NMR Spectra of Tetracyclines: Assignment of Additional Protons

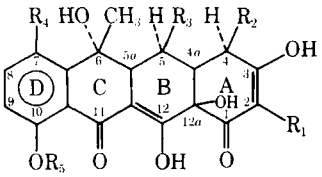
G. L. ASLESON, L. J. STOEL*, E. C. NEWMAN, and C. W. FRANK[x]

Abstract □ The assignments of four additional signals observed in the NMR spectrum of tetracycline hydrochloride were made with the help of modified tetracyclines and model compounds. The signals, all occurring downfield from 8 ppm, were caused by exchangeable protons. The signals were observed at 9.10, 9.59, 11.80, and 15.11 ppm and were assigned to the two amide protons, the 10-position hydroxyl proton, and the 12-position hydroxyl proton, respectively. The signals observed in the NMR spectra of other tetracyclines were also assigned.

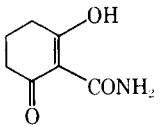
Keyphrases □ Tetracyclines—NMR spectra, assignment of additional protons □ NMR spectroscopy—assignment of additional protons in spectra of tetracyclines

NMR spectroscopy has been applied in the study of tetracyclines to elucidate stereochemical and structural features (1–6), to determine the microscopic dissociation constants (7, 8), and to monitor the kinetics of epimerization at the 4-position (9). The assignments of some protons in 16 tetracyclines were reported (10). The application of NMR to problems in tetracycline chemistry has not been extensive because the spectra obtained are fairly complex and the solubility properties of tetracyclines limit the choice of solvents. Observations of signals between 4 and 16 ppm for the exchangeable protons of tetracycline have been reported, but no assignments have been made (10).

Four exchangeable protons, all downfield from 8 ppm *versus* tetramethylsilane, were observed in the NMR spectrum of tetracycline hydrochloride (I) in deuterated dimethyl sulfoxide at 9.10, 9.59, 11.80, and 15.11 ppm (Fig. 1). Similar NMR spectra were obtained for oxytetracycline hydrochloride (II) and chlortetracycline hydrochloride (III) with signals at 9.15, 9.65, 11.75, and 15.19 ppm for the former and at 9.18, 9.67, and 12.28 ppm for the latter. The structure of the tetracycline molecule contains seven easily exchangeable protons, of which five are hydroxyl protons and two are associated with the amide functional group.



	R_1	R_2	R_3	R_4	R_5
I:	$CONH_2$	$N(CH_3)_2$	H	H	H
II:	$CONH_2$	$N(CH_3)_2$	OH	H	H
III:	$CONH_2$	$N(CH_3)_2$	H	Cl	H
IV:	CN	$N(CH_3)_2$	H	H	H
V:	CN	$N(CH_3)_2$	H	H	$SO_2C_6H_5$
VI:	$CONH_2$	H	H	H	H



VII

The variability of hydroxyl proton chemical shifts is caused principally by hydrogen bonding which, in turn, depends upon concentration, temperature, and solvent. Intramolecular hydrogen bonds are much less susceptible to change by these factors than intermolecular bonds. The reasons for a downfield shift resulting from hydrogen bonding are not completely understood, but it is generally found that the magnitude of the downfield shift increases with the strength of the hydrogen bond (11). The 6- and 12*a*-position hydroxyl protons would not be expected to have chemical shifts downfield from 8 ppm.

EXPERIMENTAL

NMR Spectra—The NMR spectra were obtained using high-resolution NMR spectrometers[1] at probe temperatures of 33 ± 1°. The chemical shift data were measured relative to tetramethylsilane[2] as an internal standard, and all values are presented as parts per million. All NMR spectra were recorded using d_6-dimethyl sulfoxide as a solvent unless otherwise noted. Deuterated solvents were obtained from commercial sources[3].

Compounds—Samples of tetracycline hydrochloride[4], oxytetracycline hydrochloride[4], dedimethylaminotetracycline[4], and chlortetracycline[5], as well as the model compounds and starting materials[6] for the syntheses of model compounds, were purchased from commercial sources.

2-Carbamoylcyclohexane-1,3-dione was synthesized by the procedure of Tomino (12). 8,9,10-Trihydroxy-1-keto-1,2,3,4-tetrahydroanthracene was synthesized by the procedure of Hochstein *et al.* (13). 2-Cyanotetracycline was prepared according to Söder and Siedel (14). The synthesis of 10-benzenesulfonyl-2-cyanotetracycline followed the procedure of McCormick *et al.* (15).

RESULTS AND DISCUSSION

A number of model compounds have structures similar to portions of the tetracycline molecule. These compounds and modified tetracyclines were investigated to aid in the assignments of the observed exchangeable protons of tetracycline. The data shown in Table I for some model compounds give the range of the chemical shift of a hydroxyl proton in phenols beta to a carbonyl, in β-diketones, and in β-triketones.

