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PHENOLICS OF FIVE YAM (*DIOSCOREA*) SPECIES

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Abstract—Cyanidin-3-glucoside, (+)-catechin and the procyanidin dimers 'B-1' and 'B-3' were identified as phenolic constituents of *Dioscorea alata* tubers, and strong evidence for the presence of a procyanidin trimer and a tetramer was found. The quantity of (+)-catechin and relative proportions of the procyanidin dimers were determined in 10 cultivars of five of the main edible yam species.

INTRODUCTION

The edible yams are major staple food crops in West Africa, world production being around 20 million tonnes [1]. The principal edible species are *Dioscorea rotundata* Poir., *D. cayenensis* Lam. and *D. alata* L. although at least 50 of the approximately 600 *Dioscorea* spp. are eaten in various parts of the world [1, 2]. Yam tubers store well, and most of the world's yam crop is consumed as a fresh product, but in some areas, especially the Western States of Nigeria, yams are traditionally processed by parboiling, sun-drying and grinding to a coarse flour [1]. Recently, there has been much interest in processing yam by more sophisticated factory-scale operations to 'instant' reconstitutable flake or powder products [3]. The tubers of many cultivars (of various species) show strong enzymic browning reactions when cut and exposed to the air. This is undesirable in yam to be consumed fresh but is more serious in the context of processing, as the final product may be greyish or brownish, instead of an organoleptically acceptable creamy white. This browning has been attributed to the oxidation of phenolic compounds under the influence of an *o*-dihydroxy-phenoloxidase on *o*-dihydroxy- or *vic*-trihydroxyphenols [4–7].

The presence of various phenolic compounds has been reported in yam tuber tissue. Cyanidin-3,5-diglucoside, cyanidin-3-monoglucoside, and cyanidin-3-rhamnoglucoside were reported from a red-fleshed form of *D. alata* in Ghana [8] while cyanidin-3-gentibioside acylated with ferulic acid, and two minor pigments of cyanidin-3-glucoside acylated with ferulic acid were isolated from a similar West Indian cultivar [9]. The isoquinoline derivative obtained from an acetone extract of *D. opposita* Thunb. (incorrectly described as *D. batatas* Decne.) was believed to have been formed by condensation between dopamine and acetone: the presence of dopamine in the same species was suggested [10]. The following phenolic compounds were reported in Indian yams: caffeic, coumaric, ferulic and sinapic acids, kaempferol, quercetin and cyanidin, all of unspecified glycosylation [11].

The main phenolic compound involved in enzymic browning in *D. alata* was reported to be a catecholamine together with two other oxidizable 'leucoanthocyanidins' [12] which absorbed at about 282 nm and, on acid hydrolysis, yielded cyanidin. A 'leucoanthocyanidin' was also identified in *D. cayenensis* [13] in addition to a catecholamine identical to that of *D. alata*. The bitter 'leucoanthocyanidin' of *D. cayenensis* is different from the four compounds which it was suggested were responsible for bitterness in *D. bulbifera*, though these also absorbed at about 280 nm [14].

The present study was initiated to obtain further information on the phenolic constituents of edible yams, particularly those compounds which may be responsible for undesirable browning reactions.

RESULTS AND DISCUSSION

Identification of phenolic constituents

The phenol content of 10 yam cultivars from five species (listed in Table 1) was measured for tubers harvested from the 1979 and 1980 planting seasons, using the Folin–Denis reagent [15]. In general the levels were similar for each species for the two seasons, the only exception being *D. cayenensis* which showed an intermediate to low phenolic content. *D. alata* and *D. bulbifera* had the highest phenolic contents, and *D. rotundata* (all cultivars) the lowest.

The phenolic constituents of *D. alata* (cv. UM 680) were examined following extraction with water or ethyl acetate, and chromatography on LH-20. Ten fractions (A–K) were found in the water extract, and 11 (L–V) in the ethyl acetate. The aqueous extract gave a similar separation pattern, in terms of absorption at 280 nm, to that of several other plant materials containing mainly flavan-3-ols and proanthocyanin polymers [16–18]. Three fractions (H, J and K) found in the aqueous extract had similar elution characteristics to three fractions (P, Q and R) from the ethyl acetate extract. The V_e/V_o ratio of R (4.73) was identical to that of (+)-catechin.

