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MINOR ALKALOIDS OF *CAMPTOTHECA ACUMINATA*

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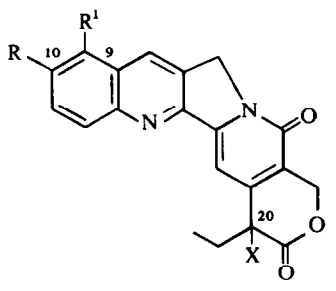
Key Word Index—*Camptotheca acuminata*; Nyssaceae; pyrrolo[3,4-*b*] quinoline alkaloids; 20-deoxycamptothecin.

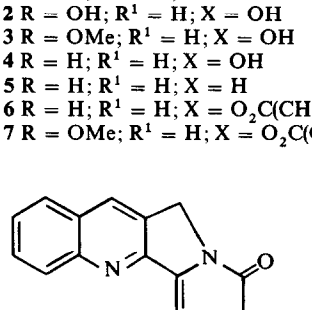
Several camptothecin alkaloidal analogs have been isolated from the leaves and bark of *Camptotheca acuminata* Decne. (Nyssaceae). In addition to the quinoline alkaloid (+)-camptothecin (**1**) [1], *C. acuminata* has yielded 10-hydroxycamptothecin (**2**) and 10-methoxycamptothecin (**3**) [2]. Camptothecin is also found in *Mappia foetida*, from which were isolated 9-methoxycamptothecin (**4**) and mappicine (**8**) [3]. All of these alkaloids continue to be of interest because of their anti-tumor properties and because of the effect of camptothecin in the inhibition of macromolecular synthesis [4].

We now wish to report the isolation from *C. acuminata* of the minor alkaloids 20-deoxycamptothecin (**5**), 20-hexanoylcamptothecin (**6**) and 20-hexanoyl-10-methoxycamptothecin (**7**).

A modification of the original extraction process, which offers higher yields in fewer steps, was employed [5]. Ground *C. acuminata* bark was extracted for one week with chloroform–ethanol. Following preliminary purification, the alkaloid containing layer was evaporated to a residue and subjected to low pressure liquid chromatography (LPLC) and PLC. This regimen yielded camptothecin at 0.012% concentration by weight compared to 0.005% in the original [5].



1 R = H; R¹ = H; X = OH
2 R = OH; R¹ = H; X = OH
3 R = OMe; R¹ = H; X = OH
4 R = H; R¹ = H; X = OH
5 R = H; R¹ = H; X = H
6 R = H; R¹ = H; X = $O_2C(CH_2)_4Me$
7 R = OMe; R¹ = H; X = $O_2C(CH_2)_4Me$



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TLC comparison to **1**, **2**, **3** and **8** eliminated known alkaloids from the search: **1**, **2**, and **3** were observed, but **8** was not detected. Remaining fluorescent fractions from LPLC, which reacted positively with ceric ammonium sulfate spray, were rechromatographed on PLC plates. An alkaloid identical chromatographically and spectroscopically to 20-deoxycamptothecin (**5**) was present at a 2.0×10^{-4}% concentration by weight. This assignment was verified by preparation of racemic **5** from **1** [6] and by oxidation of the isolated compound to **1** by the method of Winterfeldt [7]. The stereochemistry at C-20 proved, within experimental error, to be racemic, as no optical rotation was exhibited. This is an unexpected result for the putative biosynthetic precursor of camptothecin [8].

PLC also yielded a minor alkaloid present at 4.0×10^{-5}% concentration by weight, identical by NMR and IR to **6** (which had been prepared from (+)-**1**). This structural assignment was corroborated by MS. A compound corresponding by NMR and MS to **7** was isolated at 2.0×10^{-5}% concentration by weight.

EXPERIMENTAL

NMR spectra wera recorded at 90 Hz; MS were run on a Finnegan quadrupole 1015 gc/mass spectrometer interfaced to a Finnegan M6000 computer; mps are uncorr.

Extraction and purification. Ground bark of *C. acuminata* was provided by the National Cancer Institute. The bark (4.2 kg) was extracted with EtOH–$CHCl_3$ (1:1) soln in a kettle for 1 week at room temp. Evapn of the filtrate yielded 45 g of residue which was resolubilized in Me_2CO–H_2O (9:1) and washed with Skelly B. The Skelly B layer was back ext'd with H_2O and the combined Me_2CO–H_2O soln evapd to a residue (15 g). The solid was then subjected to LPLC (Si gel 60, 40–63 μm) at 120 psi, using $CHCl_3$–MeOH (96:4). LPLC fractions containing **5**, **6** and **7** were rechromatographed (20 × 20 cm plates, PF-254 Si gel). R_fs are reported for Si gel ($CHCl_3$–MeOH (96:4)) and cellulose (H_2O–HOAc (85:15)).

20-Deoxycamptothecin (**5**). 10 mg; R_f 0.69 (Si gel); R_f 0.47 (cellulose). IR ν_{max} (film) cm^{-1}: 1736, 1661, 1608. UV_{max} (EtOH) nm: 218, 253, 288, 360. ^1H NMR ($CDCl_3$): δ 1.11 (3H, *t*, H-18), 2.12 (2H, *m*, H-19), 3.63 (1H *t*, H-20), 5.31 (2H, *s*, H-5), 5.42 (2H, *dd*, J_{AB} = 16 Hz, H-17), 7.70 (1H, *s*, Ar), 7.14–8.33 (4H, *m*, Ar) and 8.40 (1H, *s*, Ar). $[\alpha]_D^{25}$ 0° (*c* 0.0025, $CHCl_3$).

