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TETRAHYDROANTHRACENE GLUCOSIDES IN CALLUS TISSUE FROM *ALOE BARBADENSIS* LEAVES

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Key Word Index—*Aloe barbadensis*; Asphodelaceae; plant tissue culture; tetrahydroanthracene glucosides; effect of light.

Abstract—Callus tissue of *Aloe barbadensis* grown in the dark produced two new tetrahydroanthracene glucosides, 3,4-dihydro-2,4,8,9-tetrahydroxy-6-methyl-1(2H)-anthracenone-4-*O*-β-D-glucopyranoside and 3,4-dihydro-2-methoxy-4,8,9-trihydroxy-6-methyl-1(2H)-anthracenone-4-*O*-β-D-glucopyranoside. The effect of light on the formation of tetrahydroanthracene glucosides in the calli is discussed.

INTRODUCTION

In an earlier paper we identified several tetrahydroanthracene derivatives from subterranean stem [1] and callus tissue [2] of *Aloe saponaria*, and discussed the biosynthetic relationship between tetrahydroanthracene derivatives and anthraquinones [3]. A recent study suggests that tetrahydroanthracene derivatives may be useful as a marker for the chemotaxonomic classification of Asphodelaceae [4]. Aloeresin E isolated from *A. barbadensis* Mill leaf showed antityrosinase [5] and topical anti-inflammatory activities [6]. The present paper describes the establishment of callus tissue from *A. barbadensis* leaf and the isolation of two new compounds (**1** and **2**) from the callus tissue. The effect of light on the formation of tetrahydroanthracene derivatives was also investigated.

RESULTS AND DISCUSSION

To induce callus of *A. barbadensis* young leaf segments were cultured either in the light or in the dark on Murashige–Skoog (MS) solid medium [7] supplemented with different combinations and concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid (NAA) and benzyladenine (BA). When the callus was cultured on MS medium supplemented with 10 mg l^{-1} NAA and 0.2 mg l^{-1} BA under continuous irradiation (fluorescence lamp 2500 l×) for 6 weeks, it grew slowly and turned dark brown, while the callus cultured under the same conditions in the dark gave a fresh yellow mass. In preliminary experiments, the phenolic components in the callus extract were examined by HPLC with photodiode-array detection [8]. The peaks in each callus extract, showing different UV-VIS spectral profiles from those of aloesin and aloin related components, had similar spectra to those of the tetrahydroanthracene derivatives aloesaponol-III and -IV glucoside, and were eluted in the range R_t 8 to 15 min. HPLC analysis of the extract showed that the most suitable conditions for formation of **1** and **2** were growth of the calli in the dark for 6 weeks on MS medium, solidified with 0.2% gelling agent, Gerlite (produced by KELCO, U.S.A.), supplemented with 10 mg l^{-1} NAA and 0.2 mg l^{-1} BA (Table 1). After subculture under the same conditions, the calli thus obtained were extracted with methanol and the extract was chromatographed on MCI-gel CHP-20P and Sephadex LH-20 columns to give **1** and **2**.

Compound **1** had a similar UV spectrum to that of aloesaponol III [1]. The HR-positive FAB mass spectrum showed the $[M+H]^+$ peak at m/z 436.1368, suggesting the molecular formula, $C_{21}H_{24}O_{10}$. By comparison of the ^1H and ^{13}C NMR spectral data of **1** with those of aloesaponol III (**3**) [1] and gasteriacenone [9], the structure of **1** was suggested to be a glucosyl compound having an additional β-hydroxy group at the C-2 position as in aloesaponol III (Tables 2 and 3). In the ^1H NMR spectrum the D-glucopyranose

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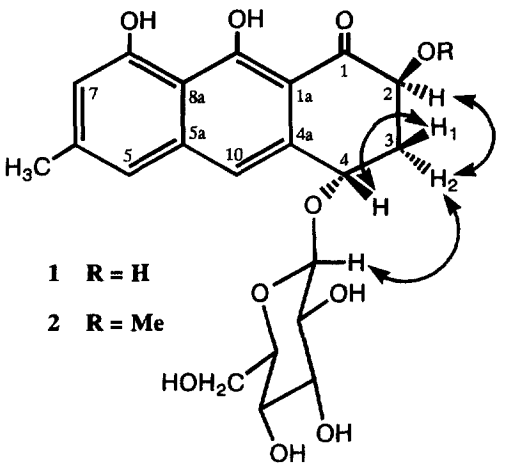
Table 1. Effect of auxins and BA on callus formation and growth in primary (a) and secondary (b) callus cultures of *A. barbadensis* leaf in the dark

