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TWO ACYLATED ANTHOCYANINS FROM *DIOSCOREA ALATA*

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Key Word Index—*Dioscorea alata*; Dioscoreaceae; tuber; cyanidin 3-*O*-gentiobioside; acylated peonidin 3-*O*-gentiobioside; sinapic acid; ^{1}H, ^{13}C NMR; FABMS; quantitative analysis.

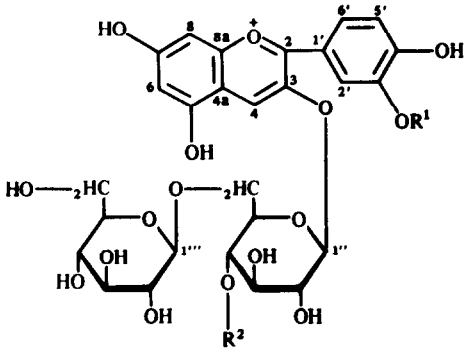
Abstract—Two new anthocyanins, cyanidin and peonidin 3-gentiobioside acylated with sinapic acid, have been isolated from the tuber of *Dioscorea alata* L. 'King yam' originated from Sri Lanka. These structures are elucidated by spectroscopic methods.

INTRODUCTION

Dioscorea alata L. is a popular edible yam; the tubers are white, but some varieties contain purple pigment. In a current survey of anthocyanins of the tubers of *D. alata*, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-diglucoside, cyanidin 3-*O*-rhamnoside, cyanidin 3-*O*-gentiobioside and cyanidin 3-*O*-glucoside acylated with ferulic acid were isolated [1, 2]. Catechin and procyanidin B-1 and B-3 were also identified from other cultivars [3]. This paper presents the isolation of two new anthocyanin diglucosides acylated with sinapic acid and the quantitative analysis of these pigments in individual organs.

RESULTS AND DISCUSSION

From *n*-butanol extract of the tuber of *D. alata* three anthocyanins **1**–**3** were isolated by repeated Sephadex LH-20 and MCI gel CHP 20P column chromatography.



1 R^1 = H R^2 = sinapoyl
2 R^1 = H R^2 = H
3 R = Me R^2 = sinapoyl

Compound **1** was obtained as a purple-red powder. The UV-visible spectrum of **1** showed that OH-3 of the aglycone is glycosylated and a cinnamic acid acyl group is present [4]. The positive FAB mass spectrum of **1** indicated a molecular ion peak at *m/z* 817 suggesting that this is composed of cyanidin, two molecules of hexose and one molecule of sinapic acid. Hydrolysis of **1** with 2 M HCl at 95° gave cyanidin, glucose and sinapic acid. The ^{13}C NMR spectrum of **1** (Table 1) clearly showed that C-6 of the glucose moiety was shifted by glycosylation; therefore, the pigment is a 3-gentiobioside. This was confirmed by partial hydrolysis of **1** with 0.6 M HCl to give **2**, which was identified as cyanidin 3-*O*-gentiobioside by TLC, FAB mass spectrum and UV-Vis [1]. The NOE between the anomeric proton (δ5.28) of one glucose moiety and H-4 of cyanidin (δ8.63) confirmed that the gentiobiose is attached to OH-3 of the cyanidin. From the COSY spectrum of **1**, the lower field signal at δ5.35 was correlated with an anomeric proton (δ5.28). Thus, the acyl group is attached to the OH-4 of the inner glucose moiety. These assignments were in good agreement with the chemical shifts of ^{13}C NMR (Table 1). The large coupling constant (J = 8 and 8 Hz, respectively) of the glucose anomeric protons appearing at δ5.28 and 4.46 indicating that **1** has the β-configuration. Thus **1** is cyanidin 3-*O*-(4″-*O*-sinapoylgentiobioside) and is named alatanin 1.

Spectral analysis and the results of hydrolysis showed that **3**, obtained as a purple-red powder, was identical to **1** except that the aglycone was peonidin. Similar arguments to those advanced above for **1** showed that **3** is peonidin 3-*O*-(4″-sinapoylgentiobioside), and is named alatanin 2.

HPLC of extracts of tubers, seedlings, nodes and stems of *D. alata* showed that the major pigment was an unknown, A, and three other unknown minor pigments, B–D were also present (Table 2). The tuber accumulated **1** 10-fold compared to the aerial parts. On the other hand, cyanidin 3-*O*-gentiobioside (**2**) accumulated in the aerial parts.

