THE CHEMICAL EXAMINATION OF MACROZAMIA SPIRALIS.

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Historical.—The family of the Cycadaceae are all tropical or subtropical plants. The genera have a very limited distribution, and are few in number, but these represent the remnants of a once extensive flora which covered the earth in the Palaeozoic and Mesozoic Eras. Scott, in his "Evolution of Plants," says that in the Secondary Floras about one plant in every three was a Cycad, and they stretched from the Equator to the Poles. They were the dominant class; there was nothing above them; they were the best thing in the way of flowering plants that their age had produced. Though these in giving rise to the Angiosperms gradually became extinct, yet from some less progressive and therefore less highly organised cycadaceous forms, we may trace through the Tertiary Era the plants which linger on to our present day. In the descendants of this ancient race of plants we still find those primitive functions and primitive structures which closely resemble those indicated in the fossils of the Carboniferous Period, and which give to the Cycads a history and an interest unique among plants.

The fossil cycads make their first appearance with the genus Pterophyllum in the Upper Carboniferous formations, and reach their maximum towards the end of the Triassic and the beginning of the Jurassic Periods. Of these ancient forms Schimper recognised 34 genera which include 278 species.

Distribution.—The Cycadaceae of the present day, according to Engler and the Index Kewensis, include only nine genera and 75 species. Four genera, including Zamia, belong to tropical America, two are confined to the African continent, and three are found in Australia. The last include Cycles which is widely distributed from India to Japan and through the Islands to Australia, one Queensland genus, and Macrozamia which is limited to Australia.
There are fourteen species of *Macrozamia*, four of which belong to New South Wales, and four to Queensland; four are common to both States, and two are found in Western Australia. They are confined to the coastal regions of our Continent, and on the Eastern side are not found beyond the Dividing Ranges. There are no native cycads in Victoria, nor in the great central deserts of Australia. *Macrozamia spiralis* has a geographical coast range of over 800 miles extending from north of Brisbane to the Victorian border.

**Evidence of Poisonous Character.**

Nearly all the cycadaceous plants are believed to contain some poisonous principle in their sap, and it is said to be concentrated in the seeds. This fact seems to be well known to all the native races in the lands where cycads are used for food. We find that they uniformly pursue an elaborate course of preparation to destroy the poisonous properties. This consists in crushing the seeds with large stones, in washing the pulp in bags laid in running water for a certain time, and lastly, in drying the mass and heating it over a fire. This method of washing and baking the pulp, as used by the Australian aborigines, is almost identical with that used by the natives of Brazil in preparing the arrowroot from the poisonous cassava—*Manihot utilissima*.

In this connection also, Greshoff describes how the natives of the Malayan Archipelago eat the seeds of the poisonous cyanogenetic plant, *Pangium edule*, but never without submitting them to the same treatment.

*Cycas revoluta*, a Japanese tree, and *Cycas cirrata*, a tropical East Indian cultivated plant, are both described as having poisonous properties. In preparing the starch or sago for food their seeds are first roasted, and then washed in running water for a long period to remove an astringent emetic substance. The aqueous extract is described as fatal to chickens. Van Dongen (23) examined the latter plant in 1903, and mentioned an amorphous glucoside, pakoein, as the poisonous principle, but apparently nothing further was done with it.

*Cycas media* of Queensland, the largest of all cycads, is also poisonous (7), and produces a kind of paralysis of the hind limbs in cattle, sheep, and horses [Pammel (28), Ewart (29)].

In Banks’ Journal (1770) there is an incident recorded, where some of Captain Cook’s men found the hulls of the nuts round a deserted camp fire of the aborigines on the coast of Eastern Australia. They were thus assured that these nuts were used as food. They found them growing in the bush and ate one or two, after which they became very ill and were violently affected with vomiting. Some of his pigs died and many others after showing very severe symptoms just recovered (1). Leichhardt also described this cycad and how the natives prepared food from it (16).

*Zamia integrifolia* of tropical Florida is also used in a similar manner for the preparation of arrowroot. American chemists have attempted to isolate the poison, but without success.

*Zamia muricata* and *Z. Fraseri* have the same poisonous seeds, which are treated in a similar manner for the preparation of their starch. The tuberous bulbs also are poisonous.

*Xanthorrhoea*, the Australian grass-tree, though a monocotyledonous plant and therefore far removed from the cycads, is reported to cause the same poisonous symptoms as the latter, when the young shoots or green buds are eaten by cattle.
Macrozamia.—All the species of this are reputed poisonous plants, and the records of their harmful nature extend from the earliest days of the colonies. Governor Phillip, in 1789, gave an account of *M. spiralis* having formed part of the diet of the Port Jackson aborigines, and of its having caused La Perouse’s sailors to become very ill with vomiting and diarrhoea after eating the nuts or kernels of the seeds (16).

In Grey’s Journal of his two Expeditions in 1837 we have a detailed account of the use of the seeds by the natives, of the careful treatment in order to remove the poisonous constituent, and of the evil effects produced by eating these seeds without this preparation. Grey found *M. Frazeri* on the Gairdner Range and Mount Horner. Several of his men ate the nuts and were taken violently ill with vomiting, vertigo and other distressing ailments, but all recovered next day (3). This “By-yu” nut of the natives he describes as a violent emetic and cathartic. The natives soak it in water, bury it in the earth till the pulp is dry, then roast it for food (2).

Mr. J. H. Maiden records the poisoning of three boys at Springsure in Queensland through eating the nuts of *Macrozamia Perowskiana* (16).

Baron von Mueller was quite convinced that all the cycadaceous plants are pervaded by a virulent poison principle, which becomes inert or is expelled by heat (6).

Moore, in describing the methods of the aborigines in preparing the starch for food, says that in the fresh state the seeds are dangerously acrid (8).

