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^{1}H- AND ^{13}C-NMR SPECTRA OF THIOCOLCHICINE AND DERIVATIVES: A COMPLETE ANALYSIS

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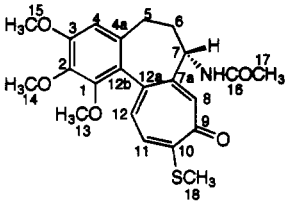
ABSTRACT.—Complete and unambiguous assignments of the ^{1}H- and ^{13}C-nmr spectra of thiocolchicine [**1**], 3-demethylthiocolchicine [**2**], and the thiocolchicosides [**3**] and [**4**] were made through extensive nmr studies, inclusive of homonuclear COSY and COSYLR, BIRDREV, APT, HETCOR, nOe difference, INEPT, and JMODXH experiments.

Colchicine is the principal alkaloid isolated from *Colchicum autumnale* L. (Liliaceae). It possesses antimitotic, antinflammatory, and antitumor effects. Among its analogues, thiocolchicine [**1**], 3-demethylthiocolchicine [**2**], and thiocolchicosides [**3**] and [**4**] are the most promising in terms of antitumor properties (1,2). Nevertheless, substantive nmr studies on these structures are lacking. There are available in the literature only a few reports concerning **1**, containing only incomplete and often ambiguous assignments (3–5). However, a complete study of the ^{1}H- and ^{13}C-nmr spectra of **1** and its derivatives would seem to be very useful because of the growing interest of medicinal chemists in antitumor products related to these structures. In this work we report a complete nmr study of thiocolchicine [**1**], 3-demethylthiocolchicine [**2**], tetra-*O*-acetylthiocolchicoside [**3**], and thiocolchicoside [**4**].

The ^{1}H-nmr spectral assignments of thiocolchicine [**1**] were completed by the analysis of one- and two-dimensional experiments, which allowed the unambiguous assignment of all of the signals. In particular, we employed homonuclear COSY (correlation spectroscopy) to observe direct couplings; COSYLR (long-range correlation spectroscopy) to observe the long-range ^{1}H-^{1}H couplings, and nOe spectra to observe nuclear Overhauser enhancements to confirm the spatial proximity of chemical groups. The spectra were recorded in $CDCl_3$ and DMSO-d_6 as solvents, between which interesting differences were noticed.

In DMSO-d_6, thiocolchicine [**1**] showed an exchangeable broad doublet at δ 8.63 (J=7.4 Hz), which was unambiguously assigned to the NH group, and was coupled to H-7 (m, δ 4.35). The COSY analysis, performed in DMSO-d_6, showed the correlation between this latter hydrogen and the two multiplets at δ 1.84 and δ 2.10, which were assigned to H-6a and H-6b. Furthermore, these signals were clearly correlated with two multiplets at δ 2.20 and δ 2.30 which were therefore assigned to H-5a and H-5b. A clear AB system (J=10.5 Hz) at δ 7.13 and 7.26, was attributed to H-12 and H-11, respectively. Indeed, the doublet at δ 7.26 showed a long-range coupling with the singlet at δ 2.40, associated with SCH_3. The sharp singlet at δ 7.02 was assigned to H-8, which showed a long-range coupling with the singlet at δ 1.80, corresponding to the methyl group of the acetamido chain. This was confirmed by its selective saturation that caused an enhancement of the signal at δ 7.02. Finally, the sharp singlet at δ 6.79 was attributed to H-4, which showed a long-range coupling with the singlet of the methoxy group at δ 3.84. This latter signal could be assigned to H-15. Methoxy group resonances (H-13, H-14) were not determined precisely by these methods. Further experiments, described below, clarified the correct assignments.

