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^{1}H- and ^{13}C-NMR SPECTRA OF CAMPTOTHECIN AND DERIVATIVES

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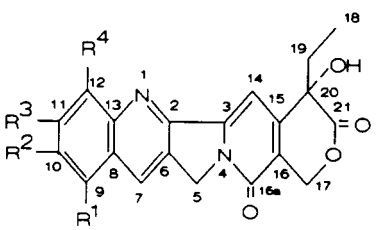
ABSTRACT.—^{1}H- and ^{13}C spectra of camptothecin, 9- and 12-nitrocamptothecins, and 10,11-methylenedioxycamptothecin are assigned from 1D and 2D nmr data.

There is current interest in the pyrroloquinoline alkaloid camptothecin [**1**] ($C_{20}H_{16}N_2O_4$), its congeners, and its synthetic analogues as antitumor agents (1) and topoisomerase I inhibitors (2,3). Although relevant chemistry is known (4–7) and ^{1}H-nmr spectra available, only an incomplete ^{13}C-nmr spectrum of **1** was available (8) at the inception of our work. More recently, ^{13}C spectra of **1** and 10-hydroxycamptothecin [**5**] have been reported by Lin and Cordell (9), with the suggestion that the earlier ^{13}C-nmr data for **1** needed revision.

Reliable assignments of ^{1}H- and most ^{13}C-nmr signals in spectra of **1** may be made with direct methods such as the attached proton test (APT) and homonuclear correlated spectroscopy (COSY). However, two pairs of aromatic protons (H-9/H-12, H-10/H-11) require care in assignment, and ten quaternary carbon atoms (seven conjugated olefinic or aromatic) pose assignment difficulties. We present here our unambiguous assignments of ^{1}H and ^{13}C spectra of **1** and of synthetic 10,11-methylenedioxycamptothecin [**2**], 9-nitrocamptothecin [**3**], and 12-nitrocamptothecin [**4**], using standard 2D nmr correlation methods, and reconcile our data with those of the prior literature (8,9).



1 $R^1=R^2=R^3=R^4=H$
2 R^2, R^3=-OCH_2O-, $R^1=R^4=H$
3 $R^1=NO_2$, $R^2=R^3=R^4=H$
4 $R^4=NO_2$, $R^1=R^2=R^3=H$
5 $R^2=OH$, $R^1=R^3=R^4=H$

EXPERIMENTAL

SPECTRAL METHODS.—Spectra were obtained using a JEOL GX270WB Fourier transform nmr spectrometer operating at 270 MHz for ^{1}H and 67.9 MHz for ^{13}C, with internal deuterium lock and TMS as internal frequency reference and analyte samples in [$^2H_6$]-DMSO, [^{2}H]-TFA, or their 1:3 admixture. Spectra acquired at 32K data points were zero-filled to 64K data points. Enhanced resolution of ^{1}H spectra was obtained with a moderate trapezoid apodization. Standard pulse sequences were used for APT (10), 2D COSY (11), and ^{1}H-^{13}C heteronuclear correlated spectroscopy (HETCORR) (12) operations. For HETCORR spectra 2048 × 256 data points were acquired and zero-filled to yield a 1024 × 512 transformed matrix, to which was applied a trapezoidal apodization. Pulse delays were optimized for one-bond ^{1}H-^{13}C correlations at $^1J_{CH}$ = 140 Hz and for two- and three-bond correlations at $^nJ_{CH}$ 5, 7, and 10 Hz, with the 7 Hz value being of greatest utility. For the least soluble samples there were 256 evolution increments with 1024 acquisitions per increment (interpulse delay 1.0 s), acquired over 83 h.

Additionally, fully coupled (FUCOUP) (13, 14) spectra were recorded to establish multiple bond connectivities for several quaternary ^{13}C atoms. Extended ^{1}H-^{1}H correlations in spectra of **1** in DMSO were also explored via the 2D HOHAHA (2D MLEV-17) experiment (15), with isotropic mixing time 256 msec optimal. All data were plotted in absolute mode.