*Macrozamia spiralis* was first examined chemically by Norrie (a Sydney pharmacist). His report was published in Dr. Milford’s paper (5), which was read before the Royal Society of New South Wales in 1876. Norrie stated (a) that the kernels of the seeds contained much starch and gluten; the soluble portion had an acid reaction, and lime water precipitated calcium oxalate; (b) that he had isolated potassium binoxalate which he stated was the poisonous substance in these nuts; and (c) that he had also observed microscopic crystals of an alkaloid in small quantity. He believed that when the nuts were heated by the natives the potassium binoxalate would be converted into carbonate, and thus rendered innocuous. Dr. Milford, in his paper, described the effects on human beings after eating the nuts, viz., the severe suffering like sea-sickness, diarrhoea, and cramps in the abdomen.

On the other hand, Dr. Bancroft stated in a Government report that the kernels contained no poison, and that extracts of the nuts produced no deleterious effect when injected into frogs and guinea-pigs. He observed that when fowls and ducks were fed at one time with a large quantity of the kernels death frequently ensued after 1 or 2 days from gastro-enteritis, caused by the indigestibility of the material. He stated that all parts of the plant are indigestible (9).

Mr. F. Turner, in 1893, described the poisonous properties of the two species *M. Miquelli* and *M. spiralis*, and the methods of the aborigines in preparing the starch for food (11).

In 1894, Govt. Vet. Surgeon Edwards, of Western Australia, wrote a report describing his experiments on feeding cattle with *Macrozamia*, and the disease produced known as rickets or “wobbles.” This is the most detailed account we have. He says the disease has been known since 1865, and is peculiar to Zamia districts. It is characterised by partial paralysis of the hind limbs, the diminished muscular power giving rise to a wobbling gait. The symptoms occurred after a
definite period according to the amount eaten. A one year calf ate 6 lbs. of leaves per day with other food, and showed symptoms on the seventh day; another ate 4 lbs. per day with wheat chaff, and became ill on the eleventh day. The author could thus induce the disease at any time and had no doubt as to the cause. After the second week the disease became thoroughly established. It did not produce death, but resulted in starvation. Edwards fed cattle with the leaves, nuts, the mucilaginous secretion, and aqueous extracts of all parts of the plant. These mucilaginous juices and extracts produced, in cattle, congestion of the fourth stomach, intestines, liver and kidneys. He concluded that the deleterious effects were due to the extremely indigestible nature of the plant, and not to any organic poison (12, 13).

Lauterer, who was experimenting in a similar way in Queensland, doubted the conclusion of Edwards, and set out to prove his assertion of the existence of a direct poison in the *Macrozamia*. In 1898, he published his results, and described the symptoms he observed of spinal meningitis or progressive paralysis (18).

Bancroft, after many trials in searching for micro-organisms in the animals affected with "wobbles," obtained invariably negative results. He described the disease as Zamia paralysis.

Lauterer stated that the leaves of *M. spiralis*, at certain times of the year, contained a considerable amount of a poisonous resin, soluble in ether. The time corresponded to the period of flowering and fruiting. The resin existed in greatest amount in the nuts, and was also present in the half subterranean stems or bulbs and the leaves.

In guinea-pigs and cats the feeding produced gastro-enteritis and death. The author could not produce "wobbles" in any animals, but stated that enteritis, through inanition, might lead to it.

Lauterer and Pound then continued their experiments, by feeding calves with chaff mixed with the cut-up leaves of *Macrozamia* in the flowering stage. The first calf ate 8 lbs. of *Macrozamia* leaves per day for 3 days, then refused to eat more, and died on the fifth day. The second calf refused to eat *Macrozamia* on the fifth day, ate lucerne for 2 days, and died on the tenth day. The third calf ate for 6 days before refusing, and lived on green pasturage till the tenth day, when it, too, died. All these animals walked slowly, and staggered from weakness. Post-mortems revealed symptoms of gastro-enteritis only, with inflamed membranes of stomach and alimentary tract (18).

Lamb, in 1895, recorded the death of a great number of cattle in North Queensland from paralysis of the hindquarters, attributed to the eating of young shoots of *M. Miquellii* (17).

Poisoning by *M. Fraseri* is recorded by Crawley in Western Australia, 1898. Twenty-four bullocks died after eating the leaves. Owing to a gradual loss of vitality, the animals lay down for a few days in a helpless and semi-paralysed condition, and finally died. On post-mortem examination, the contents of the omasum were found impacted with ingesta, which were abnormally dry. The abomasum and intestines were empty. The spinal cord and meninges were in an abnormal condition (19).

Dr. Hunt, of Queensland, carried out a series of feeding experiments in 1899. He observed that cattle fed on leaves, stem, bulb, and male and female fruits, became affected after 14 days, the ration being 2 to 4 lbs. per day. Of
the animals suffering with Zamia paralysis, some being recent acute, others old chronic cases, the author took blood, spinal and synovial fluids, and emulsion of cord, and injected these into other healthy cattle. He failed in all cases to infect the latter. He remarked that no case of disease had ever been produced by injecting hypodermically, or by internal dosing with any substance extracted or isolated from Macrozamias, but only by feeding with the plant itself. After long persistence in feeding with the plant, the practical permanence of symptoms was associated with peripheral neuritis.

Similar conclusions were arrived at by Professor Smith, of Sydney University, as a result of his experimental work.

Mr. J. H. Maiden described a convincing instance of cases of poisoning in 1895-1898. In a paddock in which Macrozamia plants were growing, stock were badly affected. The cattle were removed and the cycads cut down completely, but left lying in the paddock. Next year the stock were put back, and in six weeks were again suffering badly. Their stomachs were filled with the dried Macrozamia leaves. In 1897, the paddocks were closed again, till after one year cattle were admitted. They ate the dried withered leaves and all became ill. In 1898 the withered leaves had all disappeared, and only the roots were left, which had been dried for over 3 years. Cattle, when put in again, ate the roots, and became ill as before.

Professor J. D. Stewart, in 1899, then chief Veterinary Officer for New South Wales, conducted an enquiry into an outbreak of the disease at Moruya. A hundred head of cattle of all ages were affected. The symptoms were observed in all stages, and were described in detail and illustrated by photographs. Post-mortem examinations also were conducted. These led to the conclusion of the existence of partial motor-paralysis of the hind extremities, due to loss of nervous control over the actions of the muscles of the parts affected.