20-Hexanoyl camptothecin (**6**). 1 mg; mp 238–242°; R_f 0.77 (Si gel); R_f 0.0 (cellulose). IR ν_{max} ($CHCl_3$) cm^{-1}: 1750, 1665, 1620. IR ν_{max} (film) cm^{-1}: 1750, 1670, 1625. ^1H NMR ($CDCl_3$): δ 0.74–1.65 (13H, *m*), 1.96–2.55 (4H, *m*), 5.27 (1H, *s*, H-5), 5.53 (2H, *dd*, J_{AB} = 17 Hz, H-17) 7.20 (1H, *s*, H-14) 7.61–8.25 (4H, *m*, Ar) and 8.37 (1H, *s*, H-7). $[\alpha]_D^{25}$ −26° (*c* 0.0017, $CHCl_3$). Lack of analytic purity and possible errors in weight determination account for discrepancy with $[\alpha]_D^{25}$ of the synthetic compound. MS *m/e* (rel. int.): 446 (4) [M^+], 330 (80), 302 (80), 91 (89), 69 (100).

20-Hexanoyl-10-methoxycamptothecin (**7**). 0.5 mg; R_f 0.85 (Si gel); R_f 0.0 (cellulose). IR ν_{max} (film) cm^{-1}: 1750, 1675, 1630. ^1H NMR ($CDCl_3$): δ 0.78–2.51 (17H, *m*, resembles same region in spectrum of **6**), 3.93 (3H, *s*, MeOAr), 5.28 (1H, *s*, H-5), 5.52 (2H, *dd*, J_{AB} = 17 Hz, H-17) and 7.02–8.42 (5H, *m*, Ar). MS *m/e* (rel. int.): 476 (6) [M^+], 360 (40), 332 (48), 317 (33).

Preparation of camptothecin (**1**) *from 20-deoxy camptothecin* (**5**). In analogy to Winterfeldt [7] 3.0 mg of **5** were mixed in 1 ml DMF with 5.0 mg $CuCl_2$ and a small drop of aq. dimethylamine soln (40%). The mixture was stirred 3 hr at room temp. with O_2 bubbled through. The soln was then poured into a saturated aq. soln of NaCl and extracted with CH_2Cl_2. TLC showed spots corresponding to **5**, R_f 0.47 (Si gel) and **1**, R_f 0.43 (Si gel). The MS of this material was identical to that of **1**.

Preparation of camptothecin (**1**) *from 20-deoxycamptothecin* (**1**). **5** was prepared from **1** after M. C. Wani [6]. 20-Chlorocamptothecin in MeOH plus 5% Pd(C) was stirred under H_2 at 1 atm for 1 hr. The soln was filtered and left to stand overnight at 25°. Crystals were removed by filtering; the filtrate was concd and again allowed to crystallize. Recrystallization from MeOH–$CHCl_3$ (13:87) gave **5**. Quantitative yields of **5** were obtained when 20-chlorocamptothecin was hydrogenolysed for 10 min at 1 atm with Ra/Ni which had been activated by digestion with NaOH at 85° for 3 hr.

Preparation of 20-hexanoyl camptothecin (**6**) *from camptothecin* (**1**). A large excess of hexanoyl chloride was added to a soln of **1** in Py and CH_2Cl_2. After 6 hr of stirring at room temp. the solvents were removed by evapn and the residue chromatographed (PLC, Si gel). R_f 0.76 (Si gel); R_f 0.0 (cellulose); mp 236–241°. IR ν_{max} (film) cm^{-1}: 1750, 1670, 1625. ^1H NMR ($CDCl_3$): δ 0.75–1.63 (13H, *m*), 1.98–2.54 (4H, *m*), 5.26 (1H, *s*, H-5), 5.52 (2H, *dd*, J_{AB} = 17 Hz, H-17), 7.19 (1H, *s*, H-14), 7.60–8.25 (4H, *m*, Ar) and 8.37 (1H, *s*, H-7). MS *m/e* (rel. int.): 446 (9) [M^+], 330 (48), 302 (51), 287 (35). $[\alpha]_D^{25}$ −54° (*c* 0.0031, $CHCl_3$).

Synthesis of mappicine (**8**). **8** was partially synthesized from **1** after Govindachari *et al.* [9]. This involved reduction of **1** to camptothecin diol with $NaBH_4$, $Pb(OAc)_4$ cleavage to the keto-ester, and further reduction with $NaBH_4$ under vigorous conditions to yield **9**. Recrystallization from MeOH yielded small crystals with mp 264–6° (lit. 270–1°). ^1H NMR ($CDCl_3$): δ 0.97 (3H, *t*, *J* = 7 Hz, H-18), 1.86 (2H, *m*, H-19), 2.16 (3H, *s*, H-17), 4.89 (1H, *t*, *J* = 7 Hz, H-20), 5.12 (2H, *d*, *J* = 6 Hz, H-5), 7.44 (*s*, OH), 7.32–8.15 (4H, *m*, Ar), 7.83 (1H, *s*, H-14) and 7.96 (1H, *s*, H-7).

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