RESULTS AND DISCUSSION

Nmr data and spectral assignments for **1** and derivatives **2**–**4** in DMSO and/or TFA are summarized in Table 1. Both solvents accorded solutions of adequate concentration for 2D nmr studies, and results in either solvent were mutually

TABLE 1. ^{1}H- and ^{13}C-nmr Spectra of Camptothecin [**1**] and Derivatives **2–4**.[a]

Position	Compound **1**				
	DMSO		TFA		Observed Couplings
	δ_C	δ_H	δ_C	δ_H	
2	152.47	—	147.90	—	$^3J_{CH}$ H-5, H-7, H-14
3	145.41	—	133.59	—	$^2J_{CH}$ H-14; $^3J_{CH}$ H-5
5	50.21	5.266	54.14	5.897	$^4J_{HH}$ H-7
6	129.72	—	140.70	—	$^2J_{CH}$ H-5, H-7
7	131.45	8.671	145.87	9.483	$^4J_{HH}$ H-9; $^3J_{CH}$ H-9
8	127.87	—	132.31	—	$^3J_{CH}$ H-10, H-12
9	128.39	8.109 dd[b]	132.34	8.503 d (8.06)	$^3J_{HH}$ H-10; $^3J_{CH}$ H-7, H-11
10	127.53	7.696 ddd[b]	140.35	8.441 t (8.06)	$^3J_{HH}$ H-11; $^3J_{CH}$ H-12
11	130.26	7.852 ddd[b]	134.37	8.232 t (8.06)	$^3J_{HH}$ H-12; $^3J_{CH}$ H-9
12	128.95	8.155 dd[b]	122.78	8.534 d (8.06)	$^3J_{CH}$ H-10
13	147.87	—	141.32	—	$^3J_{CH}$ H-7, H-9, H-11
14	96.59	7.338	108.27	8.411	$^5J_{HH}$ H-17
15	149.90	—	153.74	—	$^3J_{CH}$ H-17, H-19
16	118.98	—	126.44	—	$^2J_{CH}$ H-17; $^3J_{CH}$ H-14
16a	156.73	—	160.64	—	$^3J_{CH}$ H-17
17	65.20	5.418	69.01	5.845 ABq	—
18	7.68	0.877 t (6.97)	8.47	1.213 t (7.33)	$^3J_{HH}$ H-19; $^2J_{CH}$ H-19
19	30.28	1.877 q (6.97)	33.97	2.33 q (7.33)	—
20	72.28	—	76.32	—	$^2J_{CH}$ H-19; $^3J_{CH}$ H-18
21	172.33	—	178.60	—	$^3J_{CH}$ H-17, H-19
OH	—	6.509	—	—	—
CH_2	—	—	—	—	—

[a]Signals are singlets unless otherwise designated by abbreviations, d, doublet; t, triplet; q, quartet; m, multiplet. Observed couplings from two-dimensional COSY, HETCORR, FUCOUP, and HOHAHA spectra. All one-bond ($^1J_{CH}$) couplings were observed but not recorded in table.

[b]H-9–H-12 couplings: $^3J_{9,10}=8.06$, $^3J_{10,11}=6.96$, $^3J_{11,12}=8.73$, $^4J_{9,11}=1.77$, $^4J_{10,12}=1.99$ Hz.

confirmatory. Neither residual ^{1}H nor ^{13}C signals of either solvent interfere, although the eight-line ^{13}C spectrum of TFA [δ_C 116.715 q ($^1J_{CF}$ 283 Hz), 164.042 q ($^2H_{CF}$ 43.5 Hz)] and seven-line ^{13}C spectrum of DMSO must be recognized.

All carbon and hydrogen atoms of **1** in either solvent are represented by resolved signals. The ^{1}H signals were assigned from chemical shift and multiplicity, with the exception of the coupled A-ring aromatic protons and the isolated H-5 and H-17 methylene spin systems, these assignments being made using 2D COSY, HETCORR, and FUCOUP spectra, also taking advantage of solvent-influenced chemical shifts. Our data and assignments of ^{1}H spectra for **1** in DMSO are in agreement with previous reports (9,16) with two minor differences. Our assignment of the H-10 signal at higher field (δ_H 7.696) and of H-11 at lower field (δ_H 7.852) is opposite that of Lin and Cordell (9), where H-10 at δ_H 7.71 and H-11 at δ_H 7.31 reverse this order. Additionally, in our data the H-10 and H-11 signals appear as doublet of doublets doubled, thus revealing weak four-bond couplings with the H-12 and H-9 protons, respectively; these couplings have not been previously observed.

