, and death between one and two hours.

Sample I of the seed was used in only one experiment, and in that case was more toxic than sample II. It occurred to me that this might be due to a difference in the toxicity of green seed as compared to ripe, for, owing to the method of collecting it, sample II contained more ripe seed than sample I. Fortunately, although it was late in the year (May), I was able to procure locally sufficient green berries to put the matter to a test.

At first it seemed impossible without serious loss of time to mechanically separate the small unripe seeds from the green fleshy petals, and after separating enough to find the percentage of seed present (18·2) the whole unripe fruit was administered in the form of a watery suspension. This gave the following results: 27 grm. fruit per kilogram caused death in forty-five minutes; 14·4 grm. in ninety-five minutes; 10 grm. in two hours and a half. As 10 grm. fruit contained only 1·8 grm. seed, it followed that either the unripe seed contained a very large amount of tutin, or that, at this stage, it was also present in the fleshy petals. To decide this point it was necessary to obtain a clean sample of unripe seed, and after several attempts the following method was found to be successful: Berries were dried in air at a moderate temperature and rubbed between the fingers; by this means the seeds were isolated from the remainder. The material was then put,

in small quantities at a time, into a mixture of naphtha and chloroform of such a specific gravity that the seeds floated while all the debris sank, and by skimming off and drying the seed a fine clean sample was obtained. When administered to a rabbit this gave the result shown in the table, exp. 296—viz., the percentage of tutin was no greater than the average. It follows, therefore, that at an early stage in the formation of the berry the fleshy petals contain as much tutin as other green parts of the plant—in fact, the percentage works out to the same (0·06) as was found in the green shoots of *Coriaria ruscifolia.*

It would be interesting to be able to follow the fate of the tutin in the petals. Is it transformed *in situ* into a constituent of the innocuous juice, or is it transported to other parts of the plant? If the former takes place, one is tempted to believe a ferment might be found capable of affecting the transformation, and such a ferment would be of value in destroying tutin while still in the paunch of stock poisoned by tutu. So far I have not been able to get any evidence of the presence of such a ferment.

Effects of Administration of the Oils.

Practically all parts of the tutu-plant, but especially the seeds, contain a considerable amount of a green-coloured oil—"oil of tutu"—which was believed by the earlier workers (Skey, Christie) to be or to contain the poisonous principle. That the latter supposition was the correct one was proved by Easterfield and Aston, who showed that tutin, quite apart from the oil, was sufficiently active and abundant to account for most, if not all, of the symptoms of tutu poisoning. The question still remained, however, whether the oil or oils had any action which if not toxic itself might influence the toxicity of the tutin. To throw some light on this I used the oil as obtained by extraction with mineral naphtha, which had proved itself a good solvent for oil, while it was unlikely to dissolve tutin, as this substance had been shown by Easterfield and Aston to be insoluble in benzene. Chloroform extracts were also investigated, because it was noticed that, after naphtha extraction had been carried on till the extracts were colourless, chloroform was still able to extract some green-coloured oil, probably another fraction of the mixture of oils present. The following experiments were done:—

(*a.*) 50 grm. seed (sample II) was extracted first with mineral naphtha till the extracts were colourless, then with chloroform. The chloroform-soluble part was mixed with a little alcohol and added to water, the result being a fine precipitate or suspension of the oil. This was administered to a rabbit by stomach-tube. The animal became unconscious, and remained so for about three hours. Next day it appeared to be quite well. The symptoms were probably due to the dose of alcohol, which unfortunately was not measured. No distinct tutin effects were observed.

(*b.*) A quantity of the oil extracted with naphtha was freed from all but traces of the solvent by heating it on a water bath; some olive-oil was added, and a small amount of egg-white and 1 per cent. sodium carbonate. The mixture was then emulsified by shaking, and administered by stomach-tube. No symptoms developed beyond some somnolescence. The amount of green oil given would amount to about 10 grm. = 8 grm. per kilogram for the rabbit used.

(*c.*) 50 grm. seed (sample II) was extracted with alcohol, and the residue extracted with chloroform. A considerable amount of green oil resulted. This was boiled with about a litre of water, filtered, and evaporated down on a water bath More "oil" continued to separate as evaporation proceeded, and was removed by filtration. The final result was 10 c.c. of watery extract of the "oils." Of this 5 c.c. was administered by hypodermic injection to a medium-sized rabbit. No symptoms followed.

(*d.*) 20 grm. seed (sample I), previously extracted with naphtha, was extracted with chloroform, which removed a further quantity of green oil. After driving off the chloroform the oily residue was extracted with 100 c.c. water, filtered, and concentrated to 10 c.c. Of this 5 c.c. given hypodermically produced no symptoms.

The conclusion to be drawn from these experiments is that the oil, or oils, has no toxic action. It is probable that the chloroform extracts contained some tutin, for tutin is soluble therein to a small extent, but the amount was either originally too small to produce symptoms or it underwent destruction in making the hot-water extracts.

METHODS OF EXTRACTING TUTIN.

Although at present there seems little likelihood that the pure substance, tutin, will ever be of any therapeutic or other commercial value, it may be of use to workers on the subject to add a note on the methods of extracting it. The best source of tutin is the seed—ripe or unripe. Drying in the air probably does not lead to any loss, but crushing and grinding, especially when combined with watery extraction and evaporation, lead to considerable loss. So far as my present experience goes, the best method is to extract the oils from the dried and recently crushed seed with mineral naphtha, and then extract the residue with ether. The ether-soluble material can then be again extracted with naphtha to remove more of the oils, and the result is an extremely toxic material, which can be further purified as described by Easterfield and Aston.

SUMMARY AND CONCLUSIONS.

1. No evidence was obtained of any toxic substance in the juice of the ripe tutu-fruit.

2. The green petals of the unripe fruit contain as much tutin as other green parts of the plant.

3. Both ripe and unripe seeds contain between 0·1 per cent. and 0·6 per cent. of tutin, being about double what is found in young shoots in the natural state. (When the water percentage is taken into account there is not much difference.)

4. The constituents of the seeds soluble in naphtha and chloroform (oils and resins) were not found to possess any toxic action.

5. The sugars present in the juice of the berry appear to be a mixture of dextrose and laevulose.

REFERENCES.

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