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QUINOLINE ALKALOIDS FROM *CAMPTOTHECA ACUMINATA*

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Key Word Index—*Camptotheca acuminata*, Nyssaceae; quinoline alkaloids, 22-hydroxyacuminatine, 19-hydroxymappicine

Abstract—From the seeds of *Camptotheca acuminata* a biogenetically novel, cytotoxic, quinoline alkaloid 22-hydroxyacuminatine was isolated, and its carbon framework established by spectral analysis. 19-Hydroxymappicine was also characterized.

INTRODUCTION

Previous investigations of the chemical constituents of *Camptotheca acuminata* Decne. (Nyssaceae), a tree native to China, have yielded antitumour alkaloids of the camptothecine type [1–4], and recently we have reported the isolation of several new camptothecines, new ellagic acids and indole alkaloids from this plant [5–11]. In this report, we wish to present the isolation and structure determination of two new quinoline alkaloids 22-hydroxyacuminatine (**1**) and 19-hydroxymappicine (**2**) from the seeds.

RESULTS AND DISCUSSION

Alkaloid **1** was obtained as yellow crystals, mp 258–260° (dec.), and its high resolution mass spectrum indicated the molecular formula $C_{20}H_{14}N_2O_2$ (M^+ at m/z 314.1032). The UV spectrum of this alkaloid with a maximum absorption at 380 nm and blue fluorescence under 365 nm UV light suggested that this compound might have a similar highly conjugated ring system as camptothecine (**3**). Its IR spectrum showed absorption peaks for hydroxy (3350 cm^{-1}), amide (1651 cm^{-1}), and aromatic functionalities (1610, 1595 and 1500 cm^{-1}), and the ^1H NMR spectrum displayed nine aromatic protons as two singlets (δ 7.44 and 8.66), four doublets (δ 7.81, 8.12, 8.21 and 8.31), and three triplets (δ 7.58, 7.69 and 7.86). A methylene singlet (δ 5.37) and a methylene doublet (δ 4.95, $J=5.7$ Hz) coupled to a proton triplet at δ 5.51 were also observed. The last peak was exchangeable with D_2O, indicating it to be a hydroxy group, and thus the presence of a hydroxymethyl group in **1**. Homonuclear COSY spectra of **1** indicated the following coupling patterns: the doublets at δ 7.81 and 8.31 were coupled with the triplet at δ 7.58, and the triplet at δ 7.69 was coupled with the triplet at δ 7.86 and the doublet at δ 8.12, and the latter triplet was coupled to a doublet at δ 8.21, indicating that these four protons were located at the C-9, C-10, C-11 and C-12 positions of the A-ring. The first three protons were assigned to the E-ring of the structure. The COSY spectrum of **1** also showed a long-range coupling between the two broad singlets at δ 5.37 and 8.66, indicating them to be protons at the C-5 position of the C-ring and the C-7 position of the B-ring, respectively. Comparison of the ^1H NMR data of this compound with those of camptothecine (**3**) [8, 12] (see Table 1), supported the notion that **1** has the same A, B, C, D rings as those of **3**, and that the remaining three aromatic protons and one hydroxymethyl group should be located on the E ring of **1**, which should be fully aromatic. Because those three protons were coupled to each other, the hydroxymethyl could be at the C-16 or C-19 position of the E ring. An NOE difference study indicated proximity between H-14 and CH_2OH, and established that the hydroxymethyl group should be at the C-16 position. The observation of NOE enhancement between CH_2OH and H-17 (δ 7.81) and between H-7 and H-9 (δ 8.12), and H-9 and H-10, as well as the results of the COSY spectrum led to the unambiguous assignment of the ^1H NMR spectrum of **1**. Insufficient material was available for ^{13}C NMR analysis. Compound **1** showed cytotoxic activity against the P388 and KB test systems *in vitro* with ED_{50} values of 1.32 and 0.61 μg/ml, respectively.