Assignments of ^{13}C spectra of **1** in either solvent were made from APT and 2D HETCORR pulse sequences that yielded one-, two-, and three-bond connectivities (Table 1) allowing unam-

TABLE 1. Continued.

Position	Compound **2**				
	DMSO		TFA		Observed Couplings
	δ_C	δ_H	δ_C	δ_H	
2	151.12	—	143.30	—	$^3J_{CH}$ H-7
3	146.47	—	131.86	—	$^2J_{CH}$ H-14
5	49.84	5.179 d (4.60)	53.89	5.718	$^4J_{HH}$ H-7
6	128.12	—	131.69	—	$^2J_{CH}$ H-5
7	129.91	8.437	141.68	9.036	$^4J_{HH}$ H-9
8	145.76	—	141.67	—	$^3J_{CH}$ H-12
9	102.87	7.483	106.31	7.539	$^3J_{CH}$ H-7
10	156.65[c]	—	160.77	—	$^2J_{CH}$ H-9
11	157.62[c]	—	160.45	—	—
12	104.62	7.483	106.14	8.149	$^7J_{HH}$ H-14
13	148.44	—	141.27	—	$^3J_{CH}$ H-7, H-9
14	95.66	7.234	99.21	7.680	—
15	149.83	—	153.67	—	$^3J_{CH}$ H-17, H-19
16	117.92	—	124.47	—	—
16a	158.07	—	155.31	—	—
17	65.09	5.395	68.66	5.749 ABq	—
18	7.46	0.864 t[d]	8.14	1.140 t (7.43)	$^2J_{CH}$ H-19
19	30.34	1.848 m[d]	33.58	2.151 q (7.43)	—
20	72.17	—	76.21	—	—
21	172.10	—	178.42	—	$^3J_{CH}$ H-17
OH	—	—	—	—	—
CH_2	102.28	6.265	110.47	6.429	$^5J_{HH}$ H-9

[c]May be exchanged.
[d]A_2B_3 pattern.

biguous assignments for all ^{13}C signals. Our ^{13}C assignments for thirteen ^{13}C signals referenced on solvent DMSO (δ_C 39.5) agree acceptably with those of Hutchinson *et al.* (8) similarly referenced (δ_C 39.6), and assignment differences may be reconciled upon recognition that their quaternary C-2 signal at δ_C 156.8 actually must be that of the amide carbonyl C-16a (not reported by them), that exchange of their aromatic ring C-11/C-12 and quaternary C-13/C-15 assignments is indicated, and that it be the quaternary C-2 and C-21 signals that were not observed.

Both sets of ^{13}C data appear 1.0–1.1 ppm to higher field than data of Lin and Cordell referenced on internal TMS (9). We have confirmed that the central ^{13}C signal of the solvent DMSO spectrum with internal TMS, with or without solute **1**, is at δ_C 39.5, which is thus a reliable reference point. However, the ^{13}C spectrum of **1** in Figure 1 of Lin and Cordell (9) displays solvent DMSO signals at field lower than δ_C 40 in what appears to be misregistry of spectra against internal reference TMS, perhaps accounting for the observed discepancies. We regard our data and the adjusted data of Hutchinson *et al.* (8) as the more reliable measures.

Chemical shifts of ^1H and ^{13}C signals are affected by solvent, with most ^1H signals of **1** for TFA at lower field relative to DMSO by $\Delta\delta_H$ +0.3 to +1.0 ppm. Similarly, ^1H signals of 10,11-methylenedioxycamptothecin [**2**] in TFA are at lower field ($\Delta\delta_H$ +0.1 to +0.6 ppm) than in DMSO. These solvent shifts are greater than those recorded for other alkaloids using TFA and $CDCl_3$ as solvents (17) and do not appear to be associated with protonation of

TABLE 1. Continued.