In the following year, Professor Stewart carried out feeding experiments on cattle. He gave each 2 lbs. per day mixed with chaff. Symptoms of the disease were indicated on the 23rd day, and the condition thoroughly established in all its manifestations 8 days later. This condition was shown to be permanent, and for all practical purposes incurable.

In 1906, Mann and Wallas analysed Macrozamia Fraseri, the Western Australian species, and concluded that the effects upon cattle induced by eating the plant are caused by potassium oxalate [confirming Norrie's result on M. spiralis (5)]. The authors referred to the plant but did not say whether leaves or nuts were used.

In 1912, Inspector Marks was sent to the Tabulam district, New South Wales, where in 1900 over 400 cattle had died. Heavy losses had been experienced each succeeding year till at last the leases had been given up. Zamia eaters with the confirmed habit had taught the whole herd, though plenty of green fodder was available. After some years, this neglected land was again leased for grazing and the herds were at once affected, there being many fatalities.

Dr. Cleland carried out feeding experiments on Milson Island in 1912 and 1913. The leaflets of M. spiralis were cut up small and mixed with chaff, 1 to 2 lbs. per day being given to each animal which was then well fed with other nourishing food. These experiments were of 5 months' duration, and no signs of any poisoning effects were discovered. The author's comment is that if
Macrozamia contained any actual poisonous constituent, some signs of its action would have been manifest in 5 months. Cattle in the poor Macrozamia country, however, would eat the plant in sufficient quantity to keep alive, but were not being supplied with vitamine (26).

The dietetic deficiency theory put forward by Dr. Cleland was not accepted by Professor Stewart, who has since conducted further feeding experiments. These eliminate any suspicion of lack of vitamines being the cause of the disease. He has obtained positive results in so far as the disease was established in animals receiving a "sufficient" diet, with an allowance of Macrozamia. It is understood that the details of these later feeding experiments will be published shortly.

In 1917, Mr. F. B. Guthrie analysed the nuts and leaves of M. spiralis, and the following results were published (27).

<table>
<thead>
<tr>
<th></th>
<th>Kernel</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>81.79</td>
<td>76.67</td>
</tr>
<tr>
<td>Ash</td>
<td>1.07</td>
<td>.99</td>
</tr>
<tr>
<td>Ether extract</td>
<td>.18</td>
<td>.40</td>
</tr>
<tr>
<td>Fibre</td>
<td>4.74</td>
<td>6.18</td>
</tr>
<tr>
<td>Albuminoids</td>
<td>.03</td>
<td>2.64</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>12.19</td>
<td>13.31</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

It is important to note from these figures for the kernels, after deducting the water and calculating the dry substance, that two thirds of this is starch and one quarter fibre.

**Experimental Work.**

Macrozamia spiralis Miq. grows in great abundance in certain districts, north and south of Sydney. A large stock of the leaves obtained from Bateman's Bay on the south coast of New South Wales, was made available by Professor Watt in connection with the investigation of this plant as a possible source of raw material for the manufacture of commercial alcohol.

The sample consisted of the entire rachis and leaflets, each about six feet long. Through the kindness of Mr. G. Wright, these were air-dried and put through a disintegrator in the School of Agriculture at the University. The fine dry powder thus obtained weighed 10.4 kilograms.

**Proximate Composition of the Leaves.**

A portion of the leaf-powder was dried at 100° C. for water content, then incinerated to obtain the amount of crude ash. Another portion was completely extracted successively with various solvents, in a Soxhlet extractor; the extracts were evaporated, and the residues dried at 100° and weighed. There was left an insoluble powder containing the cellulose, fibre, and other indefinite substances. The following results were obtained for the air-dried leaf-powder, and have been also calculated for the fresh and completely dried leaves.

<table>
<thead>
<tr>
<th></th>
<th>Fresh leaves</th>
<th>Air-dried</th>
<th>Dried at 100°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>60.0 %</td>
<td>9.6 %</td>
<td>—</td>
</tr>
<tr>
<td>Crude ash</td>
<td>1.7</td>
<td>3.8</td>
<td>4.2 %</td>
</tr>
<tr>
<td>Extld. by solvents</td>
<td>12.9</td>
<td>29.2</td>
<td>32.3</td>
</tr>
<tr>
<td>Insol. residue</td>
<td>25.4</td>
<td>57.4</td>
<td>63.5</td>
</tr>
</tbody>
</table>
The various organic solvents removed in solution the following amounts:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Percentage of Total Solubles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum spirit (b.p. under 50°) extracted</td>
<td>1.19%</td>
</tr>
<tr>
<td>Ether</td>
<td>1.26%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>6.04%</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>13.08%</td>
</tr>
<tr>
<td>Water</td>
<td>7.60%</td>
</tr>
<tr>
<td>Total soluble substances</td>
<td>29.17%</td>
</tr>
</tbody>
</table>

A portion of the leaves was specially tested for alkaloids by extracting with chloroform-ether-alcohol mixture. The extract, after removal of the solvents, was heated with dilute acid, and on applying the characteristic tests for alkaloids gave entirely negative results.

Another portion of the leaf-powder was treated specially for the isolation of oxalic acid or oxalates, and these were proved to be absent.

**Proximate Composition of the Nuts.**

The bright red ovules or seeds were collected and used in the fresh condition. One average ovule consisted of:

- Soft red outer cover: 10 gms.
- Hard shell: 2 gms.
- Soft white kernel: 6 gms.
- Total weight: 18 gms.

These kernels contained a harder core in the centre, and were easily cut like a potato. When they were grated down they became a sticky pulp, resembling thin dough, mixed with much mucilage. Exposed to the air, the pulp soon became dry and brittle, and was then easily powdered in a mortar. A portion of this was extracted with alcohol, which dissolved out a small amount of fixed oil, and then extracted with water. The insoluble residue from this extraction was dried and weighed. In a second portion the nitrogen was estimated by Kjeldahl's method. In a third portion a careful examination was made for salts of oxalic acid. This was subsequently repeated with a much larger sample, and the minute precipitates carefully examined under the polarising microscope, but in none of these were any of the characteristic calcium oxalate crystals seen.