It is worth noting that, using $CDCl_3$



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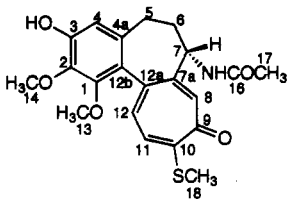

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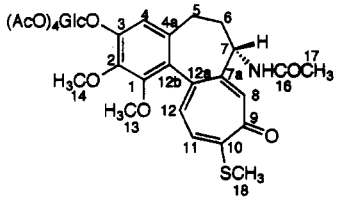

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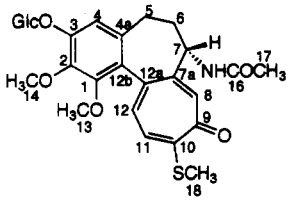

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as a solvent, there was a clear exchange in the chemical shifts of H-11 and H-12. In $CDCl_3$, in fact, H-12 was at lower field that H-11, while in DMSO-d_6 the chemical shifts were reversed. This behavior was revealed by decoupling difference spectroscopy by saturation of the signals of the AB system and the signal of SCH_3, respectively. In particular, saturation of the doublet at δ 7.08 caused an enhancement of the signal at δ 2.44 attributed to SCH_3. This permitted the assignment of the doublet at δ 7.08 to H-11 and consequently of the doublet at δ 7.31 to H-12. Also the other signals, particularly H-4 and H-8, were influenced by the solvent and showed shifts to slightly higher or lower fields. However, the difference between the chemical shifts in the different solvents was so small that it did not create any ambiguity in the ^{1}H-nmr assignments of **1** (Table 1).

The assignment of all the proton signals allowed a complete analysis of the ^{13}C-nmr spectrum of thiocolchicine [**1**]. The correct assignment of every carbon was made by performing correlation experiments of ^{13}C-^{1}H and ^{1}H-^{13}C (reverse spectroscopy) to observe the correlations via one, two, or three bonds (Table 1). By means of a ^{13}C-^{1}H correlation experiment (via J_1), we were able to assign every carbon-bearing hydrogen atom except C-13 and C-14: they showed two cross-peaks with the signals at δ 3.90 and 3.50, respectively, but they were too close together to be unambiguously assigned. The third methoxy group (C-15) was assigned without difficulty because of a clear cross-peak between the ^{1}H-nmr signal at δ 3.84 associated with H-15 and the ^{13}C-nmr signal at δ 55.7. Furthermore, it was even possible to differentiate between C-5 and C-6 because both of them showed two very evident cross-peaks with signals at δ 2.20/2.60 (H-5a and H-5b) and δ 1.84/2.20 (H-6a and H-6b), respectively.

On the basis of these results, another experiment was performed: a reverse ^{1}H-

TABLE 1. ^{1}H- and ^{13}C-Nmr Assignments of **1**.[a]

Carbon	^{1}H		^{13}C		
	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	$(J_{C,H})$[f]
1	—	—	150.3	151.6	
1a	—	—	126.2	126.2	($J_{1a,4}$=7.8) ($J_{1a,12}$=9.8)
2	—	—	140.6	142.2	($J_{2,4}$=7.8)
3	—	—	153.1	154.1	($J_{3,4}$≈2)
4	6.79	6.53	107.7	107.9	
4a	—	—	134.3	135.0	
5a	2.20	2.20	29.1[b]	30.5[b]	
5b	2.30	2.60	29.1[b]	30.5[b]	
6a	1.84	1.84	35.6[c]	36.8[c]	
6b	2.10	2.20	35.6[c]	36.8[c]	
7	4.35	4.35	51.2	52.8	
7a	—	—	151.1	152.5	($J_{7a,12}$=7.3)
8	7.02	7.39	126.6	127.2	
9	—	—	181.1	182.9	($J_{9,11}$=9.8)
10	—	—	157.2	156.8	($J_{10,11}$≈2)
11	7.26[d]	7.08[d]	127.8	128.8	
12	7.13[d]	7.31[d]	134.0	135.3	
12a	—	—	137.4	139.2	
13	3.50	3.66	60.7	61.8	
14	3.90	3.95	60.9	62.1	
15	3.84	3.91	55.7	56.6	
16	—	—	168.5	170.5	
17	1.80	1.99	22.3	23.3	
18	2.40	2.44	14.3	15.6	
NH	8.63[e]	—			

[a]Chemical shift values are reported as ppm downfield from TMS.
[b,c]Overlapped signals.
[d]AB system (J=10.5 Hz).
[e]Doublet (J=7.4 Hz).
[f]In Hz.

