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BIOTRANSFORMATION OF A GUAIANE 6,12-DIOL TO THE CORRESPONDING GUAIANOLIDE IN HYPOCOTYL CUTTINGS OF *PHASEOLUS AUREUS*

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Key Word Index—*Phaseolus aureus*; Leguminosae; guaiane 6,12-diol; guaianolide.

Abstract—The hypocotyl cuttings of *Phaseolus aureus* brought about the biotransformation of a guaiane 6,12-diol to the corresponding lactone during the formation of adventitious roots.

INTRODUCTION

The role of terpenoids and their synthetic derivatives in plant growth has attracted attention during recent years and several of the natural compounds and their synthetic analogues are capable of increasing yield [1]. As part of our extensive studies, we earlier demonstrated [2] that the presence of hydroxyl groups in these systems lowers biological activity. Notable exceptions are the alantolides, where it has been shown by us [3] that a hydroxyl group at the C-5 angular position, increases the potential for root formation. Thus, it has been established that **5** and **7** are more potent than their parent compounds **4** and **6** respectively. We then discovered, surprisingly, that the diol **1** obtained by the lithium aluminium hydride reduction of the lactone **2** was biologically active (Table 1). Since this diol should theoretically be inactive, it seemed likely that it was being transformed *in vivo* to the corresponding guaianolide **2** which in turn was responsible for root initiation. This indeed proved to be so, as shown by the experiments presented in this communication.

RESULTS AND DISCUSSION

The diol **1** was converted after seven days mainly to the lactone **2** and guaianolide **3** by the hypocotyl cuttings of *P. aureus* as revealed by HPLC. By prep. TLC **2** was isolated and identified by comparative IR studies and mmp with an authentic sample. The formation of **3** obviously involves the opening of the cyclopropane ring, an observation reminiscent of the formation of squalene from presqualene which also contains a cyclopropane moiety [4].

The lactonization of the diol **1** to the corresponding lactone **2** involves the facile and selective oxidation of the hydroxymethylene to the carboxyl group and the subsequent lactonization of the corresponding γ-hydroxy acid. Indeed lactone **2** is obtained quantitatively by the Jones chromic acid oxidation of the diol **1**. Moreover, it is known that *in vivo*, the hydroxymethylene group at C-6 in glucose is oxidized by enzymes in preference to the aldehyde and the secondary hydroxyl groups.

EXPERIMENTAL

HPLC was carried out using a Beckman gradient liquid chromatograph series 332 with a UV detector (215 nm). MeCN–H_2O (7:3) was employed as the mobile phase using an ODS column 150 × 4.6 mm and altex pre-column 45 × 4.6 mm. Relative retention times were **1** 5.30, **2** 6.77 and **3** 8.92 min.

In vivo conversion of diol **1** *to lactones* **2** *and* **3**. Diol **1** (70 mg) was administered to the hypocotyl cuttings of *Phaseolus aureus* as a 30 mg/l soln, in all using *ca* 2000 seedlings (cuttings). After 7 days at 28° when the roots initiated the total material was subjected to steam distillation. The steam distillate was extracted with Et_2O and lactone **2** was identified by TLC and HPLC studies. Lactone **3** was also identified by HPLC in addition to the parent diol. The material (50 mg) was subjected to prep. TLC when a compound, mp 42°, was obtained and identified as the guaianolide **2** by an mmp determination with an authentic sample and comparative IR spectra. In a control experimet a soln of diol **1** kept in H_2O at 28° for 7 days was recovered unchanged on steam distillation.

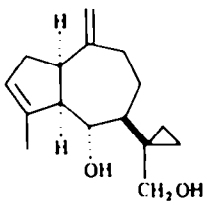
Chromic acid oxidation of **1** to **2**. To a soln of **1** (100 mg) in Me_2CO (5 ml) was added Jones chromic acid reagent until the reddish colour persisted for 30 min. It was poured into H_2O and extracted with Et_2O. The extracts were made neutral by washing with H_2O and evaporation of the solvent afforded **2** as evidenced by mmp with an authentic sample.

Biological testing. For root initiation studies on hypocotyl

Table 1. Effect of different concentrations of terpenoids on the number of roots per rooted segment produced on hypocotyl cuttings of *Phaseolus aureus* after 7 days

Treatment mg/l*	Number of roots			
	5	10	15	20
1	10.7 ± 1.4	14.3 ± 2.2	22.0 ± 1.2	22.3 ± 2.2
2	13.5 ± 2.2	18.2 ± 1.8	20.0 ± 1.4	26.7 ± 2.6
3	11.3 ± 1.9	15.8 ± 2.0	17.4 ± 2.5	20.5 ± 2.0

*Control experiment, water = 6.2 ± 0.8.



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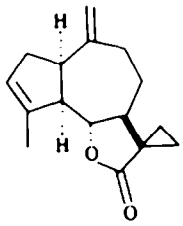

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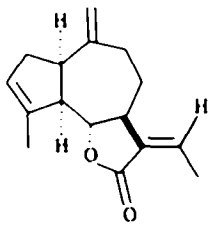

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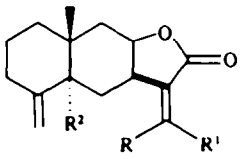

4 R = R¹ = R² = H
5 R = R¹ = H, R² = OH
6 R = R² = H, R¹ = Me
7 R = H, R¹ = Me, R² = OH

cuttings of *Phaseolus aureus* the seedlings were raised under continuous illumination. After 4 days, when the hypocotyls were 5–6 cm long, cuttings were made by excision 4 cm below the cotyledonary node leaving the cotyledonary leaves and apex intact. In all, three compounds at four concentrations (5, 10, 15 and 20 mg/l) along with H_2O as control were tested. For all treatments 10 replicates were cultured in vials each containing 30 ml of test soln. All the solns were replaced with fresh ones after 4 days. The final observations were recorded on the 7th day. The experiment was repeated × 3 at 26 ± 2°.

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