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DISTRIBUTION OF α-AMINO-β-METHYLAMINOPROPIONIC ACID IN *CYCAS*

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Key Word Index—*Cycas*; Cycadaceae; toxic amino acid; non-protein amino acid; α-amino-β-methylamino-propionic acid.

Abstract—α-Amino-β-methylaminopropionic acid, previously isolated from seeds of *Cycas circinalis*, has now been identified either free or bound in all the other nine species of this genus.

AT THE present time the Cycadales consist of about 100 species belonging to three families and nine genera. These species are the sole remaining representatives of a group of plants which constituted 80% of the world's vegetation during the Mesozoic era. They are unique morphologically and are, together with the Ginkgoaceae, the only seed plants that retain motile sperms characteristic of almost all the Cryptogams.

From seeds of several species of the Cycadales have been isolated glycosides of methyl-azoxymethanol[1,2] which show hepatotoxic and carcinogenic properties when fed orally to experimental animals. In addition to these effects, however, the ingestion of leaves of species from four genera have been reported to produce neurological disorders in cattle involving the irreversible paralysis of the hindquarters.[3]

While investigating the possibility that these plants might contain other toxic compounds Vega and Bell[4] isolated α-amino-β-methylaminopropionic acid (MeDPr), H_3C–NH–CH_2–$CH(NH_2)$–COOH from seeds of *Cycas circinalis*. This amino acid was subsequently shown to be toxic to chicks, rats and mice.[5]

The genus *Cycas* is the only genus of the family Cycadaceae.[6] It consists of nine species distributed from the islands of Madagascar and throughout S. E. Asia to the northern coastline Australia.[7]

The present paper describes the distribution and quantitative estimation of MeDPr in the seeds and leaves of the following species; *Cycas circinalis* L. (Guam), *C. revoluta* Thunb. (Japan), *C. media* R. Br. (Australia), *C. neocaledonica* Linden (from Fairchild Tropical Gardens, Miami, Florida), *C. thuarsii* R. Brown (Kenya), *C. rumphii* Miq. (Malaysia) *C. cairnsiana* L. (from Cairns Botanic Gardens, Australia), *C. siamensis* Miq. (from Fairchild Tropical Gardens, Miami, Florida) and *C. pectinata* Griff. (India). Preliminary studies have failed to show the presence of MeDPr in species of other families of the Cycadales.

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Extracts of fresh seeds and leaves were analyzed before and after hydrolysis using 2D chromatography and high voltage electrophoresis on paper. Quantitative determinations were made with an automatic amino acid analyser. MeDPr was detected in all nine species examined (Table 1), increased concentrations were found in extracts of six species after acid hydrolysis.

TABLE 1. DISTRIBUTION OF α-AMINO-β-METHYLAMINOPROPIONIC ACID IN CYCAS SPECIES
[μm (118 μg/)/g wet tissue weight]

Species	Seeds	Seed hydrolysates	Leaves	Leaf hydrolysates	Species	Seeds	Seed hydrolysates	Leaves	Leaf hydrolysates
C. circinalis	1·45	2·07	2·40	2·40	*C. rumphii*	*	*	1·02	1·12
C. revoluta	1·85	2·43	2·10	2·20	*C. cairnsiana*	0·20	0·20	0·20	0·20
C. media	0·178	0·178	0·20	0·20	*C. siamensis*	*	*	0·95	0·96
C. neocaledonica	*	*	1·07	1·07	*C. pectinata*	*	*	0·95	0·98
C. thuarsii	0·70	0·79	0·81	0·92					

* Not available for analysis.

The present investigation has revealed that MeDPr is common to all species of *Cycas* and is characteristic of the genus. The concentration of MeDPr required to produce toxicity in experimental animals suggests that it is unlikely that MeDPr is the only toxic compound in the plants. The highest concentrations of MeDPr were found in the leaves and seeds of *C. circinalis* and *C. revoluta*, both of which are reported to be neurotoxic.[8] MeDPr has not been found elsewhere in nature. Although many legumes accumulate high concentrations of α,β-diaminopropionic acid (DPr),[9] free DPr was not detected in the *Cycas* species during the present investigation.

EXPERIMENTAL

Preparation of crude extracts. Finely ground seeds (5 g) were extracted by shaking with 50% EtOH (100 ml) for 2 hr. The extraction was repeated three times and the combined extracts were concentrated to 5 ml under reduced pressure at less than 35°. A similar procedure was applied to 5 g of fresh leaves.

Hydrolysates. Hydrolysates were made by adding 0·25 ml conc. HCl to an equal vol. of the final crude extract and heating the mixture at 105° for 17 hr in an evacuated vial. The contents of the vial were then taken down to dryness repeatedly untill all the HCl was fully removed. The dried product was made up to 0·25 ml with H_2O for analysis.

Identification of MeDPr. In extracts other than those of *C. circinalis* (from which it was isolated in crystalline form) MeDPr was identified by co-chromatography and ionophoresis in the solvent systems and buffers as described before[4] using authentic MeDPr for comparison. Ninhydrin and Ehrlich's reagents prepared according to the methods of Smith,[10] were used for location of MeDPr.

Ion-exchange chromatography. Quantitative analyses were carried out on a Beckman Model 120C amino acid analyser. The resin used was sulfonated polystyrene in the Na^+ form (Beckman type PA-35) and the length of the column was 23 cm. The buffer flow rate was 50 ml/hr and the ninhydrin flow rate 25 ml/hr. The first buffer solution (pH 3·97, operating temp. of 32·5°) was followed after 185 min by a second buffer solution (pH 5·36, operating temperature of 62·5°). MeDPr emerged immediately after lysine.

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