Phytochemistry, Vol. 20, No. 7, pp. 1569–1571, 1981.
Printed in Great Britain.

0031–9422/81/071569–03 $02.00/0
Pergamon Press Ltd.

THE OCCURRENCE OF BATATASINS IN THE DIOSCOREACEAE

C. R. IRELAND, W. W. SCHWABE and D. G. COURSEY*

Department of Horticulture (Teaching), Wye College (University of London), Near Ashford, Kent, TN25 5AH, U.K.; *Tropical Products Institute, 56/62, Gray's Inn Road, London, WC1X 8LU, U.K.

(*Received* 30 *October* 1980)

Key Word Index—*Dioscorea*; Dioscoreaceae; yams; tubers; bulbils; phenols; batatasins; dormancy.

Abstract—The tubers and/or aerial bulbils of 13 species of *Dioscorea*, mostly of tropical origin, and one species each of *Rajania* and *Tamus*, were examined for the presence of batatasins. These phenolics have previously been reported in the temperate species *D. oppositа*. One or more batatasins were found in either tubers or bulbils of most species examined, including taxa from both Old and New Worlds, and with annual or perennial tubers. Batatasin I was the most widespread in occurrence, followed by B-II, while B-III, B-IV and B-V were comparatively rare. Only the bulbils of *D. oppositа* contained all five batatasins.

INTRODUCTION

The genus *Dioscorea* includes an important group of tropical food yams, the edible portion of which is the tuber or occasionally the bulbil. As the storage life of these tubers is ultimately controlled by their endogenous dormancy [1], methods of controlling dormancy are potentially of great economic importance.

Three phenolic compounds which showed growth inhibitory properties in standard bioassays, e.g. *Avena* coleoptile extension tests, were initially isolated from aerial bulbils of what was referred to as *Dioscorea batatas* Decne., correctly *D. oppositа* Thunb., and were named batatasins I, II and III (B-I, B-II and B-III) [2]. Subsequently, two further compounds, batatasins IV and V, were isolated and identified in the same species [3]. All but one of these substances have been fully characterized chemically, B-I being a trimethoxy-substituted phenanthrene and B-III, B-IV and B-V substituted bibenzyls [3–5]. The structure of B-II has yet to be established.

Batatasins appear to be associated with the control of dormancy in the bulbils of *D. oppositа* and to be related to gibberellic acid and, to a lesser extent, abscisic acid metabolism [6–8]. However, *D. oppositа* is unusual among the Dioscoreaceae in that it is a temperate plant whose dormancy occurs during a cold winter. The majority of the Dioscoreaceae, comprising several hundred species in all and including all the major economic species of yam, are tropical and their dormant phase occurs during the hot dry season [9–11].

Batatasin I has been reported in the 'rhizomes'—presumably tubers—of *D. dumetorum* (Kunth) Pax. [12] but in no other tropical species to date. A number of phenanthrene derivatives including B-I have been isolated in the temperate species *Tamus communis* [13] and naturally occurring dihydrophenanthrenes have been reported in *D. prazeri* [14] and in *D. decipiens* [15].

The present studies constitute the preliminary phase of a research programme designed to investigate the possibilities of using batatasins to control the dormancy and, therefore, influence the storage life of food yams. This initial survey was undertaken to establish whether batatasins are of widespread occurrence within the tropical Dioscoreaceae.

RESULTS AND DISCUSSION

The thin-layer characteristics and the peak fluorescent excitation and emission wavelengths of standard samples of authentic batatasins I, III, IV and V were determined and are shown in Table 1, together with the GLC relative retention times of their trimethylsilyl derivatives. A sample of B-II was not available, but the R_f value for this compound on Si gel TLC plates, determined elsewhere [3] but under similar conditions to those employed here, is included in Table 1. The much greater GLC retention time for B-I compared to B-III, B-IV and B-V and the very different fluorescent peak wavelengths reflect the different chemical nature of B-I, whereas B-III, B-IV and B-V are closely related chemical structures, B-III and B-IV being isomeric. Although the structure of B-II has yet to be determined, its TLC R_f value and colour reaction with vanillin–H_2SO_4 suggest that it is more closely related to batatasins III to V than to B-I.

Details of the plant material studied are given in Table 2. The species surveyed include all the major tropical food yams (marked ‡) as well as some wild species representative of several different taxonomic sections of the genus *Dioscorea* drawn from tropical America, Africa and Asia, in which areas the genus has different evolutionary histories. Two minor genera within the Dioscoreaceae were also included. Mature tubers were examined and also aerial bulbils of those species which readily form them.

On the basis of R_f data, colour reactions, GLC retention times and fluorescent properties, the presence of one or more of the five recognized batatasins was established in 12 species of Dioscoreaceae, and their occurrence is shown in Table 2. It will be noted that in tubers of *Dioscorea bulbifera*, *D. esculenta*, *D. minutiflora* and *D. rotundata* and in bulbils of *D. sansibarensis*, no batatasins were detected and only in the bulbils of *D. oppositа* were all five batatasins identified. Batatasin I appears to be by far the most widespread, whereas B-III, B-IV and B-V were all relatively rare. Batatasin II is apparently frequent in its

Table 1. Chromatographic and fluorescent properties of batatasins I–V

Batatasin	R_f value* and colour reaction with vanillin–H_2SO_4	GLC RR_t (min) of TMSi derivative	Fluorescence	
			Ex λ_{max}	Em λ_{max}
I (6-Hydroxy-2,4,7-trimethoxyphenanthrene)	0.49, blue	23.25	256	366
II†	0.32, pink	n.d.	n.d.	n.d.
III (3,3′-Dihydroxy-5-methoxybibenzyl)	0,13, pink-orange	3.5	276.5	303
IV (2,3′-Dihydroxy-5-methoxybibenzyl)	0.18, pink	3.25	273	299
V (2′-Hydroxy-3,4,5-trimethoxybibenzyl)	0.40, pink	4.75	275.5	300

n.d., not determined.
*Si gel G TLC (see Experimental).
†Chemical structure unknown. Sample not available but R_f data and colour reactions taken from ref. [3].

occurrence but in the absence of a standard B-II sample, this conclusion must be qualified. The apparent absence of B-I in *D. rotundata* is particularly interesting as this species is so close to *D. cayenensis*, in which B-I was detected, as to be considered by some taxonomists to be only a subspecies.