A large sample was washed in a muslin cloth under cold water, after which the white starch which deposited was dried and weighed, likewise the insoluble fibrous residue in the cloth was separated and weighed. The aqueous solution contained a considerable amount of a thick gelatinous slime, or mucilage.

**Composition of the Kernels or Seeds of the Macrozamia Nuts.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Fresh Kernel</th>
<th>Dried at 100°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>42.6%</td>
<td>-</td>
</tr>
<tr>
<td>Ash</td>
<td>1.0%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Extracted by alcohol (oil)</td>
<td>4.25%</td>
<td>7.4%</td>
</tr>
<tr>
<td>hot water</td>
<td>4.2%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Insoluble residue</td>
<td>48.0%</td>
<td>83.6%</td>
</tr>
<tr>
<td></td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1.8%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Starch</td>
<td>39.0%</td>
<td>68.0%</td>
</tr>
<tr>
<td>Residue left in cloth (fibre)</td>
<td>15.1%</td>
<td>26.3%</td>
</tr>
</tbody>
</table>
Complete Chemical Examination.

Extraction.—For the purpose of a detailed investigation of the constituents of the leaves, 6 kilograms of the air-dried plant powder were extracted with 80% alcohol, by maceration and percolation at room temperature. Three large percolators were employed. The fresh spirit was added to the first, the percolate from this run into the second, and the percolate from the second added to the third, thus making one complete extract. Each complete extract was assayed for total solids contained in it; in this way the progress of the extraction was ascertained.

1st extract, 3 litres, contained 285 gms. solid matter.
2nd " 3 " 246 "
3rd " 3 " 171 "
4th " 3 " 114 "
5th " 3 " 63 "
6th " 3 " 36 "
7th " 3 " 24 "
8th " 4 " 24 "
9th " 3 " 12 "

28 litres 975 gms.

The total solid matter, soluble in alcohol, thus obtained, represents 16% of the air-dried leaf-powder. The 28 litres of alcoholic extract consisted of a dark brown fluid, and were distilled under diminished pressure to a thick syrup. The temperature of distillation did not exceed 40° C. After removing the solvent in this way there remained in the still a thick, black, tarry product.

Distillation of the Extract in a Current of Steam.—The semi-solid mass was then distilled in a current of steam, when there was obtained 2.5 litres of aqueous distillate, and in the still an insoluble resinous mass with a large volume of hot aqueous liquid. The latter was filtered hot, the solid portion boiled in water, and decanted many times till the washings were colourless. This substance, insoluble in hot water, when dry weighed 392 gms. The filtered solution and washings were set aside to cool, and after a few days a quantity of a brown solid deposit separated, which was washed with cold water, and when dry weighed 64 gms. The following portions were obtained:

A. Volatile steam distillate 2.5 litres
B. Aqueous solution 7 "
C. Chlorophyll and insoluble resins 392 gms.
D. Brown deposit on cooling the aqueous soln. 64 "

The insoluble substances in C and D, weighing 456 gms., made up 47% of the whole alcoholic extract.

Examination of the Volatile Portion, A.

The distillate, measuring 2.5 litres, and showing a distinctly acid reaction to litmus, was shaken out with ether many times.

(1). The remaining aqueous fluid was first examined: it was still acid in reaction to litmus. When boiled with Fehling's solution it produced a slight reduction. It also reduced mercuric oxide and silver nitrate when boiled, indicating the presence of a small amount of formic acid. A little of the solution
was evaporated with sulphuric acid, when the pungent vapours of acetic acid were recognised, and on addition of alcohol, the odour of ethyl acetate was very marked.

The whole fluid was neutralised with baryta water and evaporated to dryness. This left a residue of barium salts of the organic acids weighing 0.93 gm., which was converted into barium sulphate.

0.93 gm. Ba salt yielded 0.847 gm. BaSO₄ = 91.1 %

Acetic acid requires _ = 91.3

The barium salt represents 0.54 gm. of acetic acid in the aqueous solution, with a trace of formic acid.

(2). The ether extract of the volatile distillate was agitated successively with ammonium carbonate, sodium carbonate, and sodium hydroxide until nothing further was removed in solution by each solvent. These alkaline fluids were then acidulated and extracted with ether, the solvent distilled off, and the residue converted into barium salt by titration with decinormal baryta solution. The latter was decomposed by sulphuric acid, and the barium sulphate weighed.

The ammonium carbonate extract was too small in amount for analysis, but the sodium carbonate solution gave 0.226 gm. of barium salt.

0.0465 gm. Ba salt yielded 0.032 gm. BaSO₄ = 68.80 %

Valerianic acid, C₄H₆ . COOH, requires _ = 68.73

By titration the sodium carbonate extract was found to contain 0.3 gm. of valerianic acid.

The sodium hydroxide extract was neutralised by 0.5 cc. of baryta. It left a small residue on evaporation, which possessed the odour of cresol.

The ethereal solution remaining after the treatment with alkaline liquids was dried and distilled at a low temperature. There was obtained in this way a pale yellow limpid essential oil weighing 2.15 gms. This oil possessed a strong fragrant odour like camphor, and when kept in a desiccator over sulphuric acid, it was nearly all lost by evaporation in 3 days. This exceedingly volatile oil left about 0.25 gm. of a yellow solid on spontaneous evaporation.

The volatile constituents in the steam distillate were thus identified:—

trace of formic acid . . . . . . . . . . CH₂O₂
0.54 gm. acetic acid . . . . . . . . . C₂H₄O₂
0.30 gm. valerianic acid . . . . . . . . C₅H₁₀O₂
2.15 gms. essential oils.

For comparison with these acids we may mention the historic work of Chevreul on another plant survival from the past, Ginkgo biloba, the maiden-hair tree of China and Japan. This is the sole representative now existing of the very ancient branch constituting the second Order of the Gymnosperms, and has much in common with the cycads. Chevreul and Béchamp* isolated a complete series of acids from C₁ to C₇, viz., formic, acetic, propionic, butyric, valerianic, caproic and caprylie acids. The second, fourth and sixth predominated. This result was obtained after many trials, and only after extracting a large amount, 30 kilos, was sufficient of the third, fifth and seventh acids obtained to enable them to be identified. It is quite probable that small amounts of the other acids are present in Macrozamia also, but if so they can be recognised only

*Comptes rendus de l'Acad. des Sciences, 53, 1861, 1225; Annales chim. et de phys., i., 1864, 288.
by taking a much greater quantity of material, and making this a special object of research.

