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^{13}C-NMR ASSIGNMENTS OF CAMPTOTHECINE AND 10-HYDROXYCAMPTOTHECINE[1]

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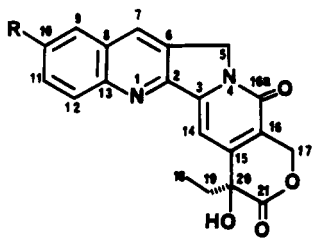
ABSTRACT.—The ^{13}C-nmr spectra of camptothecine [**1**] and 10-hydroxycamptothecine [**2**] have been assigned unambiguously through the use of APT, CSCM 1D, and selective INEPT spectroscopy.

Camptothecine [**1**] and 10-hydroxycamptothecine [**2**], two antitumor alkaloids isolated from *Camptotheca acuminata* Decne. (Nyssaceae) (1–4), have a unique quinoline skeleton with a highly conjugated five-ring system. The structure of camptothecine [**1**] was determined by X-ray analysis (1), and substantial synthetic, biosynthetic, and biological studies on **1** have been described (5). 10-Hydroxycamptothecine [**2**] is used clinically in the People's Republic of China for the treatment of stomach and liver cancers. As a result, there is currently a substantial resurgence of commercial interest in camptothecine and its analogues with a view to potentiating in vivo activity. The only prior ^{13}C-nmr analysis was obtained by comparison of natural abundance with "enriched" camptothecine (6), but several of the shifts were not unambiguously assigned because 10 of the 20 carbon atoms in **1** are quaternary carbons and some are quite proximate in chemical shift. In view of their biological significance, we present the unambiguous assignments of **1** and **2**.



1 R=H
2 R=OH

The H-9 and H-12 signals of **1** are very close to each other and were distinguished by nOe experiments, whereby through the irradiation of H-7 (δ 8.68, s), H-9 (δ 8.12, d, J = 8.5 Hz) and H-5 (δ 5.27, s) gave nOe enhancements of 5.4% and 3.3%, respectively. In the homonuclear COSY spectrum of **1**, the H-9 doublet was coupled to a triplet at δ 7.71 (J = 8.5 Hz), which should be assigned to H-10, and the H-12 (δ 8.17, d, J = 8.5 Hz) doublet was coupled to the H-11 triplet at δ 7.31 (J = 8.5 Hz). The COSY spectrum also showed the coupling between the H-19 methylene (δ 1.88, q, J = 7.2 Hz) and the H-18 methyl (δ 0.90, t, J = 7.2 Hz), and the long-range coupling between H-7 and H-5 (δ 5.27); therefore, the remaining signal at δ 5.44 (s, 2H) should be assigned to the H-17 methylene group.

The ^{13}C-nmr chemical shifts of **1** were assigned through a series of APT, CSCM 1D (7), and selective INEPT (8,9) experiments. One-bond polarization transfer experiments (CSCM 1D) confirmed C-5, C-7, C-9, C-10, C-11, C-12, C-14, C-17, C-18, and C-19 shifts by irradiation of the corresponding protons. The remaining quaternary carbons were unambiguously assigned by selective INEPT experiments, in which

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a particular proton is irradiated with a soft pulse resulting in magnetization transfer and the selective enhancement of carbon atoms three bonds away from the irradiated proton. Selective INEPT irradiation (Figure 1) of H-17 (δ 5.44) enhanced the carbon signals at δ 148.86, 157.76, and 173.43, thereby establishing C-15, C-16α, and C-21, respectively. Sometimes, $^2J_{CH}$ couplings are observed, e.g., the enhancement of C-16 in this irradiation. Care is therefore necessary to ascertain internal consistency of assignments. Irradiation of H-7 (δ 8.68) enhanced the C-2 (δ 153.46), C-13 (δ 148,85), and C-9 (δ 129.45) carbon signals, and irradiation of H-14 (δ 7.35) enhanced C-20 (δ 73.39) and C-16 (δ 120.03) carbon signals. Magnetization transfer from H-5 (δ 5.27) enhanced the C-2 (δ 153.47) and C-3 (δ 146.41) carbon signals, and also C-16a (δ 157.76), thereby distinguishing this carbon from C-15. The unambiguous assignment of the ^{13}C spectrum of **1** is shown in Table 1.