Table 1. ^{13}C and ^{1}H NMR data of compound **1** and **3** (δ ppm)*

	1		3	
Aglycone				
2	159.0	—	159.4	—
3	139.4	—	139.2	—
4	134.7	8.63 *s*	134.9	8.66 *s*
5	163.1	—	163.0	—
6	104.0	6.50 *d* (1)	105.4	6.51 *d* (1)
7	168.8	—	168.3	—
8	95.1	6.63 *d* (1)	95.3	6.68 *d* (1)
8a	157.0	—	157.2	—
4a	112.8	—	112.9	—
1′	121.2	—	121.1	—
2′	107.1	7.89 *d* (2)	107.1	8.00 *d* (2)
3′	156.2	—	158.7	
4′	147.5	—	147.6	—
5′	116.0	7.03 *d* (9)	115.9	7.03 *d* (9)
6′	118.5	8.24 *dd* (2, 9)	117.8	8.26 *dd* (2, 9)
–OMe	—	—	56.9	3.43 (3H) *s*
Glucose				
1″	104.1	5.28 *d* (8)	104.2	5.27 *d* (8)
2″	75.3	3.68 *dd* (8, 9)	75.4	3.68 *dd* (8, 9)
3″	77.0	3.47 *t* (9)	78.0	3.46 *t* (9)
4″	72.4	5.35 *br d* (9)	72.4	5.33 *br d* (9)
5″	74.7	4.04–4.10 *m*	74.7	4.00–4.05 *m*
6″	73.5	3.91 *dd* (4, 12)	73.5	3.90 *dd* (4, 12)
		4.32 *dd* (9, 12)		4.32 *dd* (9, 12)
1‴	103.3	4.46 *d* (8)	103.3	4.47 *d* (8)
2‴	75.3	3.67 *dd* (8, 9)	75.4	3.67 *dd* (8, 9)
3‴	78.3	3.79 *t* (9)	78.3	3.79 *t* (9)
4‴	70.2	3.21 *t* (9)	70.2	3.22 *t* (9)
5‴	78.0	3.84–3.89 *m*	78.0	3.85–3.89 *m*
6‴	61.1	3.86 *dd* (4, 12)	61.1	3.85 *dd* (4, 12)
		4.08 *br d* (12)		4.07 *br d* (12)
Sinapic acid				
1	124.5	—	123.8	—
2	105.4	6.24 *s*	105.4	6.23 *s*
3	149.1	—	149.1	—
4	146.0	—	146.2	—
5	149.1	—	149.1	—
6	105.4	6.24 *s*	107.1	6.23 *s*
β	147.5	7.31 *d* (16)	147.5	7.32 *d* (16)
α	117.6	6.09 *d* (16)	115.9	6.08 *d* (16)
CO	169.6	—	169.1	—
–OMe	56.1	3.45 (6H) *s*	56.1	3.46 (6H) *s*

*Measured in CD_3OD containing CF_3CO_2D.
J (Hz) in parentheses.

EXPERIMENTAL

Plant material. Small tubers of *D. alata* collected from Prof. H. P. M. Gunasera (University of Peradeniya, Sri Lanka) in 1987 were cultured *in vitro* and planted in the herbal garden of the Faculty of Pharmaceutical Sciences, Kyushu University.

General. TLC was carried out on Avicel SF cellulose with HOAc–HCl–H_2O (15:3:85) (system 1), *i*-BuOH–HOAc–H_2O (8:2:3) (system 2). HPLC was performed on a Chemkopak (Chemko) (4 × 250 mm) with a variable wave length detector (set at 525 nm). The mobile phase was H_2O–HCO_2H–MeOH (63:10:27). UV-visible spectra were measured in MeOH containing 0.01% HCl. ^{1}H and ^{13}C NMR spectra were measured at 270 and 67.8 MHz, respectively in CD_3OD containing CF_3CO_2D with TMS as int. standard. FABMS were measured in a positive mode with glycerol–HCl as the matrix. Acid hydrolysis was carried out with 2 M HCl at 95° for 1 hr. Partial acid hydrolysis was done with 0.6 M HCl at 60° for 24 hr and products were purified by TLC (System 2).

Extraction and isolation. Fresh tubers (408 g) were sliced and extracted with *n*-BuOH 6 times at room temp. The solvent was removed *in vacuo* at 50° and the extractives were dialysed using a cellulose tube. The outer soln was evapd *in vacuo* and chromatographed on a column of Sephadex LH-20 and MCI gel CHP 20P eluting with MeOH–H_2O mixture repeatedly to give **1** (450 mg), **2** (5 mg) and **3** (6 mg).

Table 2. Relative content of anthocyanins compared to **1** in various organs of *Dioscorea alata* by HPLC analysis

	Anthocyanins						
	1	**2**	**3**	A	B	C	D
Tuber	100 (0.164)*	13	17	15	—	—	—
Node	100 (0.007)*	222	—	1056	—	567	296
Stem	100 (0.004)*	313	50	469	194	131	—
Leaf	100 (0.006)*	170	119	383	264	137	81
Young leaf	100 (0.011)*	161	48	184	168	168	152
In vitro†	100 (0.013)*	238	50	614	—	317	219

*Content % (fresh weight).
†Tissue culture.

Compound **1**. Purple-red powder. λ_{max} nm (log ε): 283 (4.36), 332 (4.31), 534 (4.49). $A_{440}/A_{534}=0.24$. FABMS m/z: 817 $[M]^+$. TLC: R_f 0.37 (system 1), 0.21 (system 2). ^1H and ^{13}C NMR spectra are given in Table 1.

Compound **2** (*cyanidin* 3-O-*gentiobioside*). TLC: R_f 0.37 (system 1), 0.20 (system 2). λ_{max} nm: 282, 330, 527. $A_{440}/A_{527}=0.25$. FABMS m/z: 611 $[M]^+$, 287 (cyanidin).

Compound **3**. Purple-red powder. λ_{max} nm (log ε): 283 (4.37), 333 (4.20), 533 (4.51), $A_{440}/A_{532}=0.27$. TLC: R_f 0.37 (system 1), 0.25 (system 2). FABMS m/z 831 $[M]^+$. ^1H and ^{13}C NMR spectra are given in Table 1.

Quantitative analysis of compound **1**. Young leaves, leaves, stems, nodes, tubers and *in vitro* whole plants (5.0 g fr. wt) were homogenized and extracted with MeOH containing 1% HCl (30 ml × 4) and the solvent was evapd *in vacuo*. The residue was dissolved in MeOH containing 0.01% HCl, filtered and analysed quantitatively by HPLC. Compound **1** (0.82 mg) was dissolved in MeOH containing 0.01% HCl, and diluted stepwise; 5 μl were used for calibration of the system. From individual weight and peak height the calibration curve was made as following; 0.82 μg per 110 mm, 0.41 μg per 56 mm, 0.205 μg per 28 mm, 0.103 μg per 14 mm. The relative contents of anthocyanins contained in individual organs were calcd from the peak heights compared with that of compound **1**.

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