In addition to batatasins I to V, a number of other phenolics which showed growth inhibitory properties in a wheat coleoptile extension test were found in a number of the species examined. Work is continuing on the characterization of these additional batatasin-like substances.

Although the tuber and bulbil material analysed varied

Table 2. The occurrence of batatasins in 15 species of the Dioscoreaceae

Species and geographical area of origin	Section within *Dioscorea**		I	II	III	IV	V
Africa							
Dioscorea bulbifera L.	*Opsophyton*	Bulbil	+	–	–	–	–
		Tuber	–	–	–	–	–
‡*D. cayenensis* Lam.	*Enantiophyllum*		+	–	–	–	–
D. dumetorum (Kunth) Pax.	*Lasiophyton*		+	–	+	–	–
D. preusii Pax.	*Macrocarpea*		–	–	–	–	+
‡*D. rotundata* Poir.	*Enantiophyllum*		–	–	–	–	–
D. sansibarensis Pax.	*Macroura*	Bulbil	–	–	–	–	–
		Tuber	+	+	–	–	–
America							
D. composita Hemsl.	*Heterostemon*		+	+	–	–	–
D. floribunda Mart. et Gal.	*Heterostemon*		–	+	–	–	+
D. trifida L.f.	*Macrogynodium*		+	–	–	–	–
Rajania cordata L.	—		–	–	–	–	–
Asia							
‡*D. alata* L.	*Enantiophyllum*		+	+	–	–	–
D. bulbifera L.	*Opsophyton*	Bulbil	–	–	–	–	–
D. esculenta (Lour.) Burk	*Combilium*		–	–	–	–	–
D. minutiflora Engl.	*Enantiophyllum*		–	–	–	–	–
§*D. opposita* Thunb.	*Enantiophyllum*	Bulbil	+	+	+	+	+
		Tuber	–	+	–	–	–
Europe							
§*Tamus communis* L.	—		+	–	–	–	–

*Classification according to Burkill [10].
†Tuber examined unless otherwise stated.
‡Economically important species.
§Species native to temperate regions.

in age and was probably in different states of dormancy, i.e. in different physiological conditions, nevertheless the widespread occurrence of batatasins and batasin-like compounds is of interest and may ultimately allow for methods of control during storage using these natural chemical agents.

EXPERIMENTAL

Plant sources. Some of the plant material was of known identification from the Royal Botanic Gardens, Kew or the Jardin Botanique, Geneva. Taxonomic identification of other material was confirmed by one of us (D.G.C.).

Extraction of batatasins. Peeled tubers/bulbils were macerated in cold Me_2CO with ice and after filtration and washing, the Me_2CO was evapd *in vacuo*. The remaining aq. soln was adjusted to pH 7.5 with 1 M phosphate buffer and partitioned with EtOAc. (In the case of chlorophyll-containing bulbils, the pigment was removed before partitioning the neutral fraction by filtering off coagulated pigment in cold aq. soln, adjusting the pH to 2.8–2.5 and partitioning with Et_2O.) The neutral fractions were concd *in vacuo* and strip-loaded onto Si gel TLC plates and developed in $CHCl_3$–HOAc (19:1). The presence of phenolics was indicated colour reactions with vanillin–H_2SO_4 reagent at 128°. Batatasins were initially identified by comparison of colour reactions and R_f values with those of authentic samples of batatasins run on the same plate. Zones on TLC plates with colour reactions and R_fs characteristic of batatasins were eluted with EtOAc, purified by TLC and analysed by GLC and spectrofluorimetry.

GLC analysis of batatasins. TMSi derivatives of samples were produced by reaction with TCMS. GLC RR_fs were obtained with a 7 ft × 4 mm column packed with OV-17 (1.5%), 35 ml N_2/min, FID, isothermal 250°. Co-chromatography was used to determine whether samples and batatasin standards were equivalent, except for B-II, where no standard was available.

Spectrofluorimetric analysis of batatasins. The fluorescence characteristics of samples were obtained with a Perkin–Elmer 3000 fluorescence spectrometer with synchronous scan facility. Peak excitation and emission wavelengths were determined and synchronous scan spectra obtained, which were then compared with those for batatasin standards.

Acknowledgements—The authors wish to express their thanks to Dr. T. Hashimoto of the Institute of Physical and Chemical Research, Wako-Shi, Japan for making available authentic samples of batatasins. Further, to Dr. Linda Fellows, Ms. Ruth Henderson and Mr. B. Halliwell of the Royal Botanic Gardens, Kew; to Dr. H. C. Passam of the Tropical Products Institute but formerly at the University of the West Indies, Trinidad; to Dr. F. W. Martin, formerly of the Mayaguez Institute of Tropical Agriculture, Puerto Rico; to Dr. R. D. Cooke, currently at the Centro de Investigaciones de Tecnologia de Alimentos, Costa Rica and to Prof. J. Miège of the Jardin Botanique, Geneva for arranging for supplies of plant material. The work was financially supported by an Extra Mural Contract with the Tropical Products Institute, Overseas Development Administration.

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