^{13}C-NMR Spectroscopy of Three Tetracycline Antibiotics: Minocycline Hydrochloride, Meclocycline, and Rolitetracycline

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Abstract □ ^{13}C-NMR data and spectral assignments are presented for tetracycline antibiotics minocycline hydrochloride, meclocycline, and rolitetracycline.

Keyphrases □ Tetracyclines—^{13}C-NMR spectra, chemical shifts assigned □ ^{13}C-NMR spectroscopy—tetracyclines, chemical shifts assigned □ Antibacterials—tetracyclines, ^{13}C-NMR spectra, chemical shifts assigned

PMR and, recently, ^{13}C-NMR spectroscopy have been widely applied to the characterization of tetracycline antibiotics (1–11). ^{13}C-NMR spectral analyses have been reported for tetracycline hydrochloride (I) and base (II) and the following related hydrochloride salts: chlortetracycline (III), methacycline (IV) (6-methyleneoxytetracycline), oxytetracycline, doxycycline (6-deoxyoxytetracycline), and demeclocycline (demethylchlortetracycline) (12).

This paper reports the ^{13}C-NMR data and spectral assignments for rolitetracycline (V) (*N*-pyrrolidinomethyltetracycline), minocycline hydrochloride (VI) (7-dimethylamino-6-demethyldeoxytetracycline hydrochloride), and meclocycline (VII) (7-chloro-6-methyleneoxytetracycline). These three tetracyclines gave rise to ^{13}C-NMR signals that could be tentatively assigned by virtue of their similarity to those of the six tetracycline antibiotics examined by Asleson and Frank (12). However, because several groups of resonance lines in the three tetracyclines were sufficiently closely spaced and different from those of the previously characterized tetracyclines, further study was necessary to clarify the assignments.

EXPERIMENTAL

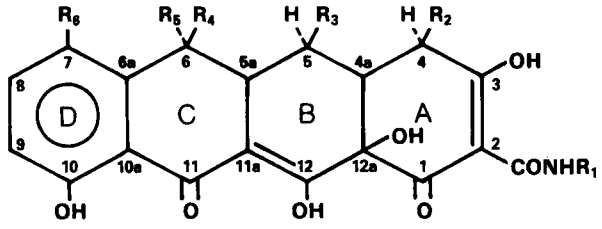
NMR Spectra—^{13}C-NMR spectra, described by 4096 data points, were obtained at 20 MHz[1]. Pulse widths of 10 μsec were used, which correspond to tip angles of 45° with 10-mm sample tubes. Single-frequency off-resonance decoupled (SFORD) ^{13}C-NMR spectra were obtained with proton-decoupling frequencies about 600 Hz upfield from tetramethylsilane and at full decoupling power levels. Spin-lattice relaxation times, T_1, were determined for two particular ^{13}C-nuclei of VII using the inversion–recovery method (13, 14).

The delay time, T, between sequences was 15 sec; the intervals between the 180 and 90° pulses, τ, were 0.1, 0.2, 0.6, 0.8, 1.2, 1.4, 1.8, 2.2, 2.8, and 4.0 sec. Approximately 0.2 *M* solutions of these tetracyclines were prepared in dimethyl sulfoxide-d_6, and ~0.1 *M* solutions were prepared in deuterium oxide. The latter were used to locate carbon signals obscured by the dimethyl sulfoxide-d_6 solvent.

Compounds—Minocycline hydrochloride, meclocyline, and rolitetracycline official and proposed FDA working standards were obtained from the National Center for Antibiotics Analysis.

RESULTS AND DISCUSSION

The ^{13}C-NMR spectrum of V (Table I), as expected, closely resembled that of II, so assignment of most resonance lines was straightforward. One notable exception in terms of chemical shift value was the amide carbon which, at 168.3 ppm, was more shielded than that of II at 172.6 ppm. Such a change in shielding for amide carbons is, however, expected upon conversion of a primary to a secondary amide (15). Four triplet signals at 22.5, 23.6, 50.1, and 56.6 ppm in an SFORD spectrum corresponded to the pyrrolidine α- and β-carbons, C-5, and the pyrrolidino *N*-methylene carbon. The pyrrolidine carbons at 23.6 and 50.1 ppm were readily distinguished from C-5 (22.5 ppm) and the pyrrolidino *N*-methylene carbon (56.6 ppm) on the basis of their double intensities, due to symmetry, in the nuclear Overhauser effect-suppressed proton noise-decoupled spectrum used to count carbon atoms (16). Moreover, the signals at 50.1 and 56.6 ppm were in the range expected for methylene carbons bonded to nitrogen and were so assigned (16).