The corresponding ^{13}C chemical shift assignments of 10-hydroxycamptothecine [**2**] were independently obtained by use of APT, CSCM 1D (7), and selective INEPT (8,9) experiments. CSCM 1D irradiation of H-5 (δ 5.23, s), H-7 (δ 8.45, s), H-9 (δ 7.28, overlapped with H-14 signal), H-11 (δ 7.44, dd, J = 9.3, 2.4 Hz), H-12 (δ 8.04, d, J = 9.3 Hz), H-14 (δ 7.28, s), H-17 (δ 5.43), H-18 (δ 1.88, q, J = 7.2 Hz), and H-19 (δ 0.91, t, J = 7.2 Hz) enhanced the corresponding carbon signals. Selective magnetization transfer from H-17 (δ 5.43) enhanced C-21 (δ 173.46), C-16a (δ 157.72), and C-15 (δ 150.29). Irradi-

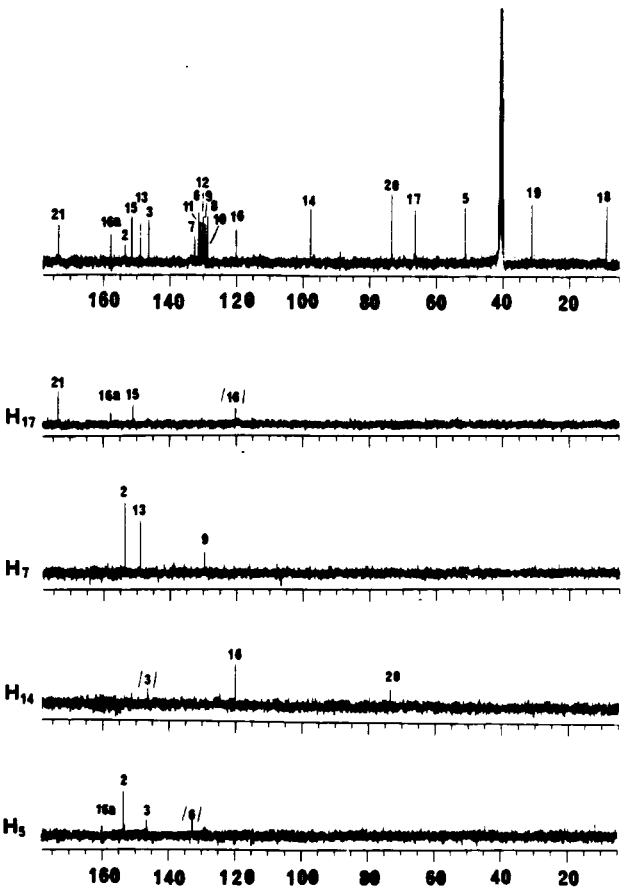

FIGURE 1. Selective INEPT spectra of **1**.

TABLE 1. ^{1}H- and ^{13}C-nmr Assignments of Camptothecine [**1**] and 10-Hydroxycamptothecine [**2**].[a]

Carbon	^{1}H nmr		^{13}C nmr		
	Compound		Compound		
	1	**2**	**1**	**1**[b]	**2**
C-2	—	—	153.47	156.8	150.28
C-3	—	—	146.41	145.4[c]	146.74
C-5	5.27 (s)	5.23 (s)	51.22	50.2	51.04
C-6	—	—	130.74	129.7	130.53
C-7	8.68 (s)	8.45 (s)	132.51	131.4[d]	130.18
C-8	—	—	128.88	127.9	130.57
C-9	8.12 (d, 8.5)	7.28 (d, 2.4)	129.45	128.4	109.67
C-10	7.71 (t, 8.5)	—	128.60	127.5	157.52
C-11	7.31 (t, 8.5)	7.44 (dd, 9.2, 2.4)	131.34	129.0	123.92
C-12	8.17 (d, 8.5)	8.04 (d, 9.3)	129.97	130.2[d]	131.50
C-13	—	—	148.85	149.9[c]	144.05
C-14	7.35 (s)	7.28 (s)	97.70	97.6	96.79
C-15	—	—	150.95	147.9[c]	150.95
C-16	—	—	120.01	119.0	118.99
C-16a	—	—	157.76	—	157.72
C-17	5.44 (s)	5.43 (s)	66.25	65.4	66.16
C-18	0.90 (t, 7.2)	0.91 (t, 7.2)	8.82	7.8	8.75
C-19	1.88 (q, 7.2)	1.88 (q, 7.2)	31.28	30.6	31.18
C-20	—	—	73.39	72.4	73.40
C-21	—	—	173.45	—	173.46