^{13}C analysis, optimized for J=3 Hz, which offered an excellent way to assign all quaternary carbons. This experiment was also useful to identify unambiguously C-13 and C-14 and to solve the problem of the assignments of H-13 and H-14 for which the one- and two-dimensional ^{1}H-nmr experiments were not successful (see above).

As stated above, H-15 was safely assigned for **1** by performing nOe experiments. In the ^{1}H-^{13}C reverse experiment (optimized for J=3 Hz), we observed a clear correlation via three bonds between H-15 and a signal at δ 153.1 which could consequently be associated with C-3. This assignment was also confirmed by a ^2J correlation between this latter carbon and H-4 at δ 6.5.

The signal at δ 140.6 was assigned to C-2 without ambiguity due to its correlation via three bonds with H-4 and the proton signal at δ 3.90 which was assigned as that of H-14. The carbon showing a J=3 Hz correlation with the last methoxy resonance at δ 3.50 appeared consequently to be C-1. Finally, with the correct assignments of these carbons and hydrogens in hand, it was possible to solve the last ambiguity at C-13 and C-14, by reinvestigating the one-bond ^{13}C-^{1}H correlations. Indeed, the observed cross-peaks between the signal of H-14 at δ 3.90 and the carbon at δ 60.9 and

between the H-13 signal at δ 3.50 and the carbon at δ 60.7, demonstrated that the first carbon was C-14 and the latter C-13. The unambiguous assignments for all carbons for compound **1** are reported in Table 1.

With the ^{1}H- and ^{13}C-nmr assignments of thiocolchicine [**1**] assured, we turned our attention to the assignments of the ^{1}H- and ^{13}C-nmr spectra of 3-demethylthiocolchicine [**2**], tetra-*O*-acetylthiocolchicoside [**3**], and thiocolchicoside [**4**]. The spectrum of **2** was very similar to that of thiocolchicine [**1**], with the only difference being the absence of an OCH_3 group. The assignments of the ^{1}H- and ^{13}C-nmr spectra were made by performing the same experiments as for **1**. These results are summarized in Tables 2 and 3.

TABLE 2. ^{1}H-Nmr Assignments of Compounds **2–4**.[a]

Proton	Compound		
	2[b]	**3**[c]	**4**[d]
4	6.55	6.86	6.87
5a	2.20	2.20	1.9–2.3[e2]
5b	2.50	2.30	1.9–2.3[e2]
6a	1.90	1.90	1.9–2.3[e2]
6b	2.20	2.20	1.9–2.3[e2]
7	4.40	4.23	4.28–4.40
8	7.03	7.03	7.03
11	7.27[f]	7.28[f]	7.28[f]
12	7.15[f]	7.15[f]	7.16[f]
13	3.54	3.55	3.55
14	3.80	3.75	3.85
17	1.86	1.83	1.86
18	2.42	2.43	2.42
NH	8.63[g]	8.63	9.63
1′	—	5.54[h]	4.94[i]
2′	—	5.15[h]	3.17–3.74[e3]
3′	—	5.42[h]	3.17–3.74[e3]
4′	—	5.04[h]	3.17–3.74[e3]
5′	—	4.17–4.35[e1]	3.17–3.74[e3]
6′	—	4.17–4.35[e1]	3.17–3.74[e3]

[a]Chemical shift values are reported as ppm downfield from TMS.
[b]Phenolic OH is a broad multiplet at δ 9.5.
[c]Acetyl groups: δ 2.03, 2.04, 2.06, 2.10.
[d]Glucoside OH: δ 5.35 (d, J=4.5 Hz), 5.14 (d, J=2.0 Hz), 5.10 (d, J=8.3 Hz), 4.65 (t, J=5.86 Hz).
[e1–3]Overlapped signals.
[f]AB system (J=10.5 Hz).
[g]Doublet (J=7.4 Hz).
[h]H-1′ (d, J=7.8 Hz), H-2′ (dd, J=9.5, 7.8 Hz), H-3′ (t, J=9.5 Hz), H-4′ (t, J=9.6 Hz).
[i]H-1′ (d, J=7.8 Hz).