Signals of identical shape were observed in the NMR spectrum of tetracycline hydrochloride at 9.10 and 9.59 ppm. The NMR spectrum of 2-carbamoylcyclohexane-1,3-dione (VII), which is similar to the A-ring in tetracycline, produced signals at 8.7 and 9.5 ppm which were assigned to the amide protons. The assignments of

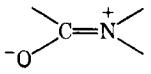
[1] Varian A-60 and HA-100.
[2] Matheson, Coleman and Bell.
[3] Stohler Isotopes and Bio-Rad Laboratories.
[4] Pfizer, Inc.
[5] American Cyanamid Co.
[6] Aldrich Chemical Co.

Table I—Chemical Shifts of Hydroxyl Protons

Compound	Parts per Million
Phenols	
1,8-Dihydroxyanthraquinone	12.0
1-Amino-4-hydroxyanthraquinone	13.9
5-Hydroxy-1,4-naphthoquinone	12.0
1,8-Dihydroxynaphthalene	11.0
1-Amino-4-hydroxy-2-methoxyanthraquinone	14.22[a] (16)
Chrysin, galangin, quercetin, kaempferol, myricetin, morin	12.40 to 12.86[b] (17)
Pinocembrin, naringenin, hesperetin, dihydroquercetin, dihydrokaempferol	11.95 to 12.20[b] (17)
β-Diketones	
2,4-Pentanedione	15.1
1-Phenyl-1,3-butanedione	16.3
1,3-Diphenyl-1,3-propanedione	17.2
1,3-(4,4′-Dibromo)diphenyl-1,3-propanedione	16.61[a] (16)
Ethyl anisoylacetate	15.3[a] (16)
β-Triketones	
2-Carbamoyl-5,5-dimethylcyclohexane-1,3-dione	18.7[c] (18)
2-Carbamoyldecalin-1,3-dione	18.98[c] (18)
3-Acetylpentane-2,4-dione	17.4[d] (19)
2-Acetyl-5,5-dimethylcyclohexane-1,3-dione	18.1[d] (19)
2-Hydrocinnamoyl-5,5-dimethylcyclohexane-1,3-dione	18.1[d] (19)
2-Cinnamoyl-5,5-dimethylcyclohexane-1,3-dione	18.6[d] (19)
Ceroptene	18.9[d] (19)

[a] *d*-Chloroform. [b] d_6-Dimethyl sulfoxide. [c] d_5-Pyridine. [d] Carbon tetrachloride.

these protons in 2-carbamoyl-5,5-dimethylcyclohexane-1,3-dione and 2-carbamoyldecaline-1,3-dione to signals observed between 9.6 and 10.3 ppm in d_5-pyridine solution were previously made (18). No signals were observed in the spectra of either 2-cyanotetracycline (IV) or 10-benzenesulfonyl-2-cyanotetracycline (V) in the 9.0–10.5-ppm range. Thus, the signals at 9.10 and 9.59 ppm in the spectrum of tetracycline hydrochloride were assigned to the amide protons. The nonequivalence of these protons can be explained by the hindered rotation about the carbon–nitrogen bond due to the contribution of the canonical form (20):



The third downfield signal in the spectrum of tetracycline hydrochloride was observed at 11.80 ppm. As shown in Table I, most phenolic protons, favorably situated for chelation with a carbonyl, have chemical shifts in the range of 12 ppm. The only tetracyclines shown in Table II that have no observed signals near 12 ppm are 10-benzenesulfonyl-2-cyanotetracycline and dedimethylaminoanhydrotetracycline (IX). The absence of this signal for 10-benzenesulfonyl-2-cyanotetracycline is easily explained because the phenolic proton has been replaced by the benzenesulfonyl ester. Thus, the signal at 11.80 ppm in the spectrum of tetracycline hydrochloride was assigned to the phenol in the 10-position. The case of dedimethylaminoanhydrotetracycline is discussed later.

The final peak was observed in the spectrum of tetracycline hydrochloride at 15.11 ppm. All tetracyclines in Table II have a signal in the 15–17-ppm range with the exception of chlortetracycline hydrochloride. The β-diketones in Table I also have their enolic proton chemical shifts in the 15–17-ppm range. Thus, the signal at 15.11 in tetracycline hydrochloride was assigned to the hydroxyl proton in the 12-position.

