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Note

Studies on phytochemicals : Part XV—Investigation on the alkaloidal constituents of Indian *Mappia foetida*†

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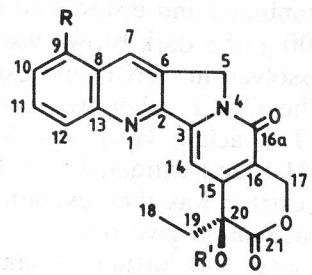
Three antitumour alkaloids, camptothecin (**1**), 9-methoxycamptothecin (**2**) and 20-*O*-acetylcamptothecin (**3**) have been isolated from the stems of Indian *Mappia foetida*. The presence of **3** in this indigenous species is reported for the first time. The ^{13}C NMR spectra of the alkaloids **2** and **3** have been studied. The effects of different solvent systems and the variation of temperature on the ^{1}H NMR spectra of these compounds (**1-3**) have been discussed.

Mappia foetida Miers (Icacenaceae) is a small tree, sometimes a shrub, abundant in the Western Ghats of India. It is also found in North Bengal and Assam, mostly on the hills[1]. The biological screening of the species has proved[2] its promising anticancer activity. In continuation of our search[3,4] for naturally occurring antitumour agents we have chemically investigated the stems of this indigeneous plant. Three alkaloids have been isolated. The first two compounds were characterised as camptothecin (**1**)[5,6] and 9-methoxycamptothecin (**2**)[6,7] by comparison of their physical and spectral properties with those reported in the literature for the compounds. The former was originally isolated[5] from the rare Chinese species *Camptotheca acuminata* and subsequently reported[6,7] from the title plant. The compound was found[5] to possess potent antitumour activity. The plant material collected by us was examined to be a convenient source of this biologically important compound. The second alkaloid, 9-methoxycamptothecin (**2**) was previously characterised[6,7] from its ^{1}H NMR spectral data. Our studies on the ^{13}C NMR spectrum of the compound (Table I) has now confirmed the reported structure. The another alkaloidal constituent of the plant, **3**, was isolated as pale yellow solid. Its structure was established as 20-*O*-acetylcamptothecin by detailed analysis of its spectral properties (UV, IR, ^{1}H NMR and mass) (vide Experimental Section). The structure was confirmed from the examination of its ^{13}C NMR spectrum (Table I). The compound was found to be identical to the product formed by treatment of camptothecin (**1**) with acetic anhydride and pyridine.



1 R= R'=H
2 R= OMe, R'=H
3 R= H, R =Ac

Table I—^{13}C NMR spectral data of 9-methoxy-camptothecin (**2**) and 20-*O*-acetylcamptothecin (**3**)

Carbon No.	δ-values of	
	2 in $CDCl_3$	**3** in DMSO & $CDCl_3$
2	150.18	148.83
3	149.45	145.88
5	50.08	49.91
6	125.95	128.46
7	130.37	131.21
8	120.54	128.21
9	154.89	128.21
10	121.47	128.03
11	127.89	130.68
12	127.44	129.53
13	146.12	145.89
14	98.06	96.03
15	152.02	152.87
16	118.52	120.33
16a	157.45	157.45
17	66.00	67.06
18	7.64	7.55
19	31.69	31.88
20	72.61	75.87
21	173.68	169.85
CH_3CO	—	167.75
OCH_3	55.76	—
CH_3CO	—	20.73

20-*O*-Acetylcamptothecin (**3**) is reported here for the first time from Indian *Mappia foetida*.

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While the isolation and characterisation of the compound was completed, this has been reported[8] from a rare Chinese variety of the plant in extremely low yield and has been proved to possess significant cytotoxic activity. However, all the spectral properties of the compound were not previously recorded[8]. The compound has now been fully characterised here with all its spectroscopic data (UV, IR, ^1H and ^{13}C NMR and mass).

While studying the ^1H NMR spectra of the alkaloids **1-3** in different solvent systems some variations in the spectra were noticed. Four different solvents, DMSO-d_6, $CDCl_3$, CH_3OH-d_4 and C_5H_5N-d_5 were used to record the spectra. In all the four solvents (single pure solvent was taken when a compound was soluble in it otherwise a mixture of solvent was used) the positions and multiplicities of the signals appearing for the protons of A-D rings of an alkaloid were almost similar through the aromatic region was more compact in C_5H_5N-d_5. However, the protons of E-ring resonated in different regions showing differences in multiplicities. In DMSO-d_6 the two protons at C-17 of camptothecin (**1**) appeared as a singlet at δ 5.39. When a few drops of $CDCl_3$ were added to this solution these two protons became non-equivalent and resonated at δ 5.71 and 5.29 (1H each, d, J = 17.0 Hz, H_2-17). The solution was heated magnetically inside the spectrometer from room temperature to 45° and the ^1H NMR spectra of the compound were recorded at different temperatures. However, the spectra did not show any change with the variation of temperature. Instead of $CDCl_3$ when a few drops of CH_3OH-d_4 were added to the solution of camptothecin (**1**) in DMSO-d_6 the two protons at C-17 also became non-equivalent appearing at δ 5.42 and 5.21 but with the lower value of the coupling constant (2.5 Hz only). The solubility of **1** in CH_3OH-d_4 is very poor at room temperature and the ^1H-NMR spectrum of the compound could not be recorded in this solvent under normal condition. In C_5H_5N-d_5 the two C-17 protons of **1** showed similar coupling to each other as they showed in the mixture of DMSO-d_6 and $CDCl_3$. The similar behaviour in the magnetic field was also observed for these two protons of 9-methoxycamptothecin (**2**) in four different solvent systems. However, in 20-*O*-acetylcamptothecin (**3**) they always appeared as non-equivalent in any solvent.