Examination of the Aqueous Solution, B.

The aqueous solution:—The voluminous washings and aqueous solutions were concentrated at a low temperature, and freed from a small amount of oil by shaking with petroleum spirit. The solution was then treated with an equal volume of 10% lead acetate solution, and the brown precipitate removed by the centrifuge and washed. The filtrate was next treated with basic lead acetate solution, and a white precipitate separated in the same way. The lead was removed from the solution by sulphuric acid and hydrogen sulphide, and the solution concentrated at 60° C. The hydrogen sulphide was removed by an air current passed through the warm solution. The black solution was shaken up repeatedly with (1) ether, (2) chloroform and (3) amyl alcohol. Ether removed about 2 gms. of a viscous resinous substance, chloroform removed only a trace, and amyl alcohol a dark brown syrup. These substances yielded nothing of a crystalline nature. They were dissolved in ether and shaken out successively with ammonium carbonate, sodium carbonate and sodium hydroxide, neutralised and again agitated with ether, but nothing could be obtained in this way.

Each of these extracts was carefully tested for alkaloids but only negative results were obtained.

The aqueous solution remaining after treatment with the above organic solvents, was distilled in a current of steam till free from amyl alcohol, and set aside. After some time crystals separated, and these were found to consist only of potassium salts. No tannin was present in this solution, and saponins were absent.

Picric acid gave a large precipitate of needle crystals of potassium salt. When boiled with potash, much ammonia was evolved, and Fehling's solution showed an immediate and strong reduction.

The osazones were next prepared from the solution, and when the product was examined under the microscope it was identified as the characteristic yellow crystals of phenyl glucosazone. These were purified by six recrystallisations from dilute alcohol, and then showed a melting point of 206° C. (corrected).

The melting point of the osazone of glucose is given as 205°. The predominating sugar is therefore glucose.

The solution also gave strong reactions for furfuraldehyde.

The total solid content of this aqueous fluid was 350 gms. or 36% of the alcoholic extract.

Examination of the lead acetate precipitates.—These lead deposits were treated with sulphuric acid and hydrogen sulphide to remove the lead, and the hydrogen sulphide boiled off. The solutions were treated with animal charcoal till nearly colourless and then concentrated. They showed no reactions with ferric chloride or sulphuric acid. When neutralised with sodium hydroxide, heavy gelatinous white precipitates were obtained, and Fehling's solution was strongly reduced.

The fluid was shaken out with ammonium carbonate, sodium carbonate and sodium hydroxide, then agitated with ether and acid, but nothing was obtained from any of these extracts in this way. The remaining solution from the normal lead precipitate, after standing some time, deposited a considerable amount of anhydrous calcium sulphate, in masses of white, needle-shaped crystals, matted together.
Examination of the Resins, C.

The resinous mass insoluble in hot water, which was left in the still after the removal of the volatile constituents by steam distillation, when dry weighed 392 gms., or 40% of the total contents of the alcoholic extract.

This substance was a dark brown powder. It was dissolved in the smallest amount of alcohol, mixed with purified sawdust and completely dried to constant weight. The dried mass thus rendered porous was transferred to a Soxhlet apparatus, and extracted successively and completely with petroleum spirit (b.p. below 50° C.), ether, chloroform, and alcohol. After each of these extracts was distilled to remove the solvent, and the residues dried at 110° and weighed, the following results were obtained:

1. Petroleum spirit extract ........ 35 gms. 8.9%
2. Ether extract ..................... 38 „ 9.7
3. Chloroform extract ............... 4 „ 1.0
4. Alcohol extract ................... 60 „ 15.4
Left unextracted ......................... 255 „ 65.0

Total .................................. 392 „ 100.0

This table shows that although 392 gms. of this mixture originally were in alcoholic solution, being extracted from the leaves with this solvent, 255 gms. had now become insoluble in that liquid, forming nearly two-thirds of the original extract.

Two factors may explain this anomaly:—The petroleum spirit extract contains all the oils and fats. Certain substances are intimately associated in the plant with these oils and are soluble in them alone. They are removed together with the oils in the extraction of the leaves by alcohol. When subsequently the oils and fats are removed by petroleum spirit these other constituents, having lost their special solvent, are now rendered completely insoluble in alcohol. In the second place the leaves were originally extracted with 80% alcohol (containing 5% wood-spirit), and in the resin analysis 98% ethyl alcohol was used.

i. Petroleum spirit extract of the Resins.—The solvent was removed by distillation, and the residue of 35 gms. was dissolved in ether leaving a small amount of insoluble brown residue, which weighed 0.2 gm. The ethereal solution was then agitated a number of times with (1) ammonium carbonate, (2) sodium carbonate, (3) sodium hydroxide, (4) water, for the separation of organic acids. The alkaline extracts were rendered acid with sulphuric, and shaken back with ether, the solvent distilled off, and the residue examined. In this way (1) ammonium carbonate yielded 0.25 gm. of a grey amorphous residue. (2) The sodium carbonate extract yielded a small quantity of a dark brown oil. At the same time there was precipitated by sulphuric acid about 10 gms. of a brown solid substance. The latter with the acid fluid was distilled in a current of steam, but from the distillate only a trace of volatile acids was obtained. The acid liquid remaining in the still, however, when titrated with baryta solution and evaporated, yielded the barium salt of acetic acid.

0.460 gm. Ba salt gave 0.410 gm. BaSO₄ = 89.2 %
Barium acetate requires .. = 91.3

The amount was equal to about 0.76 gm. of acetic acid. The brown solid substance mentioned above, of 10 gms. weight, was treated with petroleum spirit.
in which 3.3 gms. dissolved, ether dissolved 1 gm., and the remainder was soluble in alcohol only. These residues appeared to be complex mixtures of acids, and were not further examined. (3) The sodium hydroxide extract contained much chlorophyll. When acidified and shaken out with ether 0.2 gm. of substance was obtained. (4) The water extract after caustic soda treatment yielded to petroleum spirit 6.6 gms. of a white fatty substance, which was filtered and washed with cold alcohol. On recrystallisation a number of times from petroleum spirit, it showed a melting point of 47.5° C., and solidified at 46° C. The solution of this substance possessed an acid reaction.

