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CODEINE FROM CELL SUSPENSION CULTURES OF *PAPAVER SOMNIFERUM**

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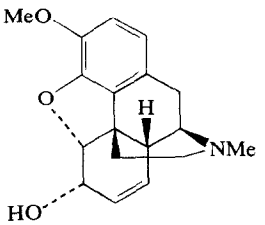
Presence of alkaloids in callus tissues of opium poppy has been reported [1–3] but no isolation and identification have been attempted. There have also been reports on the presence of phthalic acid ester in poppy tissues [4, 5]. Furuya *et al.* [6] and Ikuta *et al.* [7] reported the presence of benzophenanthridine, protopine, and aporphine type alkaloids but could not detect any synthesis of morphinan alkaloids in the callus tissues of *Papaver somniferum*. In their biotransformation experiments, Furuya *et al.* [8] indicate that the cell cultures of *Papaver somniferum* lack the ability to metabolize (*RS*)-reticuline to thebaine, codeine and morphine, but are able to metabolize (−)-codeinone to (−)-codeine. The present paper describes the synthesis of codeine **1** by cell suspension cultures of *Papaver somniferum* L. cv Marianne.



1 Codeine

The cell suspension cultures of *Papaver somniferum* cv Marianne, after incubation in 1-B5C medium for 3 weeks, were harvested and extracted for alkaloids by the procedure described below. The crude product showed presence of codeine **1** when compared with the authentic codeine on TLC. Purification of the crude product by preparative TLC yielded a compound (1.2 mg) having the same R_f (0.22) as the authentic codeine when co-chromatographed on TLC, mp 146–148° (lit. 154–155°). The MS displays a molecular ion at *m/e* 299, corresponding to $C_{18}H_{21}NO_3$, the molecular formula for codeine **1** and is identical with that of the authentic codeine. This indicates that the compound is codeine, the yield being 0.15%. Examination of the other seven fractions from preparative TLC purification by GLC with authentic morphine and thebaine as references showed neither of these alkaloids to be present. Comparison of these fractions with authentic protopine, norcodeine and papaverine on TLC showed absence of these alkaloids.

EXPERIMENTAL

Mps are uncorr. MS were recorded, using a direct insertion probe. GLC was performed on equipment with a FID using a glass column (180 × 0.2 cm) packed with 3% OV-17 on Gas-Chrom Q (80–100 mesh). Solutions of the fractions to be examined were subjected to GLC isothermally at a column temp. of 245° with He at 40 ml/min as the carrier gas. The injector and detector were at 230 and 250°, respectively.

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Cell cultures. Seeds of *Papaver somniferum* L. cv Marianne (D. J. van der Have, Netherlands) were sterilized with 20% Javex (5% Ca-hypochlorite) and germinated on wetted filter paper in Petri dishes in the dark at *ca* 25°. Hypocotyls of 5–6-day-old seedlings were isolated and explanted on nutrient medium after Gamborg *et al.* [9] with 1 mg/l. 2,4-dichlorophenoxyacetic acid (2,4-D) (1-B5) and solidified with 0.6% Difco-agar. The explants produced substantial callus and were transferred to fresh medium, 1-B5, fortified with 1 g/l. casein hydrolysate (N-Z Amine, Sheffield Co.) and 2 mg/l. kinetin. This callus material was subcultured every 4 weeks. Six-month-old callus was transferred to liquid medium and subcultured as 50 ml batches in Delong flasks on gyratory shakers (150 rpm). The resulting cell suspension was transferred to fresh medium at weekly intervals. Both callus and suspension cultures grew in continuous light, 800–1000 lx, at 25–28°. For the production of biomass and alkaloids, inoculum was collected from suspension cultures on sterile filters and *ca* 2 g of cell mass was incubated with 300 ml of B5 medium fortified with 1 mg/l. 2,4-D and 1 g/l. casein hydrolysate (1-B5C).

The suspension cultures consisted mainly of small cell aggregates, up to 1 mm in diameter. The aggregates were of little uniformity. Their pigmentation varied from dark brown to white, they contained tracheids and giant cells. The cells were rich in starch. The mitotic index of some aggregates reached 12% 5 days after transfer to fresh medium. Three per cent of mitotic figures showed polyploidy.

Isolation of codeine from the cell suspension cultures. After incubation in 1-B5C medium for 3 weeks, the medium was extracted with EtOAc (2 × 50 ml) while the cells were treated with boiling MeOH (150 ml). Both the MeOH and EtOAc extracts were combined and evapd to give a yellowish brown material which was redissolved in an EtOAc–N HCl (1:1) mixture (2 × 50 ml). The EtOAc and the N HCl layers were collected separately. The EtOAc soln was washed with N HCl (10 ml) and collected. The acidic washing was then combined with the acidic layer obtained previously and neutralized to pH 7.56 with sodium bicarbonate. After filtration, the filtrate was extracted with EtOAc (2 × 25 ml). These extracts were combined with the EtOAc extract obtained previously. Evapn yielded a yellowish brown material (97.8 mg) which was purified with PLC on Polygram® Si Gel G/UV_{254} plates (0.25)mm), using EtOAc–MeOH–NH_4OH (17:2:1) for development and $CHCl_3$–MeOH (4:1) as the eluent. This afforded codeine **1** of fair quality (1.2 mg) having the same R_f (0.22) as the authentic codeine when co-chromatographed on TLC as above, mp 145–148° (lit. 154–155°). Yield: 0.15% based on the dry cell wt (722.2 mg). MS (direct inlet) 70 eV *m/e* (rel. int.): 299 [M^+] (95), 298 [$M^+ - 1$; 19], 229 [$M^+ - 70$; 37], 214 [$M^+ - 85$; 30], 188 [$M^+ - 111$; 30], 162 [$M^+ - 137$; 100], 124 [$M^+ - 175$; 66], 115 [$M^+ - 184$; 54], 94 [$M^+ - 205$; 25], 81 [$M^+ - 218$; 36], 70 [$M^+ - 229$; 34], 59 [$M^+ - 240$; 33], 44 [$M^+ - 255$; 32], and 42 [$M^+ - 257$; 60]. Comparison of the other seven fractions from PLC using GLC with authentic morphine and thebaine as references showed neither of these alkaloids to be present. Comparison of these fractions with protopine, papaverine and norcodeine on TLC as above showed absence of these alkaloids.

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