	Auxin ppm	BA ppm	Callus formation %	Dry Weight (mg) a	b
NAA	1	0	100	25.1	183.4
	1	0.2	50	115.8	471.7
	5	0	90	53.4	1604.9
	5	0.2	100	60.3	973.8
	10	0	80	47.7	1485.2
	10	0.2	100	67.9	394.5
2,4-D	1	0	100	27.3	122.7
	1	0.2	90	63.1	273.3
	3	0	100	8.4	100.8
	3	0.2	100	30.5	289.9



$^1H-^1H$ COSY correlations shown by arrows

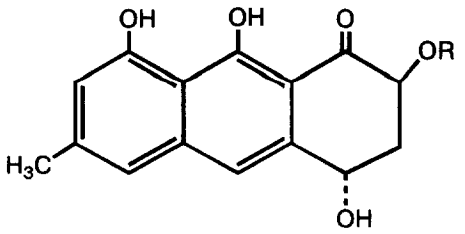
linkage in **1** was shown to be β by the coupling constant ($J = 7.6$ Hz) of the anomeric proton. The linkage of the β-D-glucopyranosyl moiety in **1** was determined

Table 3. ^{13}C NMR chemical shifts of **1** and **2** (δ in CD_3OD)

Carbon No.	**1**	**2**
1	205.7	203.4
2	69.3	79.0
3	39.9	38.2
4	67.9	66.9
5	119.7	118.6
6	143.9	144.0
7	115.8	115.7
8	158.6	158.6
9	165.8	166.3
10	123.2	123.2
1a	109.8	110.0
4a	141.7	141.7
5a	140.2	140.8
8a	115.4	115.2
2-OMe		59.1
6-Me	22.1	22.1
1′	104.3	104.2
2′	75.3	75.3
3′	77.6	77.6
4′	71.5	71.6
5′	78.7	78.7
6′	62.7	62.8

Table 2. ^1H NMR spectral data of **1** and **2** (δ in CD_3OD)

H	**1**	**2**
2	4.83 *dd* (12.4, 4.9)	4.34 *dd* (9.2, 4.3)
3_1	2.52 *ddd* (12.5, 6.8, 4.9)	2.42 *ddd* (13.0, 6.1, 4.3)
3_2	2.23 *ddd* (12.5, 12.4, 3.0)	2.32 *ddd* (13.0, 9.2, 3.7)
4	5.02 *t* (3.0)	5.03 *dd* (6.1, 3.7)
5	7.21	7.22
7	7.27	7.25
10	7.18	7.20
2-OMe		3.60
6-Me	2.46	2.45
1′	5.00 *d* (7.6)	5.00 *d* (7.9)



3 aloesaponol III R = H

4 aloesaponol IV R = OMe

to be at C-4 by proton to proton correlation (^{1}H-^{1}H COSY) experiments. On the basis of the spectral data, the structure of **1** was established as 3,4-dihydro-2,4,8,9-tetrahydroxy-6-methyl-1(2H)-anthracenone-4-*O*-*β*-D-glucopyranoside (**1**).

Compound **2** showed similar UV spectra to that of aloesaponol IV [1]. The HR-positive FAB mass spectrum showed the $[M+H]^+$ peak at *m/z* 450.1527, suggesting the molecular formula, $C_{22}H_{26}O_{10}$. The ^{1}H and ^{13}C NMR spectral data of **2** were closely related to those of aloesaponol IV (**4**) glucoside (Tables 2 and 3). The D-glucopyranose linkage in **2** was shown to be *β* by the coupling constant (J = 8.0 Hz) of the anomeric proton. On enzymatic hydrolysis **2** gave D-glucose. The linkage of D-glucose moiety in **2** was determined to be at C-4 by ^{1}H-^{1}H COSY correlation experiments. On the basis of the spectral data, the structure of **2** was established as 3,4-dihydro-2-methoxy-4,8,9-trihydroxy-6-methyl-1(2H)-anthracenone-4-*O*-*β*-D-glucopyranoside (**2**).