0.155 gm. required 4.85 ccs. of decinormal alkali to neutralise it to phenolphthalein. This gives for a monobasic acid, the molecular weight of 320. The iodine-value by Hubl's method was determined:

1. 0.1097 gm. absorbed 0.058 gm. of iodine = 52.9%  
2. 0.1012 " 0.054 "  " = 53.1

The iodine-value corresponds to 59% of oleic acid, and leaves 41% with a molecular weight approximating 374, as probably one of the higher homologues of stearic acid.

Isolation of Phytosterol.

The ethereal solution which remained from the last section, after extraction with alkalis and water, was distilled off. This residue consisted of a mixture of fats and oils, with certain unsaponifiable substances, and weighed 10 gms. It was hydrolysed by boiling for 6 hours with an alcoholic solution of potassium hydroxide, and the products of saponification brought into aqueous solution by heating on the water-bath. In this way there were separated a black insoluble unsaponified portion, and a strongly alkaline aqueous solution. The whole was cooled and agitated with ether many times until nothing further was brought out in solution.

The unsaponified portion.—The ethereal solution, dark brown in colour, contained 0.5 gm. of solid, and was distilled off leaving a residue of impure, much discoloured crystals. The latter were dissolved in alcohol and digested with animal charcoal. They were then obtained in a fairly pure condition, and were redissolved and recrystallised twice from dilute alcohol.

The crystals were pure white with glistening surfaces, but under the microscope appeared of two kinds—a few broad rectangular flakes with dome ends, and the greater portion consisting of lath-shaped forms with pointed ends.

The broad flaky crystals presented the appearance of cholesterol, with low refractive index, and with the characteristic bites out of the sides.

The lath-shaped forms resembled some phytosterol crystals.

The crystals were exceedingly soluble in chloroform, and the following specific tests were applied:

Salkowski's reaction.—A chloroform solution and concentrated sulphuric acid were mixed, when the former assumed a blood-red colour and the acid a deep-green fluorescence; the red solution when removed and evaporated slowly changed colour, through purple, violet, blue and finally colourless; on again adding sulphuric acid the original crimson colour was restored.

Liebermann's reaction.—Acetic anhydride and a drop of sulphuric acid added to a chloroform solution gave a rich rose-red colour.

Iodine and sulphuric acid gave a violet colour, changing to blue and green.

Schiff's reagent gave a reddish violet residue.
These positive reactions place the substance in the group of phytosterols.

**Purification and physical properties of the phytosterol.**—After five recrystallisations from 95 % alcohol the greater portion of the substance was obtained in one fraction which, under the microscope, showed crystals of a uniform kind, laths with pointed ends. A second small fraction contained a mixture of the phytosterol with some few crystals like cholesterol. The first fraction was used for the following determinations:

**Estimation of water of crystallisation.**

0.3301 gm. heated in the oven at 110° C. lost 0.015 gm.

Loss in weight 4.54 % water

C_{27}H_{40}O.H_{2}O contains 4.46

**Formation of acetate.**

.2657 gm. of the anhydrous crystals was boiled with acetic anhydride; the product evaporated and weighed, gave .2937 gm. of phytosterol acetate.

Increase in weight 10.6 %

C_{27}H_{46}O(CO.CH_{3}) requires 10.9

**Melting points.**—The first fraction of the phytosterol containing its water of crystallisation showed a constant melting point of 132° C. (corrected). The second fraction gave a melting point of 135° C. The phytosterol acetate crystals melted at 120° C. (corrected).

**Optical properties.**—A polarimetric determination of the substance was made with a Schmidt and Haensch polarimeter. A solution of .2657 gm. of the phytosterol crystals in 15 ccs. of ether, and equivalent to a 1.7714 % solution, was used in a 1 dem. tube. A laevo-rotation was recorded of — 0.61° at a temperature of 16° C.

The specific rotatory power, \[ \left[ \alpha \right]_{D}^{16} = -34.5. \]

This biochemical group, of which cholesterin is the best known, has been called by Abderhalden the sterins (Lehrbuch der physiol. Chemie). These appear to be intimately associated with the fats and oils in all living cells, and have certain well defined properties. Their physical constants, however, are found to vary within certain limits, showing that not one substance but several closely related compounds exist.

Cholesterin of animal tissue has been known for fifty years, but of the analogous compounds in plants, the phytosterins, our knowledge is very recent. They occur both in the free state and as esters. Chemically, they are unsaturated alcohols of high molecular weight, having the constitution of cyclic polyterpenes.

In the literature available to the author the investigation of 66 different plants includes the isolation and identification of their phytosterins. In the English literature the term phytosterols is adhered to throughout. In ultimate composition they are found to range from C_{20} to C_{30}, but two-thirds of the number have the formula C_{27}H_{46}O and the great majority possess the general formula C_{n}H_{2n-8}O. These 44 phytosterols have—

- a melting point between 130° and 138° C.
- optical rotation \( \alpha \) —30 —41
- 2 groups of acetates (1) m.p. 118 122
- (2) \( \alpha \) 125 128
Those possessing the latter constants for their acetates were distinguished by Burian as sitosterols.

The phytosterol of *Macrozamia spiralis* possesses the following physical constants:—

- Melting point, 132.0° C.
- Optical rotation, -34.5
- Acetate m.p., 120.0° C.

It therefore falls in the first of the two groups.

*Separation of Hydrocarbons.*

The alcoholic mother-liquors, left after crystallising out the phytosterols, were united, and on further concentration a small quantity of cream-coloured fatty solid was separated. This substance, when purified by digesting with animal charcoal and several crystallisations, possessed the properties of a saturated hydrocarbon, and a constant melting point of 65° C. The weight was insufficient for analysis, and was not further examined. The melting point of the paraffin triacontane, C$_{30}$H$_{62}$, as observed by Tutin, is 65° C.

