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ALKALOIDS OF *MAPPIA FOETIDA**

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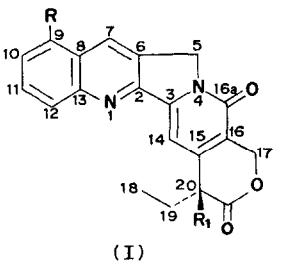
Key Word Index—*Mappia foetida*; Olacaceae; camptothecin; 9-methoxycamptothecin; alkaloid.

Abstract—Two alkaloids have been isolated from *Mappia foetida* Miers. The major alkaloid is camptothecin (Ia) and the minor alkaloid has been shown by spectral studies to be the hitherto unknown 9-methoxy-camptothecin (Ic).

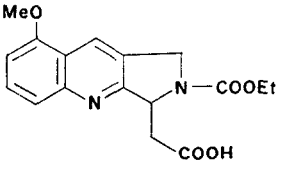
INTRODUCTION

Mappia foetida Miers (Olacaceae) is a small tree abundant in the Western Ghats of India. From the bark, stem, roots and leaves of this tree, we have isolated, besides sitosterol and lupeol, two alkaloids.[1] The major alkaloid was identified by direct comparison as the known antitumour alkaloid camptothecin (Ia)[2] whose structure was deduced by an X-ray study.[3] The minor alkaloid has been shown by spectroscopic studies to be the hitherto unknown 9-methoxycamptothecin (Ic).[4] We wish to present here experiment details of this work.



(I)

(Ia) R = H, R_1 = OH
(Ib) R = H, R_1 = OAc
(Ic) R = OMe, R_1 = OH
(Id) R = OMe, R_1 = OAc



(II)

RESULTS AND DISCUSSION

Camptothecin (Ia) has previously been isolated from the rare Chinese tree, *Camptotheca acuminata* (Nyssaceae)[2] in low yield (*ca.* 0·005%). Four syntheses of the racemic alkaloid have been reported recently,[5–8] but *Mappia foetida* represents the most convenient source

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[1] Preliminary communication, T. R. GOVINDACHARI and N. VISWANATHAN, *Indian J. Chem.* in press.
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[4] The numbering system is based on the relationship of camptothecin to indole alkaloids, see M. SHAMMA, *Experientia* **24,** 107 (1968).
[5] G. STORK and A. G. SCHULTZ, *J. Am. Chem. Soc.* **93,** 4074 (1971).
[6] R. VOLKMAN, S. DANISHEFSKY, J. EGGLER and D. M. SOLOMON, *J. Am. Chem. Soc.* **93,** 5576 (1971).
[7] M. C. WANI, J. A. KEPLER, M. E. WALL, H. F. CAMPBELL and G. A. BRINE, *8th International Symposium on the Chemistry of Natural Products*, New Delhi (February 1972).
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for the large-scale isolation of this pharmacologically interesting alkaloid which is under clinical trial for its anti-tumour and antileukemic activity.[9]

The minor alkaloid, $C_{21}H_{18}N_2O_5$, has been shown to be a methoxy-camptothecin from a study of its UV, IR, NMR and MS (see Experimental). Direct comparison showed it to be different from the known 10-methoxycamptothecin.[10] The NMR spectrum of the alkaloid in pyridine-d_5 (see Experimental) showed the presence of three adjacent hydrogens in ring *A*. The NMR spectrum of the derived acetate (Id) in DMSO-d_6 was very similar in the aromatic region to the synthetic tricyclic model compound (II),[10] the non-aromatic region being very similar to that of acetylcamptothecin (Ib). Addition of C_6D_6 to the DMSO-d_6 solution simplified the overlapping signals of the aromatic protons in such a way that the presence of three adjacent hydrogens in ring *A* could be clearly seen.