TABLE 3. ^{13}C-Nmr Assignments of Compounds **2–4**.[a]

Carbon	Compound		
	2	**3**[b]	**4**[c]
1	150.6	150.3	150.3
1a	123.5	128.0	126.4
2	139.5	141.7	141.1
3	151.2	150.8	151.0[d]
4	111.2	111.8	111.0
4a	134.2	134.3	134.0
5	28.9	29.1	29.1
6	35.6	35.3	35.5
7a	151.1	150.1	151.0[d]
8	126.6	126.5	126.5
9	181.1	181.1	181.1
10	156.9	157.7	157.3
11	133.9	127.7	127.7
12	134.2	134.1	134.1
12a	137.8	136.9	137.3
14	60.5	61.0[d]	60.9[e]
15	60.7	61.0[d]	60.9[e]
16	168.4	[f]	168.6
17	22.4	22.3	22.4
18	14.3	14.3	14.3
1′	—	97.7	100.3
2′	—	70.6	73.2
3′	—	71.8	77.1
4′	—	68.0	69.8
5′	—	70.9	76.8
6′	—	61.7	60.7

[a]Chemical shift values are reported as ppm downfield from TMS.
[b]CH_3COO-: δ 20.3.
[c]Acetyl groups: δ 2.03, 2.04, 2.06, 2.10.
[d,e]Overlapped signals.
[f]C-16 and CH_3COO-: δ 169.9, 169.6, 169.3, 169.1, 168.6.

The most relevant features of the nmr spectra of **3** and **4** compared to those of **1** and **2**, are the sugar moieties. The complete assignment of the aliphatic region was made by performing a homonuclear COSY spectrum in each case. ^{1}H- and ^{13}C-nmr assignments for the aglycone in **3** and **4** were made by comparison of the ^{1}H- and ^{13}C-nmr shifts of **1** and **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded with a Bruker

AC 200 spectrometer (^{1}H, 200.13 MHz, ^{13}C, 50.327 MHz) equipped with an Aspect 3000 data system and a Varian Gemini 200 (^{1}H, 200.00 MHz, ^{13}C, 50.00 MHz). Spectra were recorded in DMSO-d_6. For thiocolchicine, experiments were also performed in $CDCl_3$. COSY spectra were recorded with 8 scans for each 64 increments and a spectral width of 1484 Hz; relaxation delay, D1=3 sec. COSYLR spectra were recorded with 64 scans for each 128 increments and a spectral width of 1207 Hz. Delays: relaxation delay, D1=2 sec; delay for evolution of shifts and couplings, D0=0.000003; fixed delay to emphasize effect of small J, D2=0.15 (D2 ca. 0.25/J). We used sine bell multiplication in both dimensions. Nuclear Overhauser effects were observed by performing nOe difference experiments using the standard Bruker software pulse sequence. ^{13}C-Nmr shift assignments were performed by: the JMODXH sequence using relaxation delays of D1=8, D2=0.005, to switch decoupler power and a delay of 0.0074 sec, evolution period for J-modulation; the APT sequence with standard Varian software; and ^{13}C-^{1}H HETCOR recorded with 640 scans for each 64 increments and spectral widths of 10416.7 Hz (F_2) and 1719.7 Hz (F_1). Decoupler conditions: decoupler proton low power 20 DB. Inverse detection (^{1}H-^{13}C) was performed using the BIRDREV basic sequence optimized for J=3 Hz to achieve long-range couplings. D1=2 sec, D2=0.166 sec, D4=1.0 sec. Spectra were recorded with 512 scans for every 40 experiments.

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