There now remained of the unsaponified substances only an uncrystallisable dark yellow oil, weighing about 2 gms.

Something of the nature of this oil was ascertained by treating a portion with a small piece of sodium, when only a few micro-bubbles of gas were produced, even on heating to 75° C. The substance therefore was not an alcohol. This point was confirmed by acetylation of the substance: when boiled with acetic anhydride and the products separated, no gain in weight was observed. The substance therefore did not contain a hydroxyl group.

The yellow oil was next distilled under a pressure of 30 mms.; the first drop collected at 180° C., and the greater part passed over at about 220°. The distillate was a pale yellow fluid which solidified on cooling in microcrystalline needles. This clear distillate was quite solid at 16° C., and when carefully warmed became a viscous fluid at 20°; the melting point lay between 17° and 19° C. By careful bromination of the substance in solutions of carbon bisulphide, it was observed that the bromine decolourised but almost no hydrobromic acid was formed,—evidence that only addition products were present. The absence of substitution of bromine points to the absence of paraffins.

The olefine hydrocarbon, octodecylene, C$_{18}$H$_{36}$, possesses the melting point of 18° C., and boiling point 183° C., under a pressure of 30 mms.

*The Saponified Oils.*

The strong alkaline solution obtained by hydrolising the petroleum spirit extract of the resins, and after removal by ether of the unsaponified substances, was now treated for fatty acids by adding sulphuric acid and distillation in a current of steam.

The distillate was faintly acid, and contained a small amount of a greenish solid, which was filtered off and examined. The substance was recrystallised from alcohol a number of times, and then consisted of 50 mgs. of nearly white crystals in small globular masses. The melting point was 45° C. The neutralisation equivalent was determined in alcoholic solutions, the titration ended sharply and required 20 ccs. of centinormal alkali to neutralise 40 mgs., which is equivalent to a neutralisation value of 200.

Lauric acid, C$_{12}$H$_{24}$O$_2$, requires 200 and possesses a melting point of 43.6° C.
The titrated sodium salt in alcoholic solution was then precipitated by baryta, and the barium compound converted into sulphate.

0.0383 gm. Ba salt yielded 0.0162 gm. BaSO₄.

Equivalent to BaSO₄ 42.3 %

Barium laurate requires 43.5 %

The substance was therefore lauric acid, C₁₂ H₂₄ O₂

The aqueous distillate, from which the above solid lauric acid had to be filtered, was treated by shaking out with ether; but nothing was removed in solution except a trace of an acid too small to identify. The aqueous distillate was titrated with decinormal barium hydroxide, and required 42 ccs. The solution was evaporated to dryness and weighed. During the heating the strong odour of acetic acid was detected.

0.3973 gm. Ba salt yielded 0.3671 gm. BaSO₄ = 92.4 %

Barium acetate requires 91.3 %

The substance is therefore acetic acid, C₂H₄O₂.

The acid liquid remaining in the distillation flask was shaken out with petroleum spirit. This removed a dark coloured solid mass which weighed 3 gms., and consisted of the higher fatty acids including stearic and oleic acids; they were not further examined.

The same acid liquid after treatment with petroleum spirit was agitated with ether. This solvent removed about 1 gm. of solid substance in solution, which consisted of resins and resin acids.

ii. The ether extract of the Resins:—The ether extract weighing 38 gms. was examined in the following manner.

A portion of the extract was dissolved in alcohol and poured into a large volume of water, when a dark green insoluble mass separated and was deposited. The aqueous portion was a pale green colloidal solution which did not settle. The resinous contents could not be induced to separate either by spinning in a high-speed centrifuge, filtering by the suction pump, allowing to stand several weeks, or by addition of such reagents as sodium citrate, magnesium sulphate, ether or alcohol. When agitated, however, with a little dilute sulphuric acid, instant separation took place, light green resins were deposited, which were filtered off from a clear aqueous fluid, and washed with water till acid-free.

The total deposited resins were dried and extracted with prepared sawdust in a Soxhlet extractor (1) with chloroform, (2) with alcohol. From each of these extracts the solvent was removed by distillation, dried and weighed.

27 gms. soluble in chloroform.
5 gms. insoluble in chloroform, soluble in alcohol.
6 gms. insoluble in chloroform, insoluble in alcohol.

Each of these portions was examined separately in great detail. Their solutions in ether were agitated with sodium carbonate, sodium hydroxide, and water. Each solution was carefully purified by animal charcoal and evaporated spontaneously, but in no case could any crystalline substance be isolated. Amorphous residues were obtained in all cases.

The portion insoluble in chloroform but soluble in alcohol was a black brittle resinous substance; when its alcoholic solution was poured into water it assumed a brilliant pale green and bright blue fluorescence.

iii. The chloroform and iv. the alcohol extracts of the original resin.—These, following the petroleum spirit and ether extractions, were treated in the same manner as the ether soluble portion, but only amorphous resins were obtained.
The Resins deposited from Cold Water, D.

The light brown resinous powder obtained by settling the original aqueous solution of the resins, and weighing 64 gms., was dissolved in a little alcohol and mixed with prepared sawdust. The whole was then thoroughly dried, and extracted in a Soxhlet apparatus, successively with organic solvents. The yield obtained was:

- Extracted by ether: 10%
- " chloroform: 1
- " alcohol: 21
- Insoluble: 68

The brown resin contained 1.2% of inorganic salts. From each of these extracts the solvent was distilled, and the residual substance examined. Nothing crystalline was obtained from any portion, and they seemed to be composed of amorphous resins.

Feeding Experiments with Macrozamia spiralis.

The animals used were white rats. They were kept singly in metal cages, and their normal food consisted of bread, or dog-biscuit, and water.

(i.) With the fresh leaf-powder.—The powdered leaves were mixed with bread or biscuit into a paste with water. 10 gms. of leaves were thus given daily to each of four rats. In most cases the ration was finished, but at the end of two weeks they ceased to eat any more, and after starving for a number of days they were put back on normal diet.