The C_7–H, which appears in acetylcamptothecin (Ib) at δ 8·67 in DMSO-d_6, is shifted to δ 8·78 in the acetate (Id)—a shift comparable to that observed between quinoline and 5-methoxyquinoline. The C_7–H in the acetate (Id) shows allylic coupling with the methylene at C_5 and also long-range coupling (*W*-type) with C_{12}–H. Such a long-range coupling has been observed between C_4–H and C_8–H in quinoline[11] and this supports the assignment of the methoxyl to C_9 in the alkaloid.

The C.D. (in dioxane) of the new alkaloid is virtually superposable with that of camptothecin and shows that they have the same absolute configuration at the sole asymmetric centre at C_{20}.

EXPERIMENTAL

Extraction. The powdered stem (5 kg) was extracted twice with hexane. Chromatography over silica gel of the residue obtained on evaporation of hexane gave sitosterol and lupeol identified by comparison with authentic samples.

The defatted plant material was extracted thrice with Me_2CO and then twice with MeOH. The combined Me_2CO extracts were concentrated *in vacuo* and left for 1 week in the ice-chest to get the crude alkaloids as a greenish white solid (6 g). Concentration of the MeOH extracts similarly gave more of the alkaloids as a solid (3 g). The solids from the Me_2CO and MeOH extracts were combined and subjected to triangular crystallization from HOAc. The less soluble fractions gave camptothecin (5·5 g) as a pale yellow solid, m.p. 268–270° (*d*), $[\alpha]_D^{25}$ +42° (*c*, 1, $CHCl_3$–MeOH, 4:1), $[\alpha]_D^{25}$ −139·5° (*c*, 1·2, pyridine), λ_{max}^{EtOH} 218, 253, 288, 358, 370 nm (log ε 4·51, 4·34, 3·52, 4·32, 4·29), ν_{max}^{KBr} 3440, 1745, 1655, 1605, 1580 cm^{-1}. (Found: C, 68·53; H, 4·91; N, 8·15. Calc. for $C_{20}H_{16}N_2O_4$: C, 68·96; H, 4·63; N, 8·04%.) MS: *m/e* 348 (M^+, 100), 319 (41), 304 (42), 291 (31), 289 (33), 276 (25), 275 (33), 248 (57), 247 (55), 219 (48), 218 (33), 205 (16), 191 (16), 140 (16), metastable peak at 266 (348–44). The sample was identical in m.m.p., TLC (SiO_2 in $CHCl_3$–MeOH 93:7, $CHCl_3$–EtOAc–MeOH 10:1:1 and $CHCl_3$–Me_2CO–MeOH 20:2:1), IR and UV spectra with authentic natural camptothecin (Ia). It was identical in TLC with DL-camptothecin synthesized by Professor Stork.

The more soluble fraction from the fractional crystallization was chromatographed over silica gel and eluted with $CHCl_3$–MeOH (49:1). The fractions were screened by TLC and the fractions eluted before camptothecin and exhibiting a yellow UV fluorescence were pooled and repeatedly crystallized from $CHCl_3$–MeOH (1:1) to yield 9-methoxycamptothecin (Ic) (0·2 g) as light yellow crystals, m.p. 258–260° (d). $[\alpha]_D^{24}$ −77·5° (*c*, 1, pyridine), λ_{max}^{EtOH} 219, 256, 265 (*sh*), 305, 320, 358, 371 (*sh*) nm (log ε 4·53, 4·39, 4·33, 3·86, 4·05, 4·30, 4·29), ν_{max}^{KBr} 3430, 1750, 1660, 1600 cm^{-1}. (Found: C, 66·02; H, 4·86; N, 7·31. $C_{21}H_{18}N_2O_5$ requires: C, 66·66; H, 4·80; N, 7·40%.) MS: *m/e* 378 (M^+, 100), 349 (45), 334 (80), 319 (50), 306 (25), 305 (35), 304 (25), 291 (15), 278 (55), 277 (50), 262 (15), 249 (20), 248 (15), 234 (25), 219 (15), 205 (20). NMR (100 MHz, pyridine-d_5): δ 8·57 (1H, *s*, C_7–H), 8·35 (1H, broad, OH), 7·92 (1H, *d*, *J* 8 Hz, C_{12}–H), 7·89 (1H, *s*, C_{14}–H), 7·64 (1H, *t*, *J* 8 Hz, C_{11}–H), 6·87 (1H, *d*, *J* 8 Hz, C_{10}–H), 5·87 (1H, *d*, *J* 16 Hz, C_{17}–H), 5·52 (1H, *d*, *J* 16 Hz, C_{17}–H), 5·20 (2H, *s*, C_5–H), 3·92 (3H, *s*, C_9–OMe), 2·13 (2H, *q*, *J* 7 Hz, C_{19}–H), 1·14 (3H, *t*, *J* 7 Hz, C_{18}–H).

