(**2**) (31 mg), mp 145° (CH_2Cl_2–Et_2O); UV λ_{max}^{EtOH} nm: 235, 287 and 325; IR ν_{max}^{KBr} cm^{-1}: 1720, 1600 and 1460; ^1H NMR ($CDCl_3$): δ 2.35 (*s*, 3 H, OAc), 2.65 (*s*, 3 H, N-Me), 3.91 (*s*, 3 H, OMe), 3.96 (*s*, 3 H, OMe), 5.02 (*m*, 1 H, C-8 H), 6.03 (*s*, 2 H, OCH_2O), 6.98 (*d*, 1 H, J = 9 Hz, C-11 H), 7.13 (*s*, 1 H, C-4 H), 7.48 (*d*, 1 H, J = 9 Hz, C-5 H), 7.58 (*d*, 1 H, J = 9 Hz, C-12 H), 7.64 (*s*, 1 H, C-1 H) and 7.75 (*d*, 1 H, J = 9 Hz, C-6 H); MS: *m/z* 407 (M^+, 10%), 376 (5), 348 (100), 333 (34), 318 (16), 304 (16), 290 (22) and 275 (8).

A part of the EtOAc-soluble fraction (25 g) was chromatographed on a column of Si gel (1 kg) in C_6H_6 and eluted with increasing proportions of EtOAc and MeOH to afford other constituents [1–3] along with arnottianamide (**4**) (80 mg), mp 268° (MeOH), $C_{21}H_{19}NO_6$, M^+ *m/z* 381.1251; monoacetate (**5**), mp 237°, $C_{23}H_{21}NO_7$, M^+ *m/z* 423.

A part of the hexane-soluble fraction (50 g) was chromatographed on a column of Si gel (2.5 kg) in hexane and eluted with C_6H_6, EtOAc and MeOH to afford hexacosanoic acid (40 mg), mp 88° (hexane), $C_{26}H_{52}O_2$, M^+ *m/z* 396 and β-sitosterol (200 mg).

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REFERENCES

1. Sharma, P. N., Shoeb, A., Kapil, R. S. and Popli, S. P. (1979) *Indian J. Chem. Sect. B*, **17**, 299.
2. Sharma, P. N., Shoeb, A., Kapil, R. S. and Popli, S. P. (1980) *Phytochemistry* **19**, 1258.
3. Sharma, P. N., Shoeb, A., Kapil, R. S. and Popli, S. P. (1981) *Phytochemistry* **20**, 2781.
4. Dhawan, B. N., Patnaik, G. K., Rastogi, R. P., Singh, K. K. and Tandon, J. S. (1977) *Indian J. Exp. Biol.* **15**, 208.
5. Ishii, H., Ishikawa, T., Hosoya, K. and Takao, N. (1978) *Chem. Pharm. Bull.* **26**, 166.
6. MacLean, D. B., Gracey, D. E. F., Saunders, J. K., Rodrigo, R. and Manske, R. H. F. (1969) *Can. J. Chem.* **47**, 1951.
7. Ishii, H. and Ishikawa, T. (1976) *Tetrahedron Letters* 1203.
8. Francis, F. and Piper, S. H. (1939) *J. Am. Chem. Soc.* **61**, 577.

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BIOTRANSFORMATION OF THEBAINE BY CELL SUSPENSION CULTURES OF *PAPAVER SOMNIFERUM* CV. MARIANNE

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Key Word Index—*Papaver somniferum*; Papaveraceae; cell suspension culture; biotransformation; thebaine; neopine; morphinan alkaloids.

Abstract—Thebaine is biotransformed to neopine by cell suspension cultures of *Papaver somniferum* cv. Marianne grown in O-B5 medium. Results of precursor studies on these cell suspension cultures are also described.

INTRODUCTION

In our earlier paper [1], the isolation of codeine **1** from cell suspension cultures of *Papaver somniferum* L. cv. Marianne grown in 1-B5C medium was reported. Encouraged by the result, we began investigating the effects of precursors on these cell cultures. The present paper describes the results of application of codeine **1**, thebaine **2**, codeinone **3**, neopine HBr **4**, papaverine and D,L-laudanosoline HBr as precursors to these cell suspension cultures.

RESULTS

Cell suspension cultures of *Papaver somniferum* cv. Marianne were grown in 0-B5 medium (250 ml). The 0-B5 medium is the basic 1-B5 medium of Gamborg *et al.* [2] without the addition of 2,4-dichlorophenoxyacetic acid (2,4-D). After incubation with thebaine **2** (20 mg) for 3 days, the culture was harvested and extracted for alkaloids by the procedure described earlier [1]. This afforded 79.7 mg of extracted material. The mass spectrum (GC/MS) displayed a molecular ion at *m/z* 299, corresponding to $C_{18}H_{21}NO_3$ and was identical with that of authentic neopine **4**. The gas chromatogram showed that the compound had the same retention time (17.6 min) as authentic neopine. Comparison of the mass spectrum with that of codeine **1** showed that they differed in fragmentation patterns although they both displayed the same molecular ion at *m/z* 299. The compound was therefore neopine. Integration of the peak area indicated that the extracted material contained 0.79 mg of neopine, corresponding to 3.9% conversion of thebaine to neopine based on the amount of thebaine introduced. Mass spectral analysis (GC/MS) also showed the presence of thebaine (M^+ 311). Integration of the peak area representing thebaine indicated the presence of 2.9 mg of thebaine in the