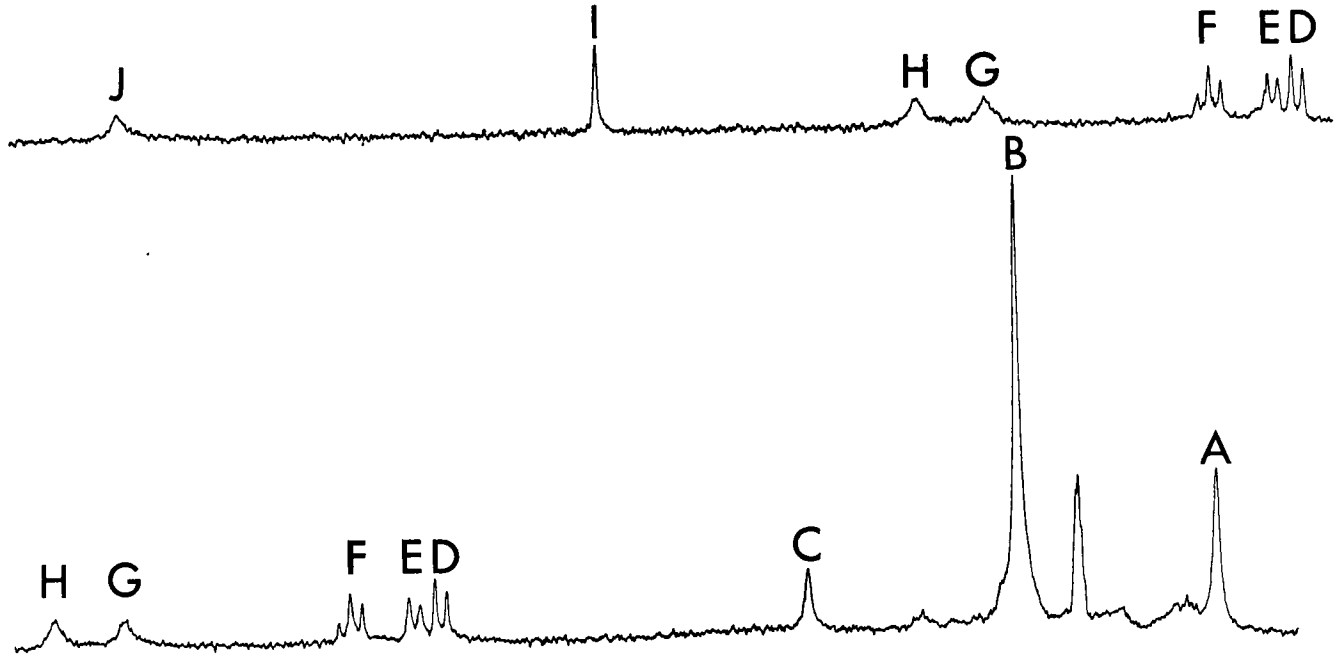


Figure 1—*NMR spectrum of tetracycline hydrochloride in* d_6*-dimethyl sulfoxide (0.20* M*). Key: A, 1.54 ppm, C-6,* CH_3*; B, 2.92 ppm, C-4,* $N(CH_3)_2$*; C, 4.38 ppm, C-4, H; D, 6.92 ppm, C-9, H; E, 7.10 ppm, C-7, H; F, 7.55 ppm, C-8, H; G, 9.10 ppm; H, 9.59 ppm; I, 11.80 ppm; and J, 15.11 ppm. See Table II for additional assignments.*

Table II—Proton Resonance Data for Tetracyclines

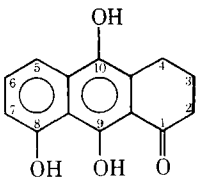
Compound	NH_1	NH_2	10-OH	12-OH	3-OH
I	9.10	9.59	11.80	15.11	—[b]
II	9.15	9.65	11.75	15.19	—[b]
III	9.18	9.67	12.28	—[b]	—[b]
IV	—[a]	—[a]	11.90	15.28	—[b]
V	—[a]	—[a]	—[a]	16.26	—[b]
VI	8.70	8.94	11.83	15.24	18.34
IX	8.85	8.98	9.90	15.29[c]	18.16

[a] Proton not present in compound. [b] Not observed in NMR spectrum. [c] 11-Position hydroxyl proton.

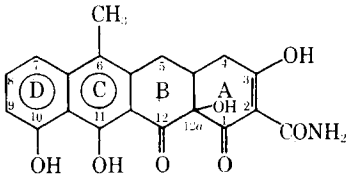
The assignments of the 10- and 11-position hydroxyl protons for dedimethylaminoanhydrotetracycline (Table II) were aided by investigation of the model compound 8,9,10-trihydroxy-1-keto-1,2,3,4-tetrahydroanthracene (VIII). Three signals for the exchangeable protons for this compound were observed at 8.60, 9.84, and 15.65 ppm. The 8.60-ppm signal was assigned to the 10-hydroxyl proton, because it is not capable of intramolecular hydrogen bonding. The 8-hydroxyl proton would not be expected to hydrogen bond intramolecularly as strongly as the 9-hydroxyl proton. Therefore, the signal at 9.84 ppm was assigned to the 8-hydroxyl proton and the signal at 15.65 ppm was assigned to the 9-hydroxyl proton. The 15.65-ppm signal fell into the range of chemical shifts for enolic protons of β-diketones, providing additional evidence for this assignment. The signals observed at 9.90 and 15.29 ppm in the spectrum of dedimethylaminoanhydrotetracycline were assigned to the 10-position and 11-position hydroxyl protons, respectively.

Finally, it must be explained why only four signals were detected in the downfield portion of the NMR spectrum of tetracycline hydrochloride instead of the predicted five. As shown in Table II, signals were observed for dedimethylaminotetracycline (VI) and dedimethylaminoanhydrotetracycline at greater than 18 ppm and were assigned to the 3-position hydroxyl proton on the basis of the data for β-triketones in Table I.

It has been observed that the presence of the dimethylamino group increases the acidity of the 3-position hydroxyl (21, 22).



VIII



IX

Therefore, the signal of the 3-position enolic proton in tetracycline hydrochloride was not observed because of rapid exchange.

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