Phytochemistry, Vol. 20, No. 6, pp. 1415–1416, 1981.
Printed in Great Britain.

0031-9422/81/061415-02 $02.00/0

SHORT REPORTS

DISTRIBUTION OF MACROZAMIN IN AUSTRALASIAN CYCADS

A. MORETTI, S. SABATO and G. SINISCALCO GIGLIANO*

Istituto di Botanica, Facoltà di Scienze, Università di Napoli, Via Foria 223, Napoli, Italy; *Orto Botanico, Facoltà di Scienze, Università di Napoli, Via Foria 223, Napoli, Italy

(*Revised received* 1 *October* 1980)

Key Word Index—Cycadales; cycads; chemotaxonomy; methylazoxymethanol glycosides; macrozamin; cycasin.

MAM (methylazoxymethanol) glycosides, a class of neurotoxic and carcinogenic compounds [1], have been found exclusively in cycads, a relict group of ancient gymnosperms. The naturally occurring MAM glycosides have the same aglycone but differ in their sugar moieties [2]. Experimental evidence shows that the aglycone is the toxic component [3].

The first biochemical isolation of a such compound, macrozamin (β-primeverosyloxyazoxymethane), was obtained from seeds of *Macrozamia spiralis* Miq. [4], an Australian cycad. The identity of its carbohydrate component was later determined as primeverose [5]. Nishida *et al.* [6] isolated, in *Cycas revoluta*, a new MAM glycoside (cycasin), chemically closely related to macrozamin except that β-D-glucose was the sugar component. Later other MAM glycosides (neocycasins) [2] with different glucose based sugar units were isolated in *C. revoluta* and *C. circinnalis* L. Recently [7], it has been stated that cycasin is characteristic of, and exclusive to, all the genera of cycads.

The present work describes the distribution and quantitative estimation of macrozamin in seeds of Australasian representatives of *Cycas*, *Bowenia*, *Lepidozamia* and *Macrozamia* (Table 1). Macrozamin occurrence has already been reported for some of the taxa mentioned [2, 8]. *Cycas* is the only genus in the Cycadaceae and consists of about 20 species distributed from Madagascar and throughout south-east Asia and tropical Australia to the western Pacific. The genera *Bowenia*, *Lepidozamia* and *Macrozamia* are in the Zamiaceae and are endemic to Australia. *Bowenia* and *Lepidozamia* consist of two species each and *Macrozamia* of fourteen species [9].

EXPERIMENTAL

Materials. Seeds of *Bowenia spectabilis* and *B. serrulata* were collected in the field by Mr. Brigden (Casuarina, Australia); seeds of *Cycas basaltica*, *C. cairnsiana*, *C. lane-poolei*, *C. pruinosa*, *Lepidozamia peroffskyana*, *Macrozamia diplomera*, *M. miquelii* and *M. moorei* were collected in the field and supplied by Terrara (Gilgandra, Australia); seeds of *C. revoluta* came from specimens grown in the Naples Botanical Garden (Italy).

Macrozamin determination. Extraction: Fresh seeds (100 mg), deprived of tegument, were ground with 50% EtOH (1.5 ml) and shaken for 2 hr at room temp. After standing for a further 17 hr, the suspension was centrifuged at 2000 rpm for 10 min. The supernatant was adjusted to 2 ml by the addition of 50% EtOH. *Two-dimensional TLC*: An aliquot (10 μl) of the extract was subjected to 2D-TLC on Si Gel G plates using *n*-BuOH–Me_2CO–H_2O (4:5:1) in the first and Me_2CO–iso-PrOH–0.1 M lactic acid (14:5:1) in the second dimension. The

Table 1. Macrozamin and cycasin percentages in ripe seeds of Australasian cycads

Species	Macrozamin % (fr. wt)*	Cycasin % (fr. wt)*
Bowenia serrulata (W. Bull) Chamberlain	4.33	0.26
B. spectabilis Hook. f.	5.04	0.42†
Cycas basaltica C. A. Gardner	0.29	0.12
C. cairnsiana F. Muell.	0.20	0.10
C. lane-poolei C. A. Gardner	0.45	0.72†
C. pruinosa Maconochie	0.33	0.10
C. revoluta Thunb.	0.26	0.28†
Lepidozamia peroffskiana Regel	1.11	0.21†
Macrozamia diplomera (F. Muell.) L. Johnson	2.41	0.16†
M. miquelii (F. Muell.) A. DC.	3.88	0.09
M. moorei F. Muell.	3.72	0.08†

* Each result is the average of three measurements.
† Data already published in ref. [7].

standard was pure macrozamin given by Professor Akira Kobayashi, Kagoshima University (Japan). The R_f values of the macrozamin in first solvent was 0.53 and in second 0.61. When treated with aniline–diphenylamine–Me_2CO–80% H_3PO_4 (4 ml: 4 g: 200 ml: 30 ml) the macrozamin gave a blue-grey colour.

Hydrolysis: Macrozamin, recovered from the plate, was hydrolysed by adding 0.5 ml 1 N HCl at 100° for 30 min.

Trimethylsilylation: The hydrolysate was dried *in vacuo* at 40° and 100 μl trimethylsilylation reagent (Sigma SIL-A) was added. It was centrifuged 10 min later and injections of 1–2 μl were made directly into the gas chromatograph.

Analysis: Gas chromatography was carried out in a 1.5 m × 4 mm glass column packed with 3% OV-1, isothermal 200°. Carrier gas was N_2 at 30 ml/min. Injector temp. and FID detector temp. were 230°. The α-xylose, β-xylose, α-glucose and β-glucose peaks of the hydrolysate and pure macrozamin were identified using xylose and glucose standards.

Cycasin determination. Cycasin was determined according to ref. [7].

Acknowledgements—We thank Professor Akira Kobayashi of Kagoshima University (Japan) for stimulating suggestions.

REFERENCES

1. Laqueur, G. L. and Spatz, M. (1968) *Cancer Res.* **28,** 2262.
2. Nagahama, T. (1964) *Bull. Fac. Agric. Kagoshima Univ.* **14,** 1.
3. Kobayashi, A. and Matsumoto, H. (1964) *Fed. Proc.* **23,** 1354.
4. Cooper, J. M. (1940) *J. R. Soc. N. S. W.* **74,** 450.
5. Lythgoe, B. and Riggs, N. V. (1949) *J. Chem. Soc.* **4,** 2716.
6. Nishida, K., Kobayashi, A. and Nagahama, T. (1955) *Bull. Agric. Chem. Soc.* **19,** 77.
7. De Luca, P., Moretti, A., Sabato, S. and Siniscalco Gigliano, G. (1980) *Phytochemistry* **19,** 2230.
8. Riggs, N. V. (1954) *Aust. J. Chem.* **7,** 123.
9. Johnson, L. A. S. (1959) *Proc. Linn. Soc. N. S. W.* **84,** 64.