Constituents of *Nothapodytes foetida*

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Five new alkaloids, along with camptothecin **1** and 9-methoxycamptothecin **2**, were isolated from a trunk bark extract of *Nothapodytes foetida* (Wight) Sleumer (Icacinaceae). The structures were elucidated by spectroscopic means as mappicine and the 9-methoxymappicine glycosides (**3–6**) and the di-*p*-coumaroylspermidine ester of a camptothecin-like compound **7**, which we have named foetidin I.

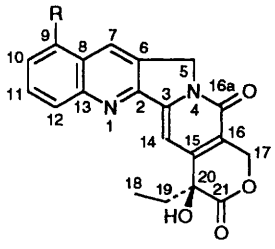
Camptothecin **1**, first isolated from the Chinese tree *Camptotheca acuminata* Decne (Nyssaceae),[1] is a heterocyclic alkaloid that demonstrates significant biological activity in various animal tumour models. It has a unique mechanism of action, producing DNA damage in the presence of topoisomerase I by binding and stabilizing a covalent DNA–topoisomerase I complex.

The antitumour activity of camptothecin resulted in Phase I clinical trials; because of its poor aqueous solubility it was evaluated as its sodium salt **1a**, but this was found to be ineffective in patients with advanced disseminated melanoma or gastrointestinal cancer. Moreover, severe toxicities were observed. In China, the sodium camptothecin **1a** has been reported to be effective in the treatment of gastric cancer, head and neck tumours and bladder carcinoma.[2]

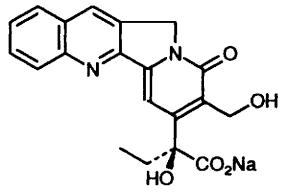
Some camptothecin derivatives (most of them are ring A modifications) showed improved antitumour activity and less bladder toxicity than camptothecin. Thus, the need to find additional water-soluble camptothecin derivatives that retain broad antitumour activity persuaded us to further investigate the natural source of camptothecin. This was particularly important when considering the low yields of the total syntheses reported to date.[3a–c]

The natural distribution of camptothecin refers to several plant families: *Camptotheca acuminata* (Nyssaceae),[1] *Nothapodytes foetida* (Wight) Sleum. (Icacinaceae),[4] *Merilliodendron megacarpum* (Helmsl.) Sleum. (Icacinaceae),[5] *Ervatamia heyneana* (Wall) T. Cooke (Apocynaceae)[6] and *Ophiorriza mungos* Linn. (Rubiaceae).[7]

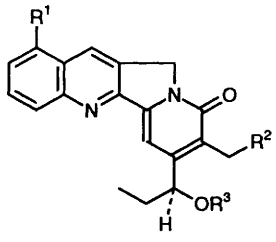
In this paper we report the chemical investigation of a methanolic extract of *Nothapodytes foetida* trunk bark.



1 R = H
2 R = OMe



1a



3 R^1 = H, R^2 = H, R^3 = β-D-Glc
4 R^1 = H, R^2 = H, R^3 = β-D-Glc$^{1\text{-}6}$Glc
5 R^1 = H, R^2 = OH, R^3 = β-D-Glc
6 R^1 = OMe, R^2 = H, R^3 = β-D-Glc$^{1\text{-}6}$Glc

Results and Discussion

A methanolic extract of *Nothapodytes foetida* bark was counter extracted first with dichloromethane and then with butanol. The dichloromethane extract contained mainly camptothecin **1** all the resonances and multiplicities associated with the A, B, C and D rings of camptothecin. The absence of the AB system at δ 5.44, the absence of the lactone carbonyl (C-21) at δ 173.45 and the presence of a singlet at δ 2.16 (3 H, 17-H) and a triplet at δ 5.14 (1 H, J = 6.7 Hz, 20-H) all suggested that compound **3** lacked the E ring of camptothecin.[9] The FAB-MS spectrum of compound **3** showed a pseudomolecular peak at M 469 $[M + H]^+$, which corresponded to the molecular formula $C_{26}H_{28}N_2O_7$ and a fragment at M 307 $[M - 162 + H]^+$

Table 1 ^{1}H NMR spectral data {300 MHz, [$^2H_6$]DMSO; δ (J)} of camptothecin **1**, 9-methoxycamptothecin **2** and compounds **3**–**7**

