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## THE UBIQUITY OF CYCASIN IN CYCADS

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Cycadales, represented today by ten genera found in all continents except Europe, together with Ginkgoales are included in the prephanerogams, a relict group of ancient gymnosperms. The presence of glycosides of MAM (methylazoxymethanol) has been reported only in the seeds of some cycads and in smaller quantity in their stems and fronds.

Cycasin is the most abundant glycoside found in *Cycas revoluta* [1,2] and *C. circinnalis* [3]; other MAM glycosides (neocycasins) occur in these plants but only in small quantities [2]. Macrozamin is a MAM glycoside found in *Macrozamia spiralis* [4] and *M. riedlei* [5]; it is probably also present in *M. moorei*, *M. pauli-guilielmi*, *M. hopei* (= *Lepidozamia hopei*), *M. douglasii* and *Bowenia spectabilis* [6].

In this work 17 species belonging to the ten genera of cycads have been analyzed for the presence of cycasin. Simultaneously, analyses were made of *Ginkgo biloba* (Ginkgoales), *Pinus canariensis* (Pinales), *Cephalotaxus harringtonia* (Cephalotaxales), *Araucaria cookii* (Araucariales) and *Marattia salicina* (Marattiales), representatives of gymnosperms and ferns related to cycads. Ripe seeds were usually used for analysis; in *Ceratozamia mexicana* and *Encephalartos umbeluziensis*, however, unpollinated ovules were examined, and in *Microcycas calocoma* and *Marattia salicina* fronds. Cycasin is present in all the cycad species examined in quantities varying between 0.01 and 0.72% (see Table 1). In *Marattia salicina* and in the other gymnosperms examined, cycasin is absent.

From the quantitative viewpoint, our results are not

completely representative, since some of the material examined was not of wild provenance. However, the cycasin percentage found in *Cycas revoluta* seeds (0.28%), coming from specimens grown in Naples Botanical Garden (Italy), is similar to values reported by Nishida *et al.* (0.28%) [1] and by Nagahama (0.296%) [2], for specimens of the same species growing in the field.

Our results show that cycasin is characteristic of and exclusive to the cycads, being present in all ten genera of this group. It is absent from other gymnosperm taxa and from the fern *Marattia salicina*. These results are of ecological interest in that the seeds of cycads are often eaten and cycasin is carcinogenic and neurotoxic [7]. It is destroyed only if the seeds are repeatedly washed and soaked, a procedure which probably liberates and activates the emulsin present in the seeds [8].

### EXPERIMENTAL

**Materials.** Seeds of *Cycas revoluta*, *Stangeria eriopus*, *Pinus canariensis* C. Sm. and *Cephalotaxus harringtonia* C. Koch, ovules of *Ceratozamia mexicana* and *Encephalartos umbeluziensis* and fronds of *Microcycas calocoma* and *Marattia salicina* Smith come from specimens grown in Naples Botanical Garden (Italy); seeds of *Cycas lane-poollei*, *Lepidozamia peroffskyana*, *Macrozamia diplomera*, *M. heteromera* and *M. moorei* were collected in the field and supplied by the Terrara firm (Gilgandra, Australia); seeds of *Bowenia spectabilis* were collected in the field and supplied by Mr. Brigden (Casuarina, Australia); seeds of *Encephalartos altensteinii* and *Zamia integrifolia* come from the cycad collection of Professor Verga (Como, Italy); seeds of *Dioon califanoi*, *D. edule*, *D. purpusii*

Table 1. Cycasin percentage in cycads

Species	Amount (%)* fr. wt	Part utilized
<i>Bowenia spectabilis</i> Hook. f.	0.42	seeds
<i>Ceratozamia mexicana</i> Brongn.	0.01	ovules
<i>Cycas lane-poolei</i> C. A. Gardner	0.72	seeds
<i>C. revoluta</i> Thunb.	0.28	seeds
<i>Dioon califanoi</i> De Luca & Sabato	0.04	seeds
<i>D. edule</i> Lindley	0.02	seeds
<i>D. purpusii</i> Rose	0.02	seeds
<i>Dioon</i> sp.†	0.03	seeds
<i>Encephalartos altensteinii</i> Lehm.	0.06	seeds
<i>E. umbeluziensis</i> R. A. Dyer	0.09	ovules
<i>Lepidozamia peroffskyana</i> Regel	0.21	seeds
<i>Macrozamia diplomera</i> (F. Muell.) L. Johnson	0.16	seeds
<i>M. heteromera</i> C. Moore	0.08	seeds
<i>M. moorei</i> F. Muell.	0.08	seeds
<i>Microcycas calocoma</i> (Miq.) A. DC.	0.05	fronds
<i>Stangeria eriopus</i> (Kunze) Nash	0.03	seeds
<i>Zamia integrifolia</i> Ait.	0.38	seeds

\* Each datum is the average of three measurements.

† From Puerto Escondido, Oaxaca, Mexico.

and *Dioon* sp. (Puerto Escondido, Oaxaca) were collected in Mexico by the authors; seeds of *Ginkgo biloba* L. and *Araucaria cookii* R. Br. come from specimens grown in Palermo Botanical Garden (Italy).

**Extraction.** The extraction of the cycasin has been made on the basis of Wells *et al.* procedure [9]. Fresh seeds (1 g) deprived of tegument (either 1 g of ovules or 1 g of fronds) were powdered and extracted in 4 ml 95% EtOH and centrifuged at 2000 rpm for 10 min. The procedure was repeated three times and the extracts were combined and adjusted to 15 ml by the addition of 95% EtOH. Androsterone (1 mg) was added as an internal standard.

**Trimethylsilylation.** Aliquots (2 ml) of the alcoholic extract were dried under vacuum at 40° and 200  $\mu$ l trimethylsilylation reagent (Sigma SIL-A) were added. Ten min later it was centrifuged and injections of 1–2  $\mu$ l were made directly into the gas chromatograph.

**Analysis.** Gas chromatography was carried out in a 1.5 m  $\times$  4 mm glass column packed with 3% OV-1, isothermal 200°. Carrier gas was N<sub>2</sub> at 30 ml/min. Injector temp. and FID detector temp. 230°. The cycasin peak has been identified utilizing

pure cycasin generously given by Professor Akira Kobayashi, Kagoshima University (Japan).

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