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FORMATION OF TETRAHYDROANTHRACENE GLUCOSIDES BY CALLUS TISSUE OF *ALOE SAPONARIA*

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

Abstract—Callus tissue of *Aloe saponaria* grown in the dark produced a new tetrahydroanthracene glucoside, 1-oxo-2-methoxy-4,8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracene (aloesaponol IV) 8-*O*-β-D-glucoside, together with known tetrahydroanthracene glucosides. The effect of light on the formation of tetrahydroanthracene and anthraquinone glucosides is discussed.

INTRODUCTION

Aloesaponarin I, laccaic acid D methyl ester and deoxyerythrolaccin isolated from the subterranean stem of *Aloe saponaria* [1] exhibited anti-microbial activity at 12.5 μg/ml (minimum inhibition concentration) [2]. The corresponding tetrahydroanthracene, aloesaponol I, however, did not show any activity. In an earlier experiment, the biosynthetic transformation of aloesaponol I to aloesaponarin I in the intact plant was discussed [3]. This paper describes the formation of tetrahydroanthracene and anthraquinone glycosides in callus tissue under the control of auxin and the structure elucidation of a new glucoside of tetrahydroanthracene, aloesaponol IV. The effect of light on the formation of tetrahydroanthracene and anthraquinone derivatives is discussed.

RESULTS AND DISCUSSION

Various organs of *A. saponaria* were investigated for callus induction in media supplemented with different combinations of 2,4-D and kinetin. The results showed that the most suitable organ was root tissue and that Murashige–Skoog medium [4], containing 1 ppm 2,4-D and 2 ppm kinetin, was the best medium for callus induction. When the callus was cultured in the same medium under continuous irradiation (fluorescent lamp, 2500 lx) for 3 months, it grew rapidly and became greenish, soft and friable. As the presence of anthraquinones was detectable by TLC monitoring, the callus was worked-up to give a small amount of chrysophanol. However, none of the tetrahydroanthracene compounds found in the subterranean stem [1] was detected.

To investigate the effect of light on the formation of tetrahydroanthracenes, the callus cultured under continuous irradiation (2500 lx) for 6 weeks was subcultured on the same medium in the dark for 9 weeks. Under these conditions there was a large increase in the fr. wt of the callus. TLC showed the presence of a new tetrahydroanthracene glucoside, **1**, together with the glucosides of aloesaponol I–III. The yield of total glucosides, on the basis of the fr. wt, was 0.016%, which is 100 times higher than that from the fresh subterranean stem [1]. On enzymatic hydrolysis, **1** gave an aglycone which was identified as aloesaponol IV. The glycoside was shown to be 8-*O*-β-D-glycopyranosyl aloesaponol IV on the basis of chemical and spectral evidence (Experimental).

In a preliminary study, the effect of either auxin or kinetin on the growth of callus in the dark was investigated (Table 1). In all media the growth of callus was extremely poor. Subsequently, the effect of a combination of auxin and kinetin for callus growth was examined. In the medium containing IAA and kinetin the higher concentration of kinetin inhibited the growth of callus. This phenomenon was also observed in the medium supplemented with NAA and kinetin (Table 1). However, the addition of 2,4-D and kinetin stimulated growth of the callus. Supplementation by a combination of 1 ppm IAA and 0.5 ppm kinetin or 0.5 ppm 2,4-D and 2.0 ppm kinetin gave the best growth. In each medium the callus tissue was pale white and friable. When the extract from each callus was analysed by TLC, no significant differences in quality were observed and **1** as well as the known tetrahydroanthracene glucosides, were detected. It is well-known

Table 1. Effect of auxins and kinetin on growth in a 9-week-old callus culture of *A. saponaria* in the dark