Compound **2** was obtained as yellow crystals, mp 245–248° (dec.), and its high resolution mass spectrum gave the molecular formula $C_{19}H_{18}N_2O_3$ (M^+ at m/z 322.1310). Like camptothecine (**3**), the UV spectrum of this alkaloid, with a maximum absorption at 367 nm and blue fluorescence under 365 nm UV light, suggested that it also had a similar highly conjugated ring system as camptothecine (**3**). Its IR spectrum also showed absorption peaks for hydroxy (3380 cm^{-1}), amide (1658 cm^{-1}), and aromatic functionalities (1577, 1560 and 1500 cm^{-1}), and the ^1H NMR spectrum displayed four coupled aromatic proton signals for the four protons of the A ring, two proton singlets for H-7 and H-14 and one methylene singlet for H-5, which are very close to those of camptoth-

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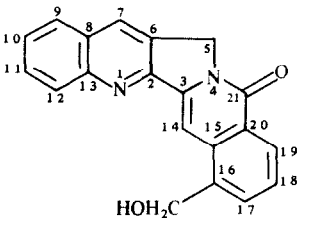
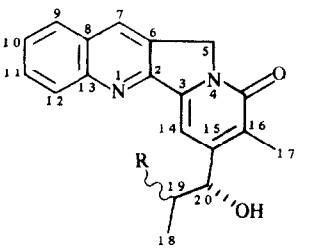


1



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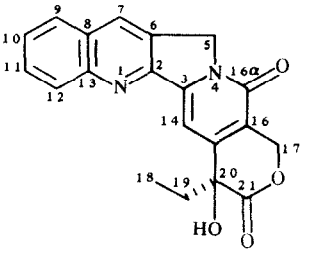
4 R = H



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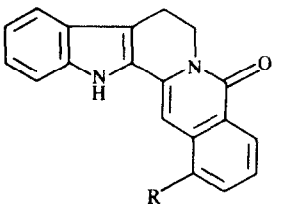

5 R = COOMe
6 R = CHO

ecine (**3**) and 22-hydroxyacuminatine (**1**) (see Table 1) The remaining signals in the ^{1}H NMR spectrum of this alkaloid were a methyl singlet at δ 2.15, a methyl doublet at δ 1 15 (J = 6 2 Hz), a methine doublet at δ 4.64 (J = 5 4 Hz) and a methine multiplet at δ 3 72 These two methine protons were coupled to each other, and the methine multiplet was coupled with the methyl doublet, which suggests the presence of the CH(OH)–CH(OH)–Me group. The observation of a strong NOE between H-14 and the CH(OH)–CH(OH)–Me unit established that this group should be located at the C-15 position and thus the methyl singlet must be located at the C-16 position of the D ring. This alkaloid shows spectral properties similar to those of mappicine (**4**) [12], which co-occurred with the camptothecines in *Mappia foetida* [13], except that this compound had one more hydroxy group in the side chain Like mappicine (**4**), this compound also shows negative Cotton effects in the region 300–400 nm, suggesting the *S*-configuration at C-20 [12]

22-Hydroxyacuminatine (**1**) lies at the circumstantial confluence of two divergent biosynthetic pathways, the oxidative rearrangement of rings A, B, C and D, as observed in the formation of camptothecine (**2**) [1], and the oxidation of ring E as found in alkaloids such as

Table 1 ^{1}H NMR assignments of compounds **1**–**4**

H	1*	2†	3†	4‡
5	5 37 (*br*, *s*)	5 22 (*s*)	5 29 (*s*)	5 18 (*s*)
7	8 66 (*br*, *s*)	8 59 (*s*)	8 70 (*s*)	8 06 (*s*)
9	8 12 (*dd*, 1 5, 7 8)	8 10 (*d*, 7 7)	8 13 (*d*, 8 5)	7 30–7.80 (*m*)
10	7 69 (*dt*, 1 5, 7 8)	7 67 (*t*, 7 7)	7 67 (*t*, 8 5)	7 30–7 80 (*m*)
11	7 86 (*dt*, 1 5, 7 8)	7 83 (*t*, 7 7)	7 87 (*t*, 8 5)	7 30–7 80 (*m*)
12	8 21 (*dd*, 1.5,7 8)	8 14 (*d*, 7 7)	8 17 (*d*, 8 5)	8 25 (*dd*, 8, 1)
14	7 74 (*s*)	7 33 (*s*)	7 34 (*s*)	7 88 (*s*)
17	7 81 (*d*, 8)	2 15 (*s*)	5 41 (AB, 16)	2 37 (*s*)
18	7 58 (*t*, 8)	1 16 (*d*, 6 2)	0 91 (*t*, 6 4)	1 16 (*t*, 7)
19	8 31 (*d*, 8)	3 72 (*m*)	1 90 (*m*)	1 88 (*m*)
20	—	4 64 (*d*, 5 4)	—	5 14 (*t*, 7)
22	4 95 (*d*, 5 7)	—	—	—
22-OH	5 51 (*t*, 5 7)	—	—	—