[9] G. H. SVOBODA, in *Pharmacognosy and Phytochemistry* (edited by H. WAGNER and L. HÖRHAMMER), p. 183, Springer, Berlin (1971).

[10] M. C. WANI and M. E. WALL, *J. Org. Chem.* **34**, 1364 (1969).

[11] W. BRÜGEL, *Nuclear Magnetic Resonance Spectra and Chemical Structures*, p. 166, Academic Press, New York (1967).

In large scale extractions, the following approximate yields were obtained (expressed as % of dry plant material): *Stem*. 0·06 (Camptothecin); 0·001 (9-Methoxycamptothecin); *Bark*. 0·08; 0·001; *Roots*. 0·1; 0·002; *Leaves*. 0·01; Traces. The crude alkaloid material showed the presence of traces of more polar compounds with blue UV fluorescence but these could not be obtained pure.

O-*Acetylcamptothecin* (Ib). Camptothecin (0·5 g) was refluxed for 24 hr with Ac_2O (10 ml) and pyridine (5 ml) and the soln kept at 60° for 72 hr more. The soln was evaporated to dryness *in vacuo* and the residue chromatographed over silica gel in $CHCl_3$. Elution with $CHCl_3$–2% MeOH gave the acetate which crystallized from $CHCl_3$–hexane as a pale yellow solid (350 mg), m.p. 288–290° (d) (lit.[2] m.p. 271–274° *d*), ν_{max}^{KBr} 1750, 1660, 1620, 1235 cm^{-1}. MS: *m/e* 390 (M^+, 25), 330 (93), 319 (8), 315 (40), 302 (100), 291 (13), 287 (57), 275 (17), 259 (8), 246 (13), 231 (5), 218 (13), 205 (12), 191 (8), 140 (7).

O-*Acetylmethoxycamptothecin* (Id). Methoxycamptothecin (0·5 g) was acetylated as above to yield, after chromatography over silica gel and elution with $CHCl_3$–2% MeOH, the acetate (285 mg), pale yellow solid (from $CHCl_3$–hexane), m.p. 231–232°, ν_{max}^{KBr} 1755, 1665, 1615, 1602 cm^{-1}. (Found: C, 65·46; H, 5·15. $C_{23}H_{20}N_2O_6$ requires: C, 65·70; H, 4·80%.) MS: *m/e* 420 (M^+, 31), 360 (58), 349 (6), 345 (31), 332 (100), 321 (15), 317 (90), 305 (19), 290 (5), 289 (5), 276 (7), 262 (4), 249 (3), 233 (5), 221 (2), 217 (2), 205 (8). NMR (100 MHz, DMSO-d_6): δ 8·78 (1H, *s*, C_7–H), 7·72 (2H, *m*, C_{12}–H, C_{11}–H), 7·12 (1H, *dd*, *J* 6, 2·5 Hz, C_{10}–H), 7·04 (1H, *s*, C_{14}–H), 5·50 (2H, *s*, C_{17}–H), 5·23 (2H, *s*, C_5–H), 4·02 (3H, *s*, C_9–OMe), 2·23 (3H, *s*, C_{20}–OCOMe), 2·13 (2H, *q*, *J* 7 Hz, C_{19}–H), 0·93 (3H, *t*, *J* 7Hz, C_{18}–H).

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