Position	Compound **3**		Compound **4**		
	TFA		DMSO		Observed Couplings
	δ_C	δ_H	δ_C	δ_H	
2	147.98	—	153.69	—	$^3J_{CH}$ H-5, H-7
3	127.05	—	144.26	—	$^2J_{CH}$ H-14; $^3J_{CH}$ H-5
5	54.11	5.946	50.42	5.324	$^4J_{HH}$ H-7
6	140.94	—	132.56	—	$^2J_{CH}$ H-5
7	130.21	10.255	126.73	9.154	$^4J_{HH}$ H-9
8	141.60	—	147.70	—	$^2J_{CH}$ H-7; $^3J_{CH}$ H-10
9	150.21	—	135.53	8.473 d (6.86)	$^3J_{HH}$ H-10
10	140.63	8.912 d (9.52)	128.69	7.993 dd (6.86, 8.67)	$^3J_{HH}$ H-11
11	137.04	8.463 dd (8.06, 9.52)	125.00	8.503 d (8.67)	
12	131.02	8.949 d (8.06)	145.72	—	$^3J_{CH}$ H-10
13	136.43	—	119.88	—	$^3J_{CH}$ H-9, H-11
14	109.37	8.463	97.27	7.405	$^5J_{CH}$ H-17
15	153.52	—	149.60	—	$^3J_{HH}$ H-17
16	124.50	—	119.88	—	$^2J_{CH}$ H-17; $^3J_{CH}$ H-14
16a	160.35	—	156.47	—	$^3J_{CH}$ H-17
17	68.68	5.825 ABq	65.09	5.42 d (6.43)	
18	8.14	1.189 t (7.33)	7.47	0.919 t (7.44)	$^3J_{HH}$ H-19
19	33.73	2.211 d (7.33)	30.42	1.886 q (7.44)	
20	76.00	—	72.07	—	$^3J_{CH}$ H-18
21	178.30	—	171.94	—	$^3J_{CH}$ H-17
OH	—	—	—	—	
CH_2	—	—	—	—	

either nitrogen atom, as neither the quinoline nor amide nitrogen of **1** is as basic as in some alkaloids. Degraded resolution of the H-9 and H-12 ^{1}H signals of **1** in TFA is accompanied by improved resolution of ^{13}C signals (except for the C-8/C-9 pair), the reverse relationship being the case for **1** in DMSO.

Proton signal multiplicity was also affected by solvent in the case of the H-17 methylene protons. The signal is a two-proton singlet for **1** in DMSO but an AB quartet for **1** in TFA. The AB quartet was also observed for DMSO solutions of **1** to which were added traces of $CDCl_3$

Solvent effects on ^{13}C spectra are more diverse, most ^{13}C signals being deshielded $\Delta\delta_C$ +0.8 to +7.3 ppm for **1** in TFA relative to DMSO but with C-6, C-7, C-10, and C-14 signals deshielded to a greater extend ($\Delta\delta_C$ +11.0 to +14.4 ppm) and C-2, C-3, C-12, and C-13 signals shielded by $\Delta\delta_C$ −4.6 to −15.5 ppm. Very similar effects were observed for ^{13}C spectra of **2**, where most ^{13}C signals were at lower field ($\Delta\delta_C$ +0.6 to +8.2 ppm), except for the greater effect on C-7 ($\Delta\delta_C$ +11.8 ppm), and with C-2, C-3, C-8, C-13, and C-16a at higher field ($\Delta\delta_C$ −2.7 to −15.7 ppm). Spectra obtained in DMSO-TFA (1:3) contained ^{1}H and ^{13}C signals generally between the extremes in neat solvent and aided in confirmation of assignments (data not shown).