^{1}H	**1**[9]	**2**	**3**	**4**	**5**	**6**	**7**
5	5.27 s	4.99 s	5.20 s	5.14 s	5.18 s	5.18 s	5.18 s
7	8.68 s	8.59 s	8.61 s	8.54 s	8.60 s	8.78 s	8.60 s
9	8.12 d (8.5)		8.07 d (8.5)	7.99 d (8.5)	8.05 d (8.5)		8.05 d (8.6)
10	7.71 t (8.5)	6.99 d (7.6)	7.65 t (7.7)	7.60 t (7.7)	7.64 t (7.7)	7.11 d (7.7)	7.65 t (7.8)
11	7.31 t (8.5)	7.62 t (7.6)	7.81 t (7.7)	7.78 t (7.7)	7.81 t (7.7)	7.73 t (7.9)	7.80 t (7.8)
12	8.17 d (8.5)	7.55 d (7.2)	8.11 d (8.5)	8.07 d (8.5)	8.10 d (8.5)	7.67 d (8.5)	8.15 d (8.6)
14	7.35 s	7.18 s	7.34 s	7.42 s	7.36 s	7.38 s	7.77 s
17	5.44 s	5.37 s	2.16 s	2.16 s	4.65 and 4.49 d (11.4)	2.16 s	5.58 and 5.41 AB system (10.6)
18	0.90 t (7.2)	0.88 t (7.4)	0.85 t (7.4)	0.88 t (7.3)	0.89 t (7.4)	0.88 t (7.4)	0.84 t (7.2)
19	1.88 q (7.2)	1.85 m	1.82, 1.72 m	1.78, 1.68 m	1.83, 1.76 m	1.78, 1.68 m	2.02, 1.90 m
20			5.14 t (6.7)	5.06 t (6.7)	5.23 t (6.7)	5.07 t (6.7)	
OMe		3.95 s				4.01 s	
MeCO							1.96 s
OH		6.49 s					
Glc 1			3.91 d (7.3)	3.97 d (7.1)	3.95 d (7.5)	3.93 d (7.1)	
Glc 1′				4.33 d (8.0)		4.32 d (7.8)	
2′–6′							6.76 d (8.6)
3′–5′							7.35 d (8.6)
7′							7.29 d (15.8)
8′							6.36 d (15.8)
10′							8.19 t (5.2)
11′							3.21 m
12′							1.74 m
13′							2.84 bt (7.2)
15′							2.84 bt (7.2)
16′							1.55 m
17′							1.46 m
18′							3.13 m
19′							8.04 t (5.2)
21′							6.35 d (15.8)
22′							7.28 d (15.8)
24′–28′							7.34 d (8.6)
25′–27′							6.76 d (8.6)

The ^{1}H and ^{13}C NMR spectra confirmed the mappicine skeleton as well as the presence of a gentiobiosyl moiety (glucose $\xrightarrow{1,6}$ glucose). Thus, compound **4** was assigned the structure of mappicine 20-*O*-β-D-gentiobioside. Again, the CD spectrum suggested a 20*S*-configuration.

Compound **5** shows an *m*/*z* pseudomolecular peak at *M* 507 $[M + Na]^+$ in the FAB-MS spectrum, corresponding to the molecular formula $C_{25}H_{28}N_2O_8$. The ^{1}H and ^{13}C NMR spectra showed the same proton signals for the A, B, C and D rings. The singlet at δ 2.16 was substituted by an AB system at δ 4.65 and 4.49 (d, J = 11.4 Hz), which suggested the presence of an oxidation at C-17. The glucosyl moiety was again linked to C-20 (^{1}H and ^{13}C resonances) which possessed the *S*-configuraton, as determined by the CD spectrum.

17-Hydroxymappicine was never isolated from a natural source, but only obtained as a racemate during the partial synthesis of mappicine from camptothecin.[10]

Compound **6** showed peculiar differences in the aromatic region of the ^{1}H NMR spectrum, in comparison with the previous isolated compounds **3**–**5**. Five resonances were readily identified instead of the usual six protons present in compounds $C_{32}H_{40}N_2O_{12}$. From this compound **4** was identified as 9-methoxymappicine 20-*O*(*S*)-β-D-gentiobioside.

