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DIHYDROPARTHENIN, A PSEUDOGUAIANOLIDE FROM TISSUE CULTURE OF *PARTHENIUM HYSTEROPHORUS*

K. K. TALWAR and P. S. KALSI

Department of Chemistry, Punjab Agricultural University, Ludhiana, India

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Key Word Index—*Parthenium hysterophorus*; Compositae, tissue culture, pseudoguaianolide; dihydroparthenin

Abstract—From the callus of *P. hysterophorus* a new pseudoguaianolide, dihydroparthenin has been isolated. Other known pseudoguaianolides have also been detected in the callus by HPLC.

INTRODUCTION

Parthenium hysterophorus is a member of the Compositae and contains a number of pseudoguaianolides of biological importance [1]. Earlier attempts have been made to establish the tissue culture of this plant to understand the biosynthesis of these sesquiterpene lactones [2] and only two compounds, coronopilin and parthenin, were detected by HPLC studies of callus extract [3]. The present communication reports the isolation and characterization of a new compound, $C_{15}H_{20}O_4$, mp 145° from callus culture.

RESULT AND DISCUSSION

An HPLC analysis of callus extract showed the presence of parthenin, coronopilin, hysterin, tetraneurin A, tetraneurin D and ambrosin in small quantities, in addition to several other unidentified compounds These compounds were extracted and separated by column chromatography and one of the compounds, mp 145°, was isolated in appreciable quantity.

This compound in its IR spectrum showed bands at 3400 (hydroxyl group) 1775 (γ-lactone) and 1725 cm^{-1} (cyclopentenone). ^1H NMR spectral features are reminiscent of parthenin with the only difference that the spectrum does not display the presence of an α-methylene-γ-lactone moiety but instead displays a doublet at δ0.9 ($J=9$ Hz). These data show that this compound is the dihydroderivative of parthenin. The other ^1H NMR spectral features are in complete accord with this structure. It is interesting that dihydroparthenin has been isolated in significant amount from the callus extract and it could be biotransformed to its dehydroderivative parthenin, a major component of the plant. Furthermore, dihydroparthenin has not been detected or isolated from the mature plant.

EXPERIMENTAL

Tissue culture Leaf blades, stem, petioles and capitula were washed quickly in 70% EtOH, rinsed twice with sterilized dist H_2O and then for 10 min with 10% commercial bleach and 2 drops of tween 20 (as wetting agent) After washing ×3 with sterilized dist water, leaves, stem and petioles were cut into 1 cm long sections and capitula were used whole

These explants were transferred to petriplates each having 25 ml of solidified 0 3% gelrite medium containing Murashige and Skoog [4] salts with 30 mg/l sucrose, 100 mg/l inositol, 0 1 mg/l 2,4-D, 0.05 mg/l kinetin and 0.1 mg/l BA along with modified Bourgan and Nitsch [5] vitamin mixture containing 2 0 mg/l glycine, 0.5 mg/l thiamine–HCl, 0 5 mg/l pyridoxine HCl, 0 5 mg/l nicotinic acid 0 5 mg/l folic acid and 0 05 mg/l biotin Media were adjusted at pH 5 7 before autoclaving. The culture were grown under fluorescent light of 1000 lux intensity with a photoperiod of 16 hr at 26±2° The callus were subcultured every 15 day on to fresh medium

Extraction and isolation Callus tissue (200 g) was extracted thoroughly with MeOH, it was concd to 50 ml and again extracted with $CHCl_3$ The $CHCl_3$ extract was directly chromatographed on silica gel using a benzene–EtOAc gradient The fraction eluted with (C_6H_6–EtOAc 9.1) afforded a crystalline compound, mp 145°, which gave a pink spot with vanillin spray (Found C, 68.01; H, 7.53 $C_{15}H_{20}O_4$ requires. C, 68 14; H, 7 63%) ^1H NMR δ0 8 (*d*, $J=9$ Hz, 3H, C-12 Me), 0 9 (*d*, $J=9$ Hz, 3H, C-10 Me), 1 2 (s, 3H, C-5 Me), 4.9 (*d*, $J=6$ Hz, 1H, C-6 proton), 6.3 (*d*, $J=6$ Hz, 1H, C-3H), 7.2 (*d*, $J=6$ Hz, 1H, C-2H)

HPLC studies HPLC was carried out using a Beckman gradient liquid chromatographic series 332 with a UV detector (215 nm) MeCN–H_2O was used as the mobile phase using an ODS column 150 × 4.6 mm and altex pre-column 45 × 4.6 mm The elution profile was 0–10 min 30% MeCN (isocratic), 10–20 min, 30–50% MeCN (linear gradient), 20–30 min, 50–70% MeCN (linear gradient) and 30–35 min, 70% MeCN (isocratic). Relative retention times were, tetraneurin D, 11 94, tetraneurin A, 12.63, hysterin, 14 32, parthenin, 15 80, coronopilin, 17 00, ambrosin 24 77, and dihydroparthenin, 32.10.

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