In addition to the couplings indicated for neat solvent solutions of **1** in Table 1, other couplings $^2J_{CH}$ C-16/H-17, C-18/H-19, and C-20/H-19 and $^3J_{CH}$ C-15/

H-17, C-15/H-19, C-20/H-18, C-21/H-19, and C-21/H-17 were observed in the FUCOUP spectrum of **1** in binary solvent DMSO/TFA solution. These data confirm in detail the assignments made otherwise and also establish that the ethyl group evinced by ^{1}H count, δ_H, signal multiplicities, and COSY correlation of H-18/H-19 is located at C-20 unambiguously, a matter not disclosed by other HETCORR data. Yet other correlations were observed from the HOHAHA spectra of **1**. In addition to correlations observed by the standard means, HOHAHA spectra revealed a four-bond ($^4J_{5,7}$) coupling between H-5 and H-7 and a five-bond ($^5J_{14,17}$) coupling between H-14 and H-17 protons as well as hydrogen bonding between the 20-hydroxyl group and moisture in solvent DMSO.

^{1}H and ^{13}C data and assignments for the derivatives **2–4** presented in Table 1 were made in like manner, ^{1}H data for solutions of **2** and **3** in TFA being in satisfactory agreement with those previously reported (18). Assignments of ^{1}H data for **2** in TFA could be made directly, but the H-9 and H-12 signals from spectra obtained in DMSO were unresolved, yielding a two-proton singlet at δ_H 7.483, thus confounding assignment of C-9 and C-12 from one-bond ($^1J_{CH}$) correlations. However, ^{13}C assignments for C-9 and C-12 were made from $^3J_{CH}$ connectivity of H-7 to one at δ_C 102.87 also coupled by one-bond $^1J_{CH}$ to the two-proton H-9/H-12 singlet, thereby identifying C-9 suitably, with assignment of C-12 by attrition.

Furthermore, for **2** the number of quaternary ^{13}C signals was increased by two, thereby posing added limitation using our approaches. Although closelying signals of quaternary C-10 and C-11 for TFA solutions may be assigned from the $^2J_{CH}$ correlation of the δ_C 160.77 signal with H-9, want of unambiguous two- or three-bond connectivities in HETCORR spectra for DMSO solutions denied unambiguous assignment of the quaternary C-10 and C-11 signals in those spectra, and the values of Table 1 may require exchange.

Spectra of the 9- and 12-nitro isomers **3** and **4** were also assigned, with TFA the better solvent for **3**, DMSO for **4**. In these cases, sufficient **4** was available for 2D nmr acquisitions, and the couplings thereby discovered are listed in Table 1. Assignments posed no problems save for the degeneracy of the quaternary C-13 and C-16 signals; however, several observed connectivities ($^3J_{CH}$ for C-13/H-9 and C-13/H-11; $^2J_{CH}$ for C-16/H-17, $^3J_{CH}$ C-16/H-14) established the assignments. Sample availability precluded assignments for **3** from 2D nmr studies, so assignments are made by analogy with **1** and 12-nitro isomer **4**. In this case assignments of ^{13}C signals for quaternary C-3 and C-16 are uncertain and may require exchange.

Substituent effects on ^{1}H- and ^{13}C-nmr spectra of **1** are revealed by data for **2–4** in Table 1 and for **5** in the prior literature (9). A-ring oxygen substitution shields vicinal H-9 and H-12 of **2** and vicinal H-9 of **5**; nitro group substitutions deshield vicinal H-10 of **3** and vicinal H-11 of **4**. Long range effects include deshielding of H-7 by the nitro group of **3** and **4** but shielding of H-14 of **2** in TFA. All A-ring substituents deshield the substituted carbon atom by large effects ($\Delta\delta_C$ + 16 to + 29 ppm) in either solvent, and the oxygen substituents of **2** and **5** shield adjacent carbon atoms ($\Delta\delta_C$ − 7 to − 27 ppm), all consistent with expectations. Nitro substituents of **3** and **4** affected adjacent carbon atoms differently, the 9-nitro group of **3** deshielding C-8, the 12-nitro group of **4** shielding adjacent C-11 and C-13. More remote effects of substitution were also observed. For **2** in either solvent the C-8 quaternary atom was deshielded; for **3** C-12 was deshielded and C-13 shielded; for **4** C-8 and C-9 were de-

shielded. Other ^{13}C signals were either unaffected by substituents or affected to a minor degree only.

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