	Auxin (ppm)	Kinetin (ppm)	Fr. wt of callus (g)
IAA	0.5	0.5	6.9
	0.5	2.0	3.0
	1.0	0.5	7.3
	1.0	2.0	3.3
	1.0	0	0.2
NAA	0.5	0.5	2.1
	0.5	2.0	1.1
	1.0	0.5	4.1
	1.0	2.0	3.3
	1.0	0	0.3
2,4-D	0.5	0.5	2.1
	0.5	2.0	7.6
	1.0	0.5	4.1
	1.0	2.0	4.4
	1.0	0	0.4
	0	1.0	0.1

that pigment formation in tissue cultures of *Morinda citrifolia* [5] and *Digitalis lanata* [6] is inhibited by addition of 2,4-D. On the other hand, it had been reported that polyketide formation in tissue cultures is not affected by 2,4-D [7]. The effect of auxins in our experiments was similar to that of auxins on the formation of anthraquinones which are derived from polyketides [8].

A characteristic alteration of the pattern in the constituents between light- and dark-grown callus was reported in *Lithospermum erythrorhizone* [9], and a quantitative change in pigment formation in callus tissue of *A. saponaria* has been described previously. The inhibitory effect of light on the accumulation of tetrahydroanthracene glucosides in the callus tissue might be related to the fact that tetrahydroanthracene derivatives are only contained in the subterranean stem of this plant. Therefore, from the finding that the formation of anthraquinone was associated with light irradiation, it was reasonable to assume that light would accelerate the metabolism of tetrahydroanthracene glucosides. To clarify the metabolic relationship between tetrahydroanthracene and anthraquinone glycosides, an experiment was carried out using a culture grown under continuous irradiation. The culture was separated into the cell bodies and the filtrate. It was clear that only the cell bodies contained pigments. Next, a dark-grown callus was cultured in liquid medium for 15 weeks under continuous irradiation to give green cells. TLC showed that the tetrahydroanthracene glucosides disappeared to be replaced by anthraquinone glucosides, one of which was identified as chrysophanol glucoside (TLC, UV of aglycone). Therefore, it is reasonable to speculate that the tetrahydroanthracene glycosides were metabolized as a result of the presence of light. The metabolism of tetrahydroanthracene glucosides in the suspension culture was also accelerated by irradiation with red (50 lx) or blue (100 lx) light.

EXPERIMENTAL

TLC (Si gel) and prep. TLC (Si gel 60, 2 mm) of tetrahydroanthracene derivatives. Glucosides: $CHCl_3$–MeOH–H_2O (7:3:0.5), EtOAc–MeOH–H_2O (20:3:2); aglycone: EtOAc–$CHCl_3$ (1:1), C_6H_6–Me_2CO (10:1). Detection: UV. TLC (Avicel SF) of glucose: BuOH–pyridine–H_2O (6:2:3, upper layer) with aniline hydrogenphthalate as spray reagent. CC was carried out on Kieselgel (0.05–0.5 mesh) and Sephadex LH-20.

Plant material and method of culture. The callus tissue used was derived from the root tip of *A. saponaria* on Murashige–Skoog (MS) medium supplemented with 1 ppm 2,4-D and 2 ppm kinetin under continuous light (2500 lx) from cool white fluorescent lamps at a temp. of 25 ± 1° for 3 months. The callus was cultured using a combination of auxin and kinetin, as shown in Table 1 in the dark at 25 ± 1°. The basal medium consisted of MS salts with the following additives (mg/l.): myoinositol (100), nicotinic acid (0.5), pyridoxine HCl (0.5), thiamine HCl (0.1), glycine (2.0), sucrose (3×10^4) and agar (8000). A voucher specimen of *A. saponaria* is available for inspection at the Herbal Garden, Kyushu University, Fukuoka, Japan.

Suspension culture. A suspension culture was initiated from the callus tissue induced in the dark and cultured in the same medium as described above without the addition of agar under continuous irradiation with white (2500 lx), blue (100 lx, 550 ± 50 nm), or red (50 lx, 600 ± 20 nm) light.