In previous experiments tetrahydroanthracene derivatives were mainly isolated from subterranean stem [1] and root of *Aloe* species [4]. The inhibitory effect of light on the accumulation of tetrahydroanthracene glucosides in the callus tissue might be related to the facts that tetrahydroanthracene derivatives are only contained in the subterranean stem and root of *Aloe* species. In the present work aloesin and tetrahydroanthracene glucosides **1** and **2** were isolated only from dark grown callus tissue of *A. barbadensis* leaf, and aloesin esters and aloin groups which are commonly found in the leaves of *Aloe* species and have antifungal activity [10] were not detected in the callus tissue. These findings show that callus tissues are active in glucosidation and do not have the acyl esterase needed to produce aloesin esters.

EXPERIMENTAL

General. O r and UV-VIS: MeOH; ^{1}H and ^{13}C NMR: TMS as int. standard; Positive FAB-MS (JEOL HX-110): glycerol as matrix; HR FAB-MS (JEOL HX-110): polyethylene glycol as matrix; CC: MCI-gel CHP 20P (75–150 *μ*m, Mitsubishi Chemical Industries) and Sephadex LH-20 (25–100 *μ*m, Pharmacia Fine Chemicals); HPLC: detection by UV-8000 UV-VIS detector (Tosoh) set at 290 nm and a Model 991J photodiode-array detector (Waters).

HPLC analysis. The column used was a Wakosil-II 5C18 HG reversed-phase column (5 *μ*m, 150 × 4.6 mm I.D., Wako Pure Chemical Industrials). The sepn was carried out at 45° using a linear gradient programme at a flow-rate of 1 ml min^{-1}; eluent MeCN–H_2O, 0–19 min, 12–23%; 19–24 min, 23–28%; 24–39 min, 28–46%.

Plant material and method of culture. Voucher specimens of the plant are deposited at the Medicinal Garden, Fukuyama University. Herbarium number 54-3. The leaves (10 cm in length) were disinfected with 75% EtOH for 1 min and rinsed once with sterilized H_2O, and then sterilized with 2% NaOCl for 10 min and washed with sterilized $H_2O \times 3$. A segment ($5 \times 5 \times 2$ mm^3) was cut off and cultured on MS solid (0.2% Gerlite) medium supplemented with 10 mg l^{-1} NAA and 0.2 mg l^{-1} BA for 8 weeks in the dark at 25°. The callus was subcultured under the same culture conditions for 4 weeks to obtain sufficient material for the experiments.

Isolation. Dried callus (200 g) was extracted with MeOH (80 g). The MeOH extract was subjected to MCI-gel CHP 20P CC using a stepwise gradient elution with H_2O–MeOH as solvent. The 40% MeOH eluate was rechromatographed over an MCI-gel CHP 20P column with 40% MeOH and a Sephadex LH-20 column with H_2O to furnish **1** (21 mg). The 50% MeOH eluate was rechromatographed over an MCI-gel CHP 20P column with 50% MeOH and a Sephadex LH-20 column with H_2O to furnish **2** (14 mg).

Compound **1**. Pale yellow powder, $[\alpha]_D^{16} -129.9°$ (MeOH; *c* 0.253). UV λ_{max}^{MeOH} nm (log *ε*): 197 (4.14), 225 (4.43), 267 (4.68), 297 (3.69), 308 (3.73) and 391 (3.99); HR-positive FAB-MS *m/z*: Found 436.1368 $[M+H]^+$ ($C_{21}H_{24}O_{10}$ requires 436.1368); ^{1}H and ^{13}C NMR: Tables 2 and 3.

Compound **2**. Pale yellow powder, $[\alpha]_D^{17} -119.3°$ (MeOH; *c* 0.247). UV λ_{max}^{MeOH} nm (log *ε*): 202 (4.36), 226 (4.57), 268 (4.73), 297 (3.86), 308 (3.90) and 395 (4.06); HR-positive FAB-MS *m/z*: Found 450.1527 $[M+H]^+$ ($C_{22}H_{26}O_{10}$ requires 450.1525); ^{1}H and ^{13}C NMR: Tables 2 and 3. Compound **2** (1 mg) dissolved in H_2O (1 ml) was hydrolysed with *β*-D-glucosidase (1 mg, Nakaraitesq) to give glucose (Avicel TLC, *n*-BuOH–pyridine–H_2O 6:2:3, with pyridine (1 drop) added to the upper layer).

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