general tetracycline structure

	R_1	R_2	R_3	R_4	R_5	R_6
I	H	$N(CH_3)_2$·HCl	H	CH_3	OH	H
II	H	$N(CH_3)_2$	H	CH_3	OH	H
III	H	$N(CH_3)_2$·HCl	H	CH_3	OH	Cl
IV	H	$N(CH_3)_2$·HCl	OH	$=CH_2$		H
V	$CH_2NC_4H_8$	$N(CH_3)_2$	H	CH_3	OH	H
VI	H	$N(CH_3)_2$·HCl	H	H	H	$N(CH_3)_2$
VII	H	$N(CH_3)_2$	OH	$=CH_2$		Cl

The carbon spectrum of VI was somewhat different from the spectra of the other tetracyclines (12) partly because VI is the only member of this family possessing methylene carbons in both the B- and C-rings—*viz.*, C-5 and C-6, and a dimethylamino group attached to the D-ring. With respect to the D-ring, the signals at 136.6 and 157.4 ppm are characteristic of quaternary carbons bonded to nitrogen and oxygen, respectively (16), and were assigned to C-7 and C-10, respectively. The two methine carbons in the D-ring, C-8 and C-9, were easily identified in an SFORD spectrum as the doublets at 115.6 and 128.5 ppm. The former was assigned to C-9 by comparison with C-9 signals of the other tetracyclines (16). The latter (C-8) was shielded by the *o*-dimethylamino group relative to the other tetracyclines, as is C-6a, to which the signal at 142.2 ppm was assigned (16).

Assignment of the triplets due to C-5 and C-6 and of the close-lying doublets due to C-4a and C-5a in the SFORD spectrum was developed in the following manner. The triplet at 33.8 ppm was assigned to C-6 because this carbon is benzylic and is expected to be more deshielded than the other methylene carbon, C-5, which appears at 29.6 ppm (16). The doublet at 35.1 ppm was assigned to C-4a by comparison with C-4a signals of the other tetracyclines (12), leaving C-5a at 31.6 ppm. With I as a model, removal of the methyl and hydroxyl groups from C-6 to produce a structure like VI is expected to have a much greater effect on C-5a, which is shielded by about 10 ppm, than on C-4a, which is essentially unchanged. The quartet at 41.2 ppm was assigned to the carbons of the D-ring dimethylamino group, and that at 44.4 ppm was assigned to those of the A-ring dimethylammonium group. The carbons of the latter group are expected to be more deshielded because of the positively charged nitrogen atom.

[1] Varian CFT-20 spectrometer.

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Table I—^{13}C-NMR Data [a] and Spectral Assignments for Several Tetracyclines [b]

Position	I[c]	II[c]	III[c]	IV[c]	V[d]	VI	VII
1	193.0	192.4	192.0	192.1	191.8	193.0	190.3
2	95.5	98.3	95.6	94.8	101.2	95.6	98.8
3	187.3	192.4	187.3	187.3	189.6	187.5	187.1
4	67.9 d	69.4 d	68.1 d	65.1 d	72.5 d	68.0 d	64.9 d
4a	34.8 d	37 d	34.9 d	41 d	38.6 d	35.1 d	40.0 d
5	27.0 t	22.4 t	27.1 t	63.9 d	22.5 t	29.6 t	65.5 d
5a	41 d	40 d	42 d	44.0 d	41.6 d	31.6 d	45.4 d
6	67.8	68.1	70.4	140.6	68.0	33.8 t	136.9
6a	148.0	148.0	143.6	142.6	148.0	142.2	138.6
7	116.9 d	116.8 d	121.2	117.2 d	116.7 d	136.6	119.6
8	136.5 d	136.4 d	139.7 d	137.0 d	135.9 d	128.5 d	137.8 d
9	115.2 d	115.4 d	118.9 d	116.4 d	115.1 d	115.6 d	118.3 d
10	161.4	161.4	160.7	160.7	161.4	157.4	159.8
10a	114.4	114.4	117.0	114.4	114.5	116.0	116.6
11	193.5	192.9	193.4	193.5	192.0	193.7	191.3
11a	106.9	105.8	106.1	105.1	105.3	108.3	105.4
12	175.1	176.8	175.7	173.6	180.1	174.3	178.2
12a	73.1	74.3	73.2	73.5	74.8	73.9	75.4
$N(CH_3)_2$	41 q	42 q	41 q	41 q	42.3 q	44.4 q	41.7 q
CONHR	172.1	172.6	172.1	171.6	168.3	171.9	170.5
CH_3	22.5 q	22.9 q	25.0 q	113.7 t[e]	22.9 q	41.2 q[f]	117.7 t[e]

[a] In dimethyl sulfoxide-d_6; parts per million from tetramethylsilane. [b] See text for numerical designation of compounds. [c] From Ref. 12. [d] Also 23.6 (t), β-pyrrolidine carbons; 50.1 (t), α-pyrrolidine carbons; 56.6 (t), CH_2N-pyrrolidine group. [e] $=CH_2$. [f] $N(CH_3)_2$.