The ^{1}H NMR spectrum of compound **7** is more complex in comparison with the aforementioned compounds. The aromatic protons of camptothecin were readily identified, as well as the two H-5 resonances; the two 17-H protons are bonded to one acetoxy group as shown by the long-range correlation between 17-H and the acetate carbonyl. Also the ethyl moiety (C-18, C-19) was present, bonded to an oxygenated carbon (C-20), as in camptothecin **1**. The two D_2O-exchangeable protons at low field (δ 8.19 and 8.04, t) could be assigned to secondary amide groups. Also, two almost equivalent *para*-substituted benzene rings were detectable [δ 6.76 (4 H, d), 7.34 (2 H, d) and 7.35 (2 H, d)]; one substituent was, for both rings, a –CH=CHCO– moiety [δ 7.29 (d), 6.36 (d), 6.35 (d) and 7.28 (d)], while the second was either a phenolic –OH or a derivative of it. In the aliphatic part of the spectrum, fourteen protons, divided into six multiplets were present; in the ^{13}C NMR spectrum, they corresponded to seven methylene carbons; two bonded to an aminic nitrogen (δ 47.23 and 45.36), two bonded to an amidic nitrogen (δ 38.41 and 36.36) and three were aliphatic (δ 26.99, 26.87 and 24.04). Simple spin–spin decoupling experiments, along with the previous observations, made it possible to assign

Table 2 ^{13}C NMR spectral data (75.43 MHz, [$^2H_6$]DMSO, δ) of camptothecin **1**, 9-methoxycamptothecin **2** and compounds **3**–**7**

^{13}C	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	153.47 (s)	152.70 (s)	153.62 (s)	153.14 (s)	153.38 (s)	153.77 (s)	153.50 (s)
3	146.41 (s)	145.65 (s)	142.80 (s)	142.54 (s)	144.55 (s)	142.63 (s)	143.56 (s)
5	51.22 (s)	50.66 (t)	50.47 (t)	50.43 (t)	50.59 (t)	50.69 (t)	50.45 (t)
6	130.74 (s)	129.04 (s)	129.97 (s)	129.81 (s)	130.19 (s)	129.22 (s)	130.33 (s)
7	131.51 (d)	126.09 (d)	131.75 (d)	131.78 (d)	131.80 (d)	126.18 (d)	131.74 (d)
8	128.88 (s)	120.11 (s)	128.11 (s)	128.02 (s)	128.88 (s)	120.08 (s)	128.23 (s)
9	129.45 (d)	155.07 (s)	128.87 (d)	128.83 (d)	129.31 (d)	155.29 (s)	128.83 (d)
10	128.60 (d)	106.08 (d)	127.67 (d)	127.64 (d)	128.20 (d)	106.01 (d)	127.78 (d)
11	131.34 (d)	130.80 (d)	130.61 (d)	130.62 (d)	130.64 (d)	130.72 (d)	130.58 (d)
12	129.97 (d)	121.30 (d)	129.21 (d)	129.10 (d)	129.87 (d)	121.28 (d)	129.40 (d)
13	148.85 (s)	148.97 (s)	148.27 (s)	148.17 (s)	148.32 (s)	149.12 (s)	148.36 (s)
14	97.70 (d)	97.04 (d)	99.20 (d)	99.62 (d)	99.41 (d)	99.44 (d)	100.68 (d)
15	150.95 (s)	150.28 (s)	149.71 (s)	150.20 (s)	152.58 (s)	150.03 (s)	159.14 (s)
16	120.01 (s)	119.34 (s)	127.25 (s)	127.04 (s)	127.81 (s)	127.06 (s)(17)[a]	123.42 (s)
16a	157.76 (s)	157.07 (s)	160.80 (s)	160.90 (s)	160.57 (s)	160.85 (s)	161.43 (s)
17	66.25 (t)	65.68 (t)	12.23 (q)	12.46 (q)	54.34 (t)	12.49 (q)	59.87 (t)
18	8.82 (q)	8.21 (q)	10.05 (q)	10.29 (q)	10.53 (q)	10.30 (q)	9.29 (q)
19	31.28 (t)	30.79 (t)	28.75 (t)	28.84 (t)	29.46 (t)	28.85 (t)	33.40 (t)
20	73.39 (s)	72.80 (s)	74.54 (d)	75.45 (d)	74.53 (d)	75.34 (d)	80.28 (d)
21	173.45 (s)	172.80 (s)					175.23 (s)
OMe		56.42 (q)					
*Me*CO							21.22 (q)
Me*C*O							170.81 (s)
1′							159.40 (s)
2′							116.18 (d)
3′							129.63 (s)
4′							126.23 (d)
5′							129.63 (s)
6′							116.18 (d)
7′							139.37 (d)
8′							119.01 (d)
9′							166.27 (s)
11′							36.36 (t)
12′							26.99 (t)
13′							47.23 (t)
15′							45.36 (t)
16′							24.04 (t)
17′							26.87 (t)
18′							38.41 (t)
20′							165.83 (s)
21′							118.68 (d)
22′							139.06 (d)
23′							126.13 (s)
24′–28′							129.56 (d)
25′–27′							116.18 (d)
26′							159.31 (s)
Glc 1			99.99 (d)	100.45 (d)	100.06 (d)	100.45 (d)	
2			73.87 (d)	73.94 (d)	73.99 (d)	73.94 (d)	
3			76.97 (d)	76.81 (d)	77.27 (d)	76.81 (d)	
4			70.60 (d)	70.56 (d)	70.63 (d)	70.56 (d)	
5			77.55 (d)	77.22 (d)	77.39 (d)	77.22 (d)	
6			61.46 (t)	68.74 (t)	61.51 (t)	68.74 (t)	
1′				103.73 (d)		103.73 (d)	
2′				73.76 (d)		73.76 (d)	
3′				76.55 (d)		76.55 (d)	
4′				70.47 (d)		70.47 (d)	
5′				77.09 (d)		77.09 (d)	
6′				61.45 (t)		61.45 (t)	