These animals were very lively and active, showing no ill effects at the end of the experiment, nor after some weeks.

(ii.) With the constituents of the ether extract.—It has been stated in the historical part above (p. 427), that Lauterer, of Queensland, separated from the leaves of Macrozamia by extraction with ether, a quantity of resin, to which he attributed the poisonous effects.

For the purpose of testing these resins a quantity of the leaves were treated in a special manner.

A quantity of the air-dried leaf-powder weighing 2.5 kilos was thoroughly extracted in large percolators with ether. After distilling off the solvent, there remained a viscous residue weighing 94 gms. (dry weight), which consisted of oils, fats and resins. Instead of using this entire extract it was further analysed by dissolving in a little alcohol, mixing with prepared sawdust, and completely drying the mass. This was extracted in a Soxhlet successively with (a) petroleum spirit, (b) ether, (c) alcohol.

The petroleum spirit extract was found to be the greatest in amount: it was further subdivided by shaking out successively with (1) sodium carbonate, (2) sodium hydroxide.

The weights of these different portions finally obtained were:

(a) Petroleum spirit extract—
- Sodium carbonate solution: 15 gms.
- Sodium hydroxide: 30 gms.
- Petrol. spirit: 20 gms.

(b) Ether extract: 24 gms.

(c) Alcohol extract: 5 gms.

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These various portions were divided into small amounts for feeding purposes, to last about two weeks. Each portion, mixed as before with about 10 gms. of bread or biscuit, was fed to a rat. Water also was given in each case.

All these extracts were readily and completely eaten. The rats apparently enjoyed the rations, and remained throughout the period of two to three weeks very active and well. These experiments show that nothing of the nature of Lauterer's poisonous resins was present.

(iii.) With the aqueous extract of the nuts.—About 1 kilo. of the seeds was made into a pulp, and extracted with 2 litres of water and 1 cc. of toluene. After 3 days, with frequent stirring, it was filtered through cloth into a tall cylinder, to allow the greater portion of the starch to deposit.

The solution thus obtained was given to two rats, the ration for each being made up of 30 ccs. of the fluid, with bread and biscuit soaked in it. This was given daily for 22 days, at the end of which period the two rats appeared quite normal and active.

The supposed poisonous principle said to be removed from the nuts by washing with water, and which would have been in the above solution, was not found in these experiments.

(iv.) With the fresh nuts.—Nuts were fed to two rats, with no other food, but with plenty of water. Both animals died of impaction within three days, and no other abnormal symptoms were detected.

In the historical record given above (p. 425), it will be noticed that animals were affected in two different ways. (1) Symptoms were rapidly manifested within the first three or four days after eating the *Macrozamia*. The animals became slow in their movements, dragged the hind limbs, and finally died of impaction. This condition would include the gastro-enteritis mentioned by certain authors. (2) Symptoms were gradually produced after three or four weeks' feeding. The condition was entirely different from the former, and was said to produce peripheral neuritis, and partial paralysis, etc. The animals, though incurable, might live on if cared for, but if not, they usually died of starvation.

The results of the experiments with the white rats were positive for the former, but entirely negative for the latter condition.

The symptoms described for the real *Macrozamia* poisoning are characterised by the slow onset of the disease, but when fully established, the disease has not been associated with any very definite pathological changes. These symptoms, in a general sense, have also been observed after animals have fed for long periods on certain other plants, for example, the Grasstrees, the Darling Peas, *Lathyrus*, *Loxo* weeds, etc., and in none of these has any active poisonous chemical compound been identified.

This chemical investigation has shown that the *Macrozamia* contains no active poisonous principle which could be isolated or identified, or any individual constituent which could be associated with the disease.

**Summary.**

*Macrozamia spiralis*, which grows abundantly along the East Coast of New South Wales, has been regarded as a poisonous plant from the earliest days of the Colony.

A complete summary of its poisonous record is given.

The chemical composition of its leaves is characterised by a large amount of amorphous resins.
The following constituents were identified:
Formic, acetic, valerianic and laurie acids.
Oleic, stearic, and higher fatty acids.
A very volatile essential oil.
A phytosterol with m.p. 132°, opt rot. — 34.5, and m.p. of acetate 120° C.
A paraffin, with the properties of triacontane C\textsubscript{30}H\textsubscript{62}, and an olefine having the properties of octodecylene.
The nuts contained 30% of starch, and much mucilage.
In the feeding experiments, white rats were given, with their ordinary food, (1) the crushed fresh leaves, (2) the grated seeds, (3) the rich, fatty, and resinous components extracted from the leaves by ether, and which Dr. Lauterer stated contained the poisonous principle, (4) the aqueous extracts of the leaves, and the seeds, by which the aborigines believed the poison was removed.
The animals showed no signs of being affected after three weeks' feeding, the material was apparently not poisonous to white rats. With careless feeding the animals are easily killed by impaction, which is due to the fibrous nature of the material.

References to the Poisonous Properties of Australian Cycads
(Arranged in chronological order of publication.)

9. Bancroft.—The Queenslander, 1890 (thro. Turner (11).)
10. ————Brisbane Courier, Oct. 29, 1892.
11. Turner.—Agric. Gaz. N.S.W., iv., 1893, 158.
15. ———— viii., 1897, 20.
16. ———— x., 1899, 738, 1259.
17. Lamb.—Agric. Gaz. N.S.W., vi., 1895, 505.
23. Van Dongen.—1903, thro. Wehmer's Die Pflanzenstoffen.
THE CHEMICAL EXAMINATION OF MACROZAMIA SPIRALIS.


27. **Guthrie.**—Agric. Gaz. N.S.W., xxviii., 1917, 625, 865; [p. 625 also quoted in Turner (11).]


References to Gums and Starch.


32. **Lauterer.**—Macrozamia gum. Chem. and Drug. of Australasia, 1890.

33. **Wagner.**—The Zamia Palm of N.S.W. and Q. Starkefabrikation, 1886.


Petrie, J M. 1920. "The chemical examination of Macrozamia spiralis."
Proceedings of the Linnean Society of New South Wales 45, 424–442.
https://doi.org/10.5962/bhl.part.19553.

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