*Recorded in DMSO-d_6, chemical shift values are reported as δ values (ppm) from internal TMS at 300 MHz, signal multiplicity and coupling constants (Hz) are shown in parentheses

†Recorded in DMSO-d_6 at 400 MHz.

‡Recorded at 100 MHz in pyridine-d_5 [12]

oxogambirtannine (**5**) and naucleficine (**6**), which has also been isolated from this plant [10], where the E ring is of the yohimbane type [14, 15]. It is reasonable to speculate that **5** or **6** could be a biosynthetic intermediate *en route* to **1**.

EXPERIMENTAL

General Mp uncorr. ^{1}H NMR and homonuclear COSY spectra were recorded in $CDCl_3$, using TMS as the int standard Low resolution MS 70 eV.

Extraction and isolation of compounds **1** *and* **2**. The powdered seeds (100 kg) of *Camptotheca acuminata* were percolated with EtOH and followed by evapn, filtration, extraction with $CHCl_3$ and $CHCl_3$–EtOH successively, and crystallization from $CHCl_3$–MeOH, to yield camptothecine (30 g) and 10-hydroxycamptothecine (2 g) [6] After removal of the remaining camptothecines and acidic compounds, the mother liquor (*ca* 500 g) was dissolved in $CHCl_3$, and the $CHCl_3$ soln was extracted 3 × with 10% NaOH soln, washed with H_2O, dried over Na_2SO_4, and evapd to give a residue The residue (25 g) was chromatographed on silica gel (silica gel 60H, Merck, 1 kg), and eluted with $CHCl_3$ and $CHCl_3$–Me_2CO (7:3) The fractions were examined by TLC and combined, **1** and **2**, together with several indole alkaloids [10], were obtained from the $CHCl_3$–Me_2CO fractions.

22-*Hydroxyacuminatine* (**1**). Yellow crystals from Me_2CO (6 mg), mp 258–260° (dec.); UV λ_{max} nm (log ε) 222 (4.45), 251 (4.51), 274 sh (4 00), 285 (4.00), 313 sh (3.91), 365 sh (4.21) and 380 (4 23); IR ν_{max}^{KBr} cm^{-1}· 3350, 1651, 1610, 1595, 1500; ^{1}H NMR, see Table 1; MS *m/z* (rel. int): 314 (M^+, 100), 313 (40), 298 (16), 285 (40), 268 (18), 255 (24), 242 (10), 227 (10), 218 (8), 202 (14), 169 (12), 157 (14), 149 (14), 128 (60) and 113 (35); HRMS *m/z* M^+ 314.1032 for $C_{20}H_{14}N_2O_2$ (Δ -2.3 mmu).

19-*Hydroxymappicine* (**1**). Yellow crystals from Me_2CO (8 mg), mp 245–248° (dec.), UV λ_{max} nm (log ε): 218 (4 54), 245 (4.37), 253 (4 39), 293 (3 76), 314 sh (3.76), 332 sh (3.95) and 367 (4.25), IR ν_{max}^{KBr} cm^{-1}: 3380, 1658, 1577, 1560, 1500, ^{1}H NMR, see Table 1; MS *m/z* (rel. int.): 322 (M^+, 52), 305 (14), 291 (11), 278 (100), 277 (40), 263 (70), 249 (50), 248 (28), 235 (30), 221 (45), 219 (45), 205 (26) and 181 (26), HRMS *m/z* M^+ 322 1310 for $C_{19}H_{18}N_2O_3$ (Δ -0 7 mmu), CD (CH_3CN) $\Delta\varepsilon$ (nm +4 85 (221), +0 75 (244), +0 41 (277), +0.28 (303), −0 08 (315), −0.44 (328), −0.70 (365), −0 56 (384) and 0 (410)

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