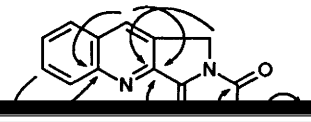
[a] Proton to which the corresponding carbon is long-range correlated.

recorded with several matrices). A peak at *M* 438, which corresponded to the fragment $[C_{25}H_{32}N_3O_4]^+$, supported the presence of a di-*p*-coumaroylspermidine moiety, the peaks at *M* 349 $[C_{20}H_{17}N_2O_4]^+$ and 305 $[349 - CO_2]^+$ were characteristic of camptothecin **1**.

No long-range coupling between the carbonyl at δ 175.23 (C-21) and any proton of the coumaroyl moiety was found; thus, the site of bonding of the side chain on the camptothecin-like skeleton could not be determined by NMR. The C-20 hydroxy could be the only alternative candidate for binding the side chain through an ether bond, but the chemical behaviour of compound **7** and some chemical shift consideration of its ^1H NMR spectrum, strongly support the indicated structure. When compound **7** was left at room temp. for 72 h in a buffered solution (pH 4.5), it gave camptothecin **1** almost quantitatively. Such behaviour was more consistent with a phenol ester linkage than with a phenol ether. The two coumaroyl moieties were inequivalent both in the ^1H and ^{13}C NMR spectra; all the resonances for one of them were slightly deshielded in comparison with the other; the major difference was displayed by the two amidic –NH– protons which resonated at δ 8.19 and 8.04. This was consistent with a decreased electron-

donating effect by the phenolic oxygen, which could be explained if it formed an ester linkage with C-21.

Finally, starting from the more deshielded amidic –NH– and following the coupling pattern throughout the spermidine chain, the site of bonding of the triamine onto the first coumaroyl unity (C-1 ⟶ C-9′) was determined. The most significant ^{1}H–^{13}C long range correlations are shown in Fig. 1.



were achieved along F_1 before full matrices transformations. ROESY experiments were run using the time-shared spin-lock sequence with a mixing time of 0.4–0.6 s. 1K data memory was used in the F_2 dimension and 256 t_1 increments; zero-filled to 1024 before Fourier transformation. Two 2D heterocorrelated spectra *via* $^{1}J_{C-H}$ were run; one for aromatic carbons (95–145 ppm) and one for aliphatic carbons (5–65 ppm). 2K data points were used along F_2 and 256 t_1 increments of 256 transients each were collected; zero-filling until 1K was performed along F_1 before full matrix transformation. Analogous conditions were adopted in the FLOCK experiment where a value of 7 Hz