Formation of chrysophanol in the light. Fresh green callus tissue (42 g) was harvested after 12 weeks. The MeOH extract was subjected to CC on Amberlite XAD-2 followed by Sephadex LH-20 to give a small amount of chrysophanol, which was identical with an authentic sample (TLC, UV).

Formation of glucosides of aloesaponol I–III in the dark. A pale yellow callus tissue (370 g) cultured in the dark and showing a strong yellow fluorescence was harvested after 9 weeks. The MeOH extract (10 g) was subjected to CC on Amberlite XAD-2 using H_2O and MeOH as solvents, successively. The MeOH eluate (0.3 g) was chromatographed on Sephadex LH-20 eluted with Me_2CO followed by Si gel eluted with 10% MeOH–EtOAc to afford a mixture of glucosides (0.06 g, yield 0.016%). The glucoside mixture was separated by prep. TLC to give glucosides of aloesaponol I–III. Each glucoside was identified by: (1) co-chromatography with a standard sample on TLC; and (2) hydrolysis with crude hesperidinase to give the aglycone which was identified by co-chromatography (TLC) with an authentic specimen.

Identification of **1** (*aloesaponol IV* 8-O-β-D-*glucopyranoside*). Compound **1**, pale yellow fluorescent powder, $[\alpha]_D^{18} - 34.1°$ (MeOH; *c* 0.27), positive colour reaction to $FeCl_3$ reagent. IR ν_{max}^{KBr} cm^{-1}: 3400, 1600; UV λ_{max}^{MeOH} nm (log ε): 266 (4.22), 297 (3.80), 306 (3.80), 320 (sh), 395 (3.80); UV $\lambda_{max}^{MeOH-AlCl_3}$ nm: 278, 315, 450; ^{1}H NMR 100 MHz (Me_2CO-d_6, TMS as int. standard): δ 1.18 and 1.40 (2H, –CH–C<u>H</u>$_2$–CH–, *J* = 16, 4 Hz), 2.44 (3H, Me), 3.58 (3H, MeO), 4.30 (1H, C<u>H</u>OMe), 5.00 (C<u>H</u>OH), 5.06 (1H, anomeric, *J* = 6 Hz), 7.28 (3H, aromatic), 15.00 (OH). Compound **1** (10 mg) was dissolved in 0.01 M Pi buffer (pH 6.3, 3 ml) and hydrolysed with crude hesperidinase (10 mg) at 37° for 24 hr. The reaction mixture was extracted with EtOAc and the EtOAc extract chromatographed on Si gel to give an aglycone which was identical with authentic aloesaponol IV (TLC, UV and ^{1}H NMR). From the aq. layer glucose was detected on Avicel TLC. Therefore, **1** was aloesaponol IV β-D-glucoside. The glucose linkage in **1** was established by spectral study of the acetate as follows. Compound **1** (3.5 mg) was acetylated in the usual way followed by chromatography on Si gel (C_6H_6–Me_2CO, 5:1) to give a yellow amorphous acetate, MS (*m/z*): 660 $[M]^+$, 483 $[M - 3 \times MeCOO]^+$; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3200 (OH), 1750, 1620 (chelated C=O); UV λ_{max}^{MeOH} nm (log ε): 265 (4.10), 300 (3.70), 310 (3.70), 385 (3.50); $\lambda_{max}^{MeOH-AlCl_3}$ nm: 270, 385, 450; ^{1}H NMR ($CDCl_3$ 100 MHz TMS as int. standard): δ 1.26 (2H, –CH_2–), 2.00–2.20 (5 × Ac), 2.46 (3H, Me), 3.66 (3H, MeO), 6.30 (1H, C<u>H</u>OAc, *m*), 7.00, 7.12, 7.22 (1H, each, aromatic), 14.80 (OH). These data showed that **1** was the 8-*O*-β-glucoside of aloesaponol IV.

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