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ORIENTALIDINE AND ISOTHEBAINE FROM CELL CULTURES OF *PAPAVER BRACTEATUM*

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Key Word Index—*Papaver bracteatum*; Papaveraceae; cell cultures; alkaloids; orientalidine; isothebaine.

Abstract—Orientalidine, isothebaine and sanguinarine were isolated from callus cultures of *Papaver bracteatum* when grown on M & S medium with various hormonal supplements. The derived suspension cultures yielded orientalidine and sanguinarine.

INTRODUCTION

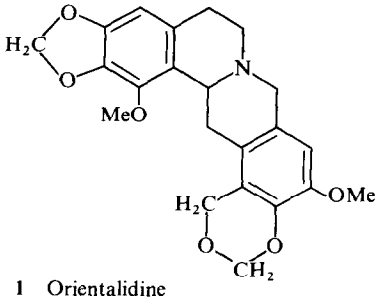
Cell cultures of *Papaver bracteatum* have been shown to contain only traces of thebaine [1] along with the benzophenanthrenes, protopines and aporphines [2]. Thebaine has also been detected in cultures from plants of Arya II population [3]. Amino acid precursor feeding had little effect on thebaine levels [4]. Seeds of *P. bracteatum* (Arya II) were found to contain thebaine 0.05%, but the dried callus and suspension cultures yielded no trace of thebaine. This paper describes cultures able to synthesize orientalidine (**1**) and isothebaine (**2**).

RESULTS AND DISCUSSION

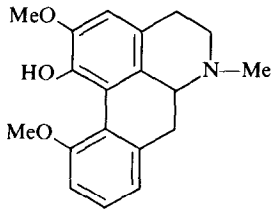
Callus cultures grown on Murashige and Skoog's medium with the addition of 1 mg/l. 2,4-D yield orientalidine and isothebaine. When subcultured onto medium containing in addition 0.1 mg/l. kinetin the growth rate increases, sanguinarine (**3**) is produced and isothebaine disappears. Reduction of the 2,4-D concentration to 0.5 mg/l. or 0.25 mg/l. results in the production of the same alkaloids. Addition of 10 mg/l. of ascorbic acid gives a cream-coloured callus containing the same two alkaloids. Furthermore, the addition of 10 mg/l. of cinnamic acid, whilst inhibiting growth, had no effect on the alkaloids.

When callus grown on medium supplemented with 1 mg/l. of 2,4-D alone is subcultured onto medium containing in addition 0.1 mg/l. kinetin + 1% polyvinyl-pyrrolidine the alkaloids orientalidine, isothebaine and sanguinarine are produced. In all cases the agar media contained traces of the alkaloids found in the callus.

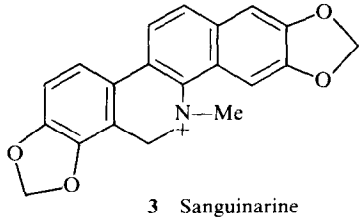
When sixth generation original callus was transferred into liquid medium containing 1 mg/l. 2,4-D and 1% Polyclar AT, only orientalidine and sanguinarine were detected. Addition of adsorbent polystyrenes to the media



1 Orientalidine



2 Isothebaine



3 Sanguinarine

resulted in decreased darkening of cells but no detectable increase in alkaloidal levels.

All extracted cells were found to contain sanguinarine alone after further extraction of the acid hydrolysed cell marc.

EXPERIMENTAL

Cell cultures. Seeds of *Papaver bracteatum* Lindl. Arya II were surface sterilized in 100 vol. H_2O_2 containing 1% Triton X for 2 min, then germinated on wet filter paper in Petri dishes in the dark at 25°. Hypocotyls of 5-day-old seedlings were explanted onto Murashige and Skoog's revised Tobacco medium (M & S) [5] containing 1 mg/l. 2,4-D. Further additions of 0.1 mg/l. kinetin, 10 mg/l. ascorbic acid, 1% acid washed Polycar AT and 1% were incorporated in subsequent media. The callus growth in darkness at 25° was cream in colour and subculturing was undertaken every 4 weeks with the addition of kinetin and ascorbic acid to the medium. Suspension cultures were initiated by transferring 6th generation callus portions to 250 ml conical flasks containing 50 ml liquid medium, and shaken at 150 rpm on an orbital shaker. Suspensions were maintained by shaking at 100 rpm in a 12 hr dark/12 hr light sequential regimen, and subcultured weekly.

Extraction procedures. Callus cultures were extracted by separation of the callus from medium, grinding with 5% HOAc, and filtering under vacuum. The filtrate was basified by addition of 10% NH_4OH to pH 9 and extracted by shaking with 3 × 50 ml portions $CHCl_3$. Pooled extracts were evapd to dryness under vacuum. Suspension cultures were first separated by filtration, and the cells treated as for callus. Media were basified and then extracted. Cell marcs were hydrolysed by refluxing with 2 N HCl at 100° for 5 min. Preliminary identification of alkaloids was by Si gel TLC developed in (a) Me_2CO–toluene–EtOH–NH_4OH (40:40:12:2.5), and (b) C_6H_6–Me_2CO–MeOH (7:2:1). Isolation was undertaken by chromatographing the extracts on 2 mm thick Si gel G layers in the above systems. Visualization was effected by Dragendorff's reagent and the alkaloids isolated from the adsorbent by elution with $CHCl_3$–MeOH (1:1) and the extracts evapd to dryness under N_2. Alkaloids were identified by comparison of TLC R_f values (systems (a) and (b)) and MS data with those of standard samples. TLC identification yielded the following R_fs in two systems, isothebaine 0.70 (a), 0.41 (b); orientalidine 0.82 (a), 0.78 (b); sanguinarine 0.93 (a), 0.91 (b). MS 70 eV, *m/z* (rel. int.): isothebaine 311 (M^+ 100), 310 ($M^+ - 1$, 62), 296 (33), 294 (55), 280 (42); orientalidine 397 (M^+ 65), 206 (47), 205 (29), 204 (41), 193 (29), 192 (53), 164 (35), 163 (35), 162 (100); sanguinarine 332 (10), 317 (100).

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