Solubility of camptothecin (**1**) and its derivatives is a real problem and different compounds are soluble in different solvents, sometimes at room temperature and sometimes only after warming or heating. Our present studies will give an idea of the effects of different solvent systems and the variation of temperature on the ^1H NMR spectra of such compounds which are very important for their characterisation.

Experimental Section

Melting points were determined in a Buchi-510 instrument and are uncorrected. IR spectra were recorded on Nicolet 740 spectrophotometer, UV on Shimadzu 240 spectrophotometer, ^1H and ^{13}C NMR on Varian Gemini 200 MHz spectrometer and mass on VG Micromass 7070 H (70 eV). Optical rotations were determined with a Jasco DIP 360 digital polarimeter. Column chromatography was performed on silica gel (BDH, 100-200 mesh) and TLC with silica gel GF-254. The TLC spots were detected under UV light and in an iodine chamber.

Plant material. The stems of *Mappia foetida* were collected from Maharashtra near Kolhapur. A voucher specimen (Mf-S) has been preserved in our laboratory.

Extraction of plant material and isolation of alkaloids. The shade-dried and powdered stems (1 kg) of *Mappia foetida* were extracted thrice with CH_3OH at room temperature (each extraction for 5 days using 4 L of solvent). The total extract was concentrated and chromatographed over silica gel using C_6H_6, EtOAc and MeOH as the eluents. Mixtures of some oily compounds and of some steroids and terpenoids were eluted with C_6H_6. 20-*O*-Acetylcamptothecin (**3**) was eluted with C_6H_6-EtOAc (4:1) and purified by crystallisation from hexane. The earlier fractions of EtOAc afforded 9-methoxycamptothecin (**2**), m.p. 258-59° (d) ($CHCl_3$-MeOH), $[\alpha]_D^{25}$ −77.2° (c, 0.9813, C_5H_5N), yield 120 mg (0.012%) and the later fractions produced camptothecin (**1**), m.p. 270-71° (d) (glacial HOAC), $[\alpha]_D^{25}$ + 42.5° (c, 1.1264, $CHCl_3$-MeOH 4:1), yield 812 mg (0.0812%). The physical and spectral properties of **1** and **2** were found to be identical to those reported[5-7] in the literature for the compounds.

20-O-Acetylcamptothecin (3). M.P. 274-275° (d) (hexane), $[\alpha]_D^{25}$ + 26.4° (c, 0.1132, $CHCl_3$-MeOH 4:1), yield 72 mg (0.0072%); UV (ethanol) : 218, 250, 288, 360 and 368 nm (log ε 4.41, 4.25, 3.50, 4.08 and 4.05); IR (KBr) : 1738, 1660, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 8.38 (1H, s, H-7), 8.21 (1H, dd, J = 8.0 and 1.2 Hz, H-12), 7.92 (1H, dd, J = 8.0 and 1.2 Hz, H-9), 7.81 (1H, td, J = 8.0 and 1.2 Hz, H-11), 7.64 (1H, td, J = 8.0 and 1.2 Hz, H-10), 7.20 (1H, s, H-14), 5.65 and 5.41 (1H each, d, J = 17.0 Hz, H_2-17), 5.39 (2H, s, H_2-5), 2.24 and 2.11 (1H each, q, J = 7.5 Hz,

H_2-19), 2.22 (3H, s, OAc), 0.98 (3H, t, J = 7.5 Hz, Me-18); MS (%) : m/z 390 (M^+, 10), 330 (68), 315 (38), 302 (100), 287 (65), 246 (22), 218 (24), 191 (19).

Acetylation of camptothecin (1). Camptothecin (**1**) (100 mg) was added to acetic anhydride (2 mL) and pyridine (1 mL). The mixture was warmed at 40° for 2 hr and kept at room temperature for 2 days. Work-up in the usual procedure followed by crystallisation of the product from hexane afforded pale yellow needles, m.p. 273-74° (d), yield 82 mg. The compound was found to be identical to naturally occurring 20-*O*-acetylcamptothecin (**3**) in all respects (m.p., m.m.p. co-TLC and spectral properties, ^{1}H-NMR and mass).

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