Pooled fractions from several columns, after rechromatography on LH-20, were further investigated. Peaks D, E, F, G, J and L, M, N, O and Q were small (in terms of

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Table 1. The phenolic content of yams

Yam species	Cultivar or Nigerian local name	Phenolic content (mg (+)-catechin equivalent/100 g fresh yam) 1979	1980	(+)-Catechin content (mg/kg)	Peak area (cm^2) B-1	B-3
D. alata L.	Ominelu	408	513	7.55	11.55	3.15
D. alata L.	UM 680	371	304	4.30	4.55	1.66
D. bulbifera L.	Adu	423	427	68.44	1.95	16.60
D. cayenensis Lam.	Oku	27	83	1.67	1.98	0.98
D. dumetorum Pax.	Ona	86	74	0.66	0.51	0.75
D. rotundata Poir.	Abii	26	12	0.61	0.48	0.42
D. rotundata Poir.	Ekpe	24	19	n.d.	0.75	0.46
D. rotundata Poir.	Nwopoke	45	69	1.20	0.13	1.18
D. rotundata Poir.	Obiaoturugo	27	31	0.26	0.66	0.36
D. rotundata Poir.	Okwocha	31	29	0.23	0.63	0.31

their absorbance at 280 nm), and were discarded. Ferric chloride [19] was used to obtain an indication of the presence of phenols; fractions C, F and Q–V gave the blue colour characteristic of phenols.

Identification of (+)-catechin and procyanidins in ethyl acetate extract

Fractions R, S, T, U and V were tested with a number of reagents [19–23] after two-dimensional paper chromatography. The characteristics of fraction R were similar to those of authentic (+)-catechin, the reaction with 2,4,6-trinitrophenol being specific for catechins. The absorption spectra of fractions R, S, T, U and V were recorded, and found to have maxima at 281–284 nm in methanol containing 0.1 % v/v hydrochloric acid, unchanged by the addition of aluminium chloride or sodium acetate–boric acid. (+)-Catechin has an absorption maximum at 281 nm. Addition of sodium methoxide produced bathochromic shifts of 10–11 nm in the sample, and of 14 nm with catechin, from which it may be deduced that these fractions contained catechin or flavan-3,4-diol.

Several authors [24–26] have noted that, with the exception of flavans, phenolic compounds exist as glycosides rather than as aglycones in plant tissues. The R_f value, after acid hydrolysis, of R was 0.68, about the same as the R_f value of authentic (+)-catechin. The R_f value of the red solutions of the 'aglycones' obtained by acid hydrolysis of fractions S, T, U and V were similar (0.47–0.51), whilst the absorption spectra showed maximum absorption between 545 and 548 nm. The pigment present was identified as cyanidin on the basis of visible max, R_f and various colour reactions including Hayashi's test [27]. These results suggest that fractions S, T, U and V contain procyanidins, giving cyanidin chloride on hydrolysis with hydrochloric acid (see below for further evidence).

Confirmation of the occurrence of cyanidin-3-glucoside in yams

Rasper and Coursey [8] reported the presence of cyanidin-3-glucoside in *D. alata*, and it was suspected that fraction C of the LH-20 chromatogram of the aqueous extract, which was red, contained this compound. The aglycone produced by hydrolysis of this fraction with hydrochloric acid was cyanidin chloride (identified as above). Paper chromatography indicated that the principal sugar liberated on hydrolysis was glucose. Paper chromatographic data on the colour reactions and R_f values in five solvents, when compared with literature values [25, 26, 28], confirm that fraction C contained cyanidin-3-monoglucoside.

Confirmation of the occurrence of (+)-catechin in yams

Using reverse-phase HPLC, fraction R and authentic (+)-catechin had almost identical retention times, and when chromatographed together, a single peak emerged, using the solvent system of Hoefler and Coggon [29], and also using phosphate buffer. The TMS derivative of fraction R co-chromatographed with that of authentic (+)-catechin, and the breakdown patterns on mass spectrometry were virtually identical.

