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8-HYDROXYDIHYDROCHELERYTHRINE AND ARNOTTIANAMIDE FROM ROOTS OF *TODDALIA ASIATICA**

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Key Word Index—*Toddalia asiatica*; Rutaceae; roots; benzophenanthridine alkaloid; 8-hydroxydihydrochelerythrine; secobenzophenanthridine alkaloid; arnottianamide; hexacosanoic acid; β-sitosterol; structural analysis.

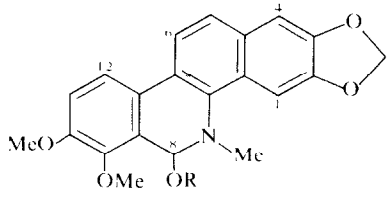
Abstract—In addition to hexacosanoic acid, β-sitosterol and arnottianamide, 8-hydroxydihydrochelerythrine, a new benzophenanthridine alkaloid has been isolated as its acetyl derivative from the roots of *Toddalia asiatica*.

INTRODUCTION

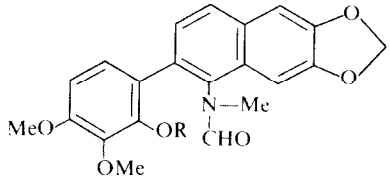
Our continued efforts to isolate the active constituents [1–3] responsible for conferring the diuretic activity [4] to *Toddalia asiatica* led to the chemical investigation of its polar fractions. Accordingly, CC of the BuOH-soluble fraction gave a residue, which was acetylated and purified by prep. TLC to afford the new base as its acetate whose structural elucidation is discussed in the present communication.

RESULTS AND DISCUSSION

The base acetate, mp 145°, $C_{23}H_{21}NO_6$, M^+ *m/z* 407, possessed UV and IR spectral features similar to 8-methoxydihydrochelerythrine (**3**) [5]. The presence of an acetyl function (ν_{max}^{KBr} cm^{-1}: 1720; δ 2.35, *s*, 3 H) and its location at C-8 was ascertained by the study of its mass spectrum which showed a base peak at *m/z* 348, originating presumably by the loss of the C-8 substituent of 59 mu (CH_3COO) from the molecular ion at *m/z* 407 [6]. The structure (**2**) in agreement with preceding observations is fully sustained by the ^1H NMR spectrum detailed in the Experimental. This constitutes the first report of the occurrence of an 8-hydroxylated benzophenanthridine base (**1**) in nature.



1 R = H
2 R = Ac
3 R = Me



4 R = H
5 R = Ac

The EtOAc-soluble fraction on CC over Si gel afforded another base in 0.0023% yield, mp 268°, $C_{21}H_{19}NO_6$, M^+ *m/z* 381.1251, which was identified as arnottianamide (**4**) on the basis of its spectral analysis and that of its acetate (**5**). This unusual amidic base has been previously reported from *Xanthoxylum cuspidatum* Champ. and *X. arnottianum* Maxim. [7] and its occurrence along with other benzophenanthridine bases bears biogenetic significance.

β-Sitosterol and hexacosanoic acid, mp 88°, $C_{26}H_{52}O_2$, M^+ *m/z* 396 [8] were isolated from the hexane-soluble fraction.

EXPERIMENTAL

All mps are uncorr. The ^1H NMR spectra were recorded at 90 MHz using HMDS as an int. standard and MS using a direct inlet system.

Isolation of constituents. Air-dried, powdered roots of *T. asiatica* Lamk. (6 kg) were percolated with 95% EtOH (3 × 7 l.). The residue (800 g) obtained after removal of solvent under red. pres. was diluted with H_2O and defatted with hexane (4 × 500 ml) to afford a hexane-soluble residue (98.5 g). After extracting the defatted material with 2 N HCl, the remainder was treated with EtOAc and BuOH to furnish residues (160 g) and (11.2 g), respectively.

A part of the BuOH-soluble fraction (10 g) was chromatographed on a column of Si gel (400 g) in C_6H_6 and eluted with increasing proportions of EtOAc and MeOH to afford a residue (150 mg) (EtOAc–MeOH, 7:3). The latter on acetylation at room temp. (pyridine–Ac_2O) yielded a solid (140 mg). This was purified by prep. TLC on Si gel (MeOH–EtOAc, 1:4) to afford 8-acetoxydihydrochelerythrine

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(**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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