As anticipated, the carbon spectrum of VII was similar to spectra of III and IV. Like VI, the methine carbons, C-8 and C-9, were readily identified in an SFORD spectrum and assigned to the doublets at 137.8 and 118.3 ppm, respectively, by comparison with corresponding carbon signals of III. Further examination permitted differentiation of the doublet due to C-4 from that of C-5, these two signals being observed at 64.9 and 65.5 ppm, respectively. However, the components of the low-field doublet were relatively sharp while those of the high-field doublet exhibited considerable splitting due to long-range coupling, *i.e.*, two bonds or more. Since C-4 should be coupled to the six methyl protons of the 4-dimethylamino group and C-5 does not appear to have as extensive coupling possibilities, the signal at 64.9 ppm was assigned to C-4. Moreover, the doublets assigned to C-4 in I, III, IV, and VI all displayed similar long-range coupling compared with other doublet signals.

Examination of a fully coupled spectrum of meclocycline allowed the signal of C-7 to be distinguished from that of C-10a. Two singlet signals were observed at 116.6 and 119.6 ppm in the SFORD spectrum and were ascribed to the quaternary carbons C-7 and C-10a. In the fully coupled spectrum, however, the low-field signal appeared as a doublet of doublets, with coupling constants of 4 and 7 Hz, while the high-field signal was observed as a 7-Hz doublet. Since both carbons should exhibit *trans*-oriented three-bond couplings to H-9 and since C-7 should be coupled to H-8 as well, the four-line signal at 119.6 ppm was assigned to C-7 and the doublet at 116.6 ppm was assigned to C-10a. The remaining doublets at 40 and 45.4 ppm were assigned to C-4a and C-5a, respectively, by comparison with analogous carbon signals of the other tetracyclines (12). In these compounds, C-4a resonances were consistently ~5 ppm upfield from those attributed to C-5a carbons; in IV, these two carbons appeared at 41 and 44 ppm, respectively.

Several lines of reasoning led to the assignment of signals at 136.9 and 138.6 ppm to C-6 and C-6a, respectively. First, in the SFORD spectrum the low-field signal was considerably broader than the high-field one, which was only 1 Hz wider than the corresponding resonance line in the proton noise-decoupled spectrum. Inspection of VII revealed that C-6a should be *trans*-coupled to one exocyclic methylene proton and *cis*-coupled to the other and should exhibit a *trans*-oriented three-bond coupling to H-8. While C-6 ought, in principle, to be coupled to these same exocyclic methylene protons, the olefinic *cis* and *trans* three-bond couplings of C-6a should be larger and result in a wider signal for this carbon in the SFORD spectrum (17–19). Therefore, the low-field signal was tentatively assigned to C-6a.

Second, a selective heteronuclear decoupling experiment was carried out in which H-8, at 7.67 ppm, was irradiated while an otherwise fully coupled spectrum of VII was obtained. The signal at 138.6 ppm (tentatively assigned to C-6a) appeared to sharpen compared with the corresponding signal in a control, fully coupled spectrum, while that at 136.9 ppm apparently remained unchanged. Since C-6a is expected to show a larger coupling to H-8 than is C-6 (*trans*-oriented three-bond coupling *versus* allylic-type four-bond coupling) (17–19), the low-field signal was assigned to C-6a, consistent with the first results.

Third, spin-lattice relaxation times, T_1, were determined for the two signals. The resonance at 136.9 ppm had a T_1 value of 0.7 sec while that of the 138.6-ppm signal was 2.5 sec. Examination of a Dreiding model of VII demonstrated that C-6 is situated approximately 2 Å from both the two exocyclic methylene protons and H-5a while C-6a is located about 2.5 Å from the nearer exocyclic methylene proton and about 3 Å, or greater, from H-9, H-5a, and the further exocyclic methylene proton. Due to the substantial decrease in effectiveness of the dipole–dipole relaxation mechanism with increasing internuclear distance (13), C-6 would be expected to have a shorter T_1 value than C-6a. Therefore, the signal at 136.9 ppm was assigned to C-6, again consistent with the initial assignment.

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