[a]Recorded in DMSO-d_6. Chemical shifts are reported in δ values downfield from internal TMS.
[b]Data taken from Hutchinson *et al.* (6).
[c,d]These assignments may be interchanged.

ation of H-5 (δ 5.23) enhanced C-7 (δ 130.18), C-2 (δ 150.29), and C-3 (δ 146.74), and irradiation of H-7 (δ 8.45) enhanced C-2 (δ 150.28), C-9 (δ 109.67), C-5 (δ 51.04), and C-13 (δ 144.05). The latter resonance was also enhanced by the irradiation of H-11 (δ 7.44). Irradiation of H-12 (δ 8.04) enhanced C-10 (δ 157.52) and C-8 (δ 130.53), and irradiation of H-14 (δ 7.28) and H-9 (δ 7.28, both signals are overlapped) enhanced C-20 (δ 73.40), C-16 (δ 118.99), and C-2 due to the irradiation of H-14, as well as C-13 (δ 144.05), C-11 (δ 123.92), and C-7 (δ 130.18) due to the irradiation of H-9. Comparison of the ^{13}C data of **1** and **2** showed that the data were consistent, and the unambiguous assignment of the ^{13}C-nmr spectrum of **2** is shown in Table 1.

Comparison of the ^{13}C-nmr shifts with those in Hutchinson *et al.* (6) (Table 1) showed that the chemical shifts for C-2, C-6, C-11, C-12, C-13, and C-15 should be revised. Carbons 16a and 21 were not observed previously.

EXPERIMENTAL

^{1}H-nmr spectra were determined on a Varian XL-300 instrument operating at 300 MHz. Standard Varian pulse programs were used for homonuclear COSY and NOE difference spectra. ^{13}C nmr and APT were obtained on the same instrument at 75.4 MHz. CSCM 1D and selective INEPT spectra were obtained on a Nicolet NMC-360 spectrometer operating at 90.8 MHz. Data sets of 16K covering a spectral width of 10,000 Hz were acquired. Proton pulse widths were calibrated by using a sample of HOAc in 10% C_6D_6 ($^{1r}J = 6.7$ Hz) in a 5-mm nmr tube (10). The radio frequency field strength for the soft proton pulse was on the order of 25 Hz for these experiments. For aromatic protons 8 Hz and 6 Hz were used as $^{3}J_{C\text{-}H}$ and for aliphatic protons, 6 Hz and 4 Hz. The compounds were obtained from the seeds of *C. acuminata* (11).

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LITERATURE CITED

1. M.E. Wall, M.C. Wani, C.E. Cook, K.H. Palmer, A.T. McPhail, and G.A. Sim, *J. Am. Chem. Soc.*, **94**, 3888 (1966).
2. M.C. Wani and M.E. Wall, *J. Org. Chem.*, **34**, 1364 (1969).
3. L.-T. Lin, T.-J. Chao, and J.-S. Hsu, *Acta Chim. Sin.*, **35**, 227 (1977).
4. J.-S. Hsu, T.-J. Chao, L.-T. Lin, and C.-F. Hsu, *Acta Chim. Sin.*, **35**, 193 (1977).
5. J.-C. Cai and C.R. Hutchinson, *Alkaloids (N.Y.)*, **21**, 101 (1983).
6. C.R. Hutchinson, A.H. Heckendorf, P.E. Daddona, E. Hagaman, and E. Wenkert, *J. Am. Chem. Soc.*, **97**, 5609 (1974).
7. S.K. Sarkar and A. Bax, *J. Magn. Reson.*, **62**, 109 (1985).
8. A. Bax, *J. Magn. Reson.*, **57**, 314 (1983).
9. G.A. Cordell, *Korean J. Pharmacogn.*, **19**, 153 (1988).
10. A. Bax, *J. Magn. Reson.*, **52**, 76 (1983).
11. L.-Z. Lin and G.A. Cordell, *Phytochemistry*, **28**, 1295 (1989).

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