Confirmation of the presence of procyanidins in yams

Fractions S, T, U and V and authentic samples of procyanidin polymers B-1, B-2, B-3, B-4 and C-1 were treated with toluene-α-thiol, followed by a further reaction with Raney nickel. The R_f values of fractions S and T before treatment were almost identical with those of B-1 and B-3, respectively. On degradation with toluene-α-thiol, the fractions S, T, U and V and the procyanidin polymers B-1 and B-3 gave two spots, one of which had similar R_f values to those of (+)-catechin. The corresponding R_f values of the reaction products of B-2, B-4 and C-1 refer to (−)-epicatechin, as expected. The products of the degradation reaction of procyanidin polymers with the toluene-α-thiol are (+)-catechin or (−)-epicatechin from one half of the molecule [16, 30] and the stereospeciically formed thioethers from the other half of the molecule. The R_f values of the second components of the reaction mixtures of fractions S, T, U, V, B-2, B-3, B-4 and C-1 are attributed to these thioethers. Desulphurization of the mixture with Raney nickel yields

the flavan-3-ol; the results obtained, however, indicate that the desulphurization was incomplete.

As expected, the treatment of fractions S, T, U and V with hydrochloric acid at 60°, gave different products depending on the acid strength [16]. The R_f values of spots seen on HPLC plates for fractions treated with 0.1 M hydrochloric acid are indicative of the liberation of flavan-3-ol, possibly (+)-catchin. Fraction V may be a trimer of R_f 0.46, and the spot of R_f 0.64 which appears may be a dimer. Similarly U may be a tetramer of R_f 0.32, which on treatment with 0.1 M hyrochloric acid may give rise to a trimer of R_f 0.45, and a dimer of R_f 0.64. Treatment of fractions S, T, U and V with 5 M hydrochloric acid yielded red coloured solutions, which, in each case, showed only one red spot on chromatograms, typical of cyanidin. A subsidiary peak at 460–464 nm noted in the visible max is produced by phlobaphene usually associated with the formation of cyanidin from procyanidin polymers or flavan-3,4-diols [16, 26, 30]. From these results, it is suggested that *D. alata* contains the procyanidin dimers B-1 and B-3, and a procyanidin trimer and a tetramer.

Estimates of (+)-catechin content and relative proportions of procyanidins in yams

HPLC was used to estimate the (+)-catechin content of the remaining yam cultivars (Table 1). In the absence of samples of B-1 and B-3 sufficiently pure for use as HPLC standards, the peak areas of these two compounds are given. The actual (+)-catechin contents shown in Table 1 are much lower than the values given in Table 1 for the Folin–Denis reacting material present in the extracts, expressed as catechin equivalents, as the extract contains many substances.

D. bulbifera contains the highest concentration of (+)-catechin, and contains relatively more B-3 than B-1. This species is also known to have a high level of polyhydric phenol oxidase, and to show a severe browning reaction when cut [5]. The two cultivars of *D. alata* also contain more (+)-catechin than the other three species, but show more B-1 than B-3. The relative concentrations of B-1 or B-3 and the content of (+)-catechin in *D. cayenensis*, *D. dumetorum* and *D. rotundata* are all much lower than in *D. bulbifera* or *D. alata*. It will be noted that one cultivar of *D. rotundata* appeared to lack (+)-catechin, whilst another cultivar containing more (+)-catechin than the other four *D. rotundata* cultivars also had a relative preponderance of B-3 over B-1 [5].

The conclusions of this work are that *D. alata* contains (+)-catechin, the procyanidin dimers B-1 and B-3, and two other procyanidins, most probably a trimer and tetramer. The presence of cyanidin-3-monoglucoside was confirmed. Tubers of nine other yam cultivars from five edible species contained the procyanidin dimers and eight contained (+)-catechin, which was not detected in one cultivar of *D. rotundata*. These results should be useful in extending studies of browning in yams caused by the oxidation of polyhydric phenols.

EXPERIMENTAL

The phenolics were identified by standard spectroscopic and chromatographic methods. Further details can be obtained in ref. [31].

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