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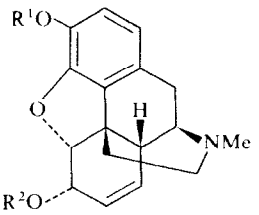
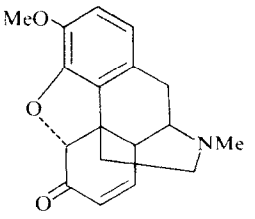


1 R^1 = Me, R^2 = H
5 R^1 = R^2 = H



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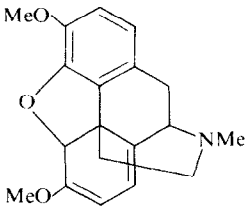

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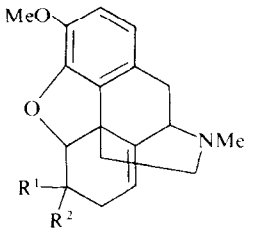

4 R^1 = H, R^2 = OH
6 R^1 = R^2 = O

material. The result showed that about 16 mg of thebaine had been catabolized.

Under the same condition, codeinone **3** (20.5 mg) was fed to the cell suspension cultures. Purification of the crude extract (136 mg) by prep. TLC afforded a yellow material (4.2 mg). The mass spectrum (GC/MS) displayed a molecular ion at *m/z* 299, corresponding to $C_{18}H_{21}NO_3$, the molecular formula for codeine and was identical with that of authentic codeine. It was estimated from the gas chromatogram that the material contained 0.29 mg of codeine. The result indicated the reduction of codeinone to codeine as found by Furuya *et al.* [3] in earlier biotransformation experiments.

Codeine, neopine HBr, papaverine and D,L-laudanosoline HBr were fed to the cell suspension cultures respectively under the same conditions, but were not metabolized. The material obtained from the cell suspension cultures grown in 0-B5 medium without the precursors showed no trace of morphinan alkaloids as revealed by GLC.

DISCUSSION

We have studied the effect of precursors on the cell suspension cultures of *Papaver somniferum* cv. Marianne grown in 0-B5 medium and concluded that only thebaine and codeinone gave rise to neopine and codeine respectively. The fact that codeine was not metabolized to morphine **5** whereas codeinone was transformed to codeine indicates that this cell line lacks the ability to demethylate codeine to morphine. This also accounts for the fact that only codeine was isolated from the 3-week-old cell suspension cultures of *Papaver somniferum* cv. Marianne grown in 1-B5C medium as reported earlier [1]. Conversion of thebaine to neopine instead of neopinone **6** indicates that the transformation in plant cell cultures does not necessarily follow the biosynthetic pathway for conversion of thebaine to neopinone in *Papaver somniferum* plants as established by Parker *et al.* [4].

EXPERIMENTAL

Mass spectra were recorded on a Finnigan Model 330 GC/MS system with an Inco Model 2300 data. The transfer line and the jet separator were maintained at *ca* 280° and the GC injector temp. was kept at 275°. The flow rate of He was 30 ml/min. The ionization voltage was 70 eV and scanning was done repetitively at 2.5 sec/scan. The glass column (1.8 m × 2 mm) was packed with 3% OV-17 on Chromosorb W (80/100 mesh). GC was performed on a gas chromatograph equipped with FID using a capillary column (WCOT, 8.5 m) packed with OV-101. A splitless injection system was employed with 2 μl as the injection volume for the sample examined. Solutions of the samples to be examined were subjected to GC as a column temperature programed 150–240° at 4°/min with He at 100 ml/min as the carrier gas. The injector and detector were at 200° and 250°, respectively.

Biotransformation of thebaine to neopine. After incubation of thebaine (20.0 mg) for 3 days in 250 ml of a 3-week-old cell suspension culture grown in 0-B5 medium the supernatant was extracted with EtOAc (2 × 150 ml) while the cells were treated with boiling MeOH (400 ml). Both MeOH and EtOAc extracts were combined and evaporated to give a yellowish material which was redissolved in an EtOAc–1 N HCl (1:1) mixture (2 × 100 ml). The EtOAc and the 1 N HCl layers were collected separately. The EtOAc soln was washed with 1 N HCl (20 ml) and collected. The acidic washing was then combined with the acidic layer obtained previously and treated with $NaHCO_3$ to pH = 7.55. After filtration, the filtrate was extracted with EtOAc (2 × 100 ml). The extracts were combined with the EtOAc extract obtained previously. Evaporation yielded a yellowish brown material (79.7 mg). The mass spectrum (GC/MS) displayed molecular ion at *m/z* 299, corresponding to $C_{18}H_{21}NO_3$ and was identical with that of the authentic neopine. MS (GC/MS) 70 eV *m/z* (rel. int.): 299 [M^+] (100), 284 [$M^+ - 15$; 14], 254 [$M^+ - 45$; 69], 243 [$M^+ - 56$; 7], 242 [$M^+ - 57$; 8], 225 [$M^+ - 74$; 10], 152 [$M^+ - 14$; 11], 128 [$M^+ - 171$; 15], 127 [$M^+ - 172$; 19], 115 [$M^+ - 184$; 15], 94 [$M^+ - 205$; 8], 77 [$M^+ - 222$; 15], 70 [$M^+ - 299$; 15], 58 [$M^+ - 242$; 11], 44 [$M^+ - 255$; 34], and 42 [$M^+ - 257$; 70].

Biotransformation of codeinone to codeine. Codeinone **3** (20.5 mg) was incubated for 3 days in 250 ml of a 3-week-old cell suspension culture grown in 0-B5 medium and worked up as above. Purification of the extract (136 mg) with PLC on Polygram® Si gel/UV_{254} plates (0.25 mm), using EtOAc–MeOH–NH_4OH (17:2:1) for development and $CHCl_3$–MeOH (8:2) as eluent afforded a yellow material (4.2 mg). The mass spectrum (GC/MS) was that of codeine.

The other precursors, codeine (20.5 mg) neopine HBr (20.7 mg), papaverine (20.0 mg) and D,L-laudanosoline HBr (20.8 mg) were incubated with the cell suspension cultures under the above conditions, but were not metabolized.

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REFERENCES

1. Tam, W. H. J., Constabel, F. and Kurz, W. G. W. (1980) *Phytochemistry* **19**, 486.
2. Gamborg, O. L., Miller, R. A. and Ojima, K. (1968) *Exp. Cell Res.* **50**, 151.
3. Furuya, T., Nakano, M. and Yoshikawa, T. (1978) *Phytochemistry* **17**, 891.
4. Parker, H. I., Blaschke, G. and Rappoport, H. (1972) *J. Am. Chem. Soc.* **94**, 1286.

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N(1)-ACETYL-*N*(1)-DEOXYMAYFOLINE FROM *MAYTENUS BUXIFOLIA*

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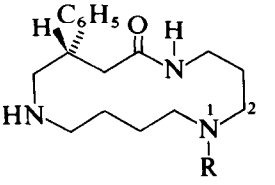
Key Word Index—*Maytenus buxifolia*; Celastraceae; spermidine alkaloid; *N*(1)-acetyl-*N*(1)-deoxymayfoline.

Abstract—A new alkaloid, *N*(1)-acetyl-*N*(1)-deoxymayfoline was isolated from *Maytenus buxifolia* growing in the vicinity of Santiago de Cuba. Plants of the same species obtained from other regions of Cuba, however, contain only mayfoline.

From *Maytenus buxifolia* collected in the Province of Matanzas (Cuba), in the vicinity of Lomas de Galindo, only the spermidine alkaloid mayfoline (**1**) could be detected [1]. The occurrence of the alkaloid in this species was confirmed in the present investigation using plants from Canasí in the same province. However, plants of the same species obtained from Santiago de Cuba, Oriente, contain another alkaloid, for which the structure **2** was proved.

The IR, UV and ^1H NMR spectra indicated an aromatic partial structure; absorption at 705 cm^{-1} corresponds to a mono-substituted C_6H_6 ring. Absorption maxima at 1660 and 1629 cm^{-1} are in accordance with the presence of amide groups and the band at 1560 cm^{-1} indicates a secondary amide. The elemental composition was shown to be $C_{18}H_{27}N_3O_2$ by high resolution MS. The fragmentation pattern is similar to that of mayfoline [1], thus proving the same skeleton. The *N*-acetyl group replacing the hydroxyl group of mayfoline gives rise to two singlets in the ^1H NMR spectrum at 2.10 and 2.12 ppm (two conformers). The ^1H NMR spectrum also shows the presence of a partial structure NH–CH(Ph)–CH_2 [2] thus proving the *N*(1)-position of the acetyl group. The similarity of the ORD curves of mayfoline [1] and of the new alkaloid proves the S-configuration for the latter compound.

As the plants of both populations were harvested at different seasons, the question of the existence of chemical races [3] in this species remains open, but it should be mentioned that both populations also differ in their morphology.



1 R = OH
2 R = Ac

EXPERIMENTAL

Plant material. Plants containing *N*(1)-acetyl-*N*(1)-deoxymayfoline were collected in March 1980 in Cuba, Oriente, in the vicinity of Santiago de Cuba, Morro Castle; plants containing mayfoline were collected in Sept. 1980 in Cuba, Province of Matanzas, Canasí. The plants were identified by Lic. Pedro Herrera, Havana. Voucher specimens are retained in the