FORMATION OF TETRAHYDROANTHRACENE GLUCOSIDES BY CALYX TISSUE OF ALOE SAPONARIA

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Abstract—Calyx tissue of Aloe saponaria grown in the dark produced a new tetracyclanthracene glucoside, 1-(3,4-dimethoxy-4,8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracen-9-yl)-2,3,5,6-tetrahydroanthracene-9-glucoside, together with known tetracyclanthracene glucosides. The effect of light on the formation of tetracyclanthracene and dihydroanthracene glucosides is discussed.

INTRODUCTION

Aloe saponaria L., hexacyclic D-methyl ether and desacyclanthracene isolated from the subterranean latex of Aloe saponaria [1] exhibited anti-microbial activity at 12.5 µg/ml (minimum inhibition concentration) [2]. The corresponding tetrahydroanthracene, aloesaponin I, however, did not show any activity. In an earlier experiment, the biosynthetic transformation of aloesaponin I to aloe saponin I in the intact plant was discussed [3]. This paper describes the formation of tetracyclanthracene and dihydroanthracene glucosides in calyx tissue under the control of auxin and the synergistic elimination of a new glucoside of tetracyclanthracene, aloesaponin IV. The effect of light on the formation of tetracyclanthracene and dihydroanthracene glucosides is discussed.

RESULTS AND DISCUSSION

Various organs of A. saponaria were investigated for inclusion 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 illumination (fluorescent lamp, 2500 lux) for 3 months, it grew rapidly and became greenish, soft and fluffy. As the presence of anthraquinones was detectable by TLC monitoring, the callus was used to grow small amounts of dry callus. However, none of the tetracyclanthracene compounds found in the subterranean latex [1] was detected.

To investigate the effect of light on the formation of tetracyclanthracenes, the callus cultured under continuous illumination (2500 lux) for 6 weeks was subcultured on the same medium in the dark for 9 weeks. Under these conditions there was a 10% increase in the fr wt of the callus. TLC showed the presence of a new tetracyclanthracene glucoside, I, together with the glucosides of aloesaponin I and II. The yield of the glucoside on the basis of the fr wt was 0.01% which is 100 times higher than that from the fresh subterranean latex [1]. On thin-layer chromatography, I gave an aglucone which was identified as aloesaponin IV. The glucoside was shown to be 1-(3,4-dimethoxy-4,8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracen-9-yl)-2,3,5,6-tetrahydroanthracene-9-glucoside on the basis of chemical and spectral evidence (Experimental).

In a preliminary study, the effect of mixtures of auxin and kinetin on the growth of callus in the dark was investigated (Table I). At low auxin 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 1 ppm 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 II). However, the addition of 2,4-D and kinetin stimulated growth of the callus. Supplementation by a concentration of 1 ppm IAA and 0.5 ppm kinetin or 0.1 ppm 2,4-D and 2.0 ppm kinetin gave the best growth. In each medium the callus tissue was pink, white and fibrous. When the extract from such callus was analyzed by TLC, no significant differences in quantity were observed and as well as the known tetracyclanthracene glucosides, were detected. It is well-known

Table 1. Effect of auxins and kinetins on growth in a 2,4-D and NAA in the dark

<table>
<thead>
<tr>
<th>Auxin (ppm)</th>
<th>Kinetin (ppm)</th>
<th>Fr. wt of callus (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.5</td>
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<td>0.5</td>
</tr>
<tr>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>1.6</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>6.0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>NAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
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<tr>
<td>1.0</td>
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</tr>
<tr>
<td>1.6</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>6.0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>2,4-D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
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<tr>
<td>0.5</td>
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<tr>
<td>1.0</td>
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<tr>
<td>6.0</td>
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</tr>
</tbody>
</table>
that pigment formation in tissue cultures of Morinda
chiropeia [5] and Dugesia tenea [6] is inhibited by
addition of 2,4-D. On the other hand, it had been reported
that polyphenol 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 anthra-
quinones which are derived from polyphenols [8].
A characteristic alteration of the pattern in the con-
stituents between light- and dark-grown callus was re-
ported in Lycopersicon esculentum [9], and a quantita-
tive change in pigment formation in callus tissue of A.
θ-θ-8 has been described previously. TheURITY
the inhibitory effect of light on the accumulation of teratrol-
ol anthraquinones in the callus tissue might be related
the fact that teratrololanthraquinone derivatives are
only contained in the subterranean stem of this plant.
Therefore, from the finding that the formation of anthra-
quinones was associated with light irradiation, it was
reasonable to assume that light would accelerate the
metabolism of teratrololanthraquinones. To clarify
the metabolic relationship between teratrolol-
anthraquinones and anthraquinones glycosides, an experi-
ment was carried out using culture grown under controlled
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 cul-
tured in liquid medium for 15 weeks under continuous
irradiation to give green cells. TLC showed that the
teratrololanthraquinones glycosides disappeared to be
replaced by anthraquinones glycoside, one of which was
determined as chrysophanol glycoside (TLC, UV, 0.05 g of
alcohol). Therefore, it is reasonable to speculate that the
antheraquinones glycosides were metabolized as a result of
the presence of light. The metabolism of teratrololanthra-
nquinones glycosides in the suspensor culture was
also accelerated by irradiation with red (600 nm) or
blue (450 nm) light.

EXPERIMENTAL

TLC (sil gel and prep. TLC (sil gel 60, 3.5 mm) of teratrol-
anthraquinones derivatives, 2-C-CH3-Malonol [10] (3:1:3),
EIOH-MeOH-water (20:3:2) aglucone EIOAc-
CH2OH (1:1); C18 H32016 [10] has been described previously. UV TLC (sil S 8) of glucose and pyranose HPLC was carried out using a diode array spectrophotometer at 280 nm.

Materials and methods of culture. The callus tissue used was
obtained from the apex of A. θ-θ-θ grown on Morinda-glyco-
mic medium supplemented with 1 ppm 2,4-D and 2 ppm kinetin
under continuous light (1000 lux) from cool white fluorescent lamps. The callus was maintained by subculture every 4 weeks. The separation of the callus was performed using a combination of monos and kinesin, as shown in Table 1. Growth of the excised callus was maintained in a medium with the following additions (mg per 100 ml): myo-inositol (10), nicotinic acid (10) and pyridoxine. HCl (50), thiamine HCl (40), glycine (20). In order to make the culture homogeneous and 

Suspensin was used as a suspensor culture to prevent the growth of new callus tissues from the callus tissues harvested in the dark and cultured in the same medium as described above without the addition of auxin. The cultures in which the growth of new callus tissues was inhibited were subjected to the anthraquinones determination of the whole callus tissue harvested after 12 weeks. The EIOAc extract was subjected to TLC on Amliter X-2 before being subjected to a small amount of chrysophanol, which was detected
by a standard sample. TLC (UV, 0.05 g of silica gel 60, 3.5:1
MeOH, CH3CO2H, CH3OH (20:3:2) followed by 103 MeOH
dissolved in the 30:80, 50:80, 80:80 (v/v) and 95 MeOH
UV detection at 280 nm. TLC was developed at 200 MeOH
(20:3:2) for 24 hrs. Reaction mixture was extracted with EIOAc
extract of the 0.05 g of silica gel which was subjected to anthraquinone isolation (UV, 0.05 